

**KARPAGAM ACADEMY OF HIGHER EDUCATION**

(Deemed to be University)

(Established Under Section 3 of UGC Act 1956)

Coimbatore - 641021.

(For the candidates admitted from 2017 onwards)

DEPARTMENT OF BIOCHEMISTRY**SUBJECT : MEMBRANE BIOLOGY AND BIOENERGETICS****SEMESTER : I****SUBJECT CODE: 18BCU103****CLASS: I B.Sc., BIOCHEMISTRY****Course objectives**

- This course elucidates in detail the structural and functional importance of biomembranes in cellular existence.
- The course provides an in-depth understanding on the concept of sub-cellular compartmentalization within a cell and its biological implications.

Course outcome

- The course gives a sound knowledge on structure, organization and constitutional dynamics of biomembrane.
- The course lights the importance of photosynthetic system in Biomembranes.

Unit 1

Biomembranes, membrane structures and membrane dynamics: Composition of biomembranes- prokaryotic, eukaryotic, neuronal and sub-cellular membranes. Study of membrane proteins. Fluid mosaic model with experimental proof. Monolayer, planer bilayer and liposomes as model membrane systems. Polymorphic structures of amphiphilic molecules in aqueous solutions- micelles and bilayers. CMC, critical packing parameter. Membrane asymmetry. Macro and micro domains in membranes. Membrane skeleton, lipid rafts, caveolae and tight junctions. RBC membrane architecture. Lateral, transverse and rotational motion of lipids and proteins. Techniques used to study membrane dynamics - FRAP, TNBS labeling etc. Transition studies of lipid bilayer, transition temperature. Membrane fluidity, factors affecting membrane fluidity.

Unit 2

Membrane transports: Thermodynamics of transport. Simple diffusion and facilitated diffusion. Passive transport- glucose transporter, anion transporter and porins. Primary active transporters- P type ATPases, V type ATPases, F type ATPases. Secondary active transporters- lactose permease, Na⁺-glucose symporter. ABC family of transporters- MDR, CFTR. Group translocation. Ion channels- voltage-gated ion channels (Na⁺/K⁺ voltage-gated channel), ligand-gated ion channels (acetyl choline receptor), aquaporins, bacteriorhodopsin. Ionophores - valinomycin, gramicidin.

Unit 3

Vesicular transport, membrane fusion and bioenergetics: Types of vesicle transport and their function- clathrin, COP I and COP II coated vesicles. Molecular mechanism of vesicular transport. Membrane fusion. Receptor mediated endocytosis of transferrin. Laws of thermodynamics, state functions, equilibrium constant, coupled reactions, energy charge, ATP cycle, phosphorylation potential, phosphoryl group transfers. Chemical basis of high standard energy of hydrolysis of ATP, other phosphorylated compounds and thioesters. Redox reactions, standard redox potentials and Nernst equation. Universal electron carriers.

Unit 4

Oxidative phosphorylation: Mitochondria. Electron transport chain- its organization and function. Inhibitors of ETC and uncouplers. Peter Mitchell's chemiosmotic hypothesis. Proton motive force. Fo F1ATP synthase, structure and mechanism of ATP synthesis. Metabolite transporters in mitochondria. Regulation of oxidative phosphorylation. ROS production and antioxidant mechanisms. Thermogenesis. Alternative respiratory pathways in plants.

Unit 5

Photophosphorylation : General features of photophosphorylation, historical background, Hills reaction, photosynthetic pigments, light harvesting systems of plants and microbes and resonance energy transfer. Bacterial photophosphorylation in purple bacteria, Green sulfur bacteria and *Halobacterium salinarum*. Photophosphorylation in plants - structure of chloroplast, molecular architecture of Photosystem I and Photosystem II, Z-scheme of photosynthetic electron flow, oxygen evolving complex and action of herbicides. Cyclic photophosphorylation and its significance. Photo inhibition. Evolution of oxygenic photosynthesis.

REFERENCES

Nelson, D.L. and Cox, M.M., W.H.Freeman., Lehninger: Principles of Biochemistry (2013) 6th ed., and Company (New York), ISBN:13: 978-1-4641-0962-1 / ISBN:10:1-4641-0962-1.

Lodish, H., Berk, A., Kaiser, C.A., Krieger, M., Bretscher, A., Ploegh, H., Amon, A. and Scott, M.P., Molecular Cell Biology (2013) 7th ed., W.H. Freeman & Company (New York), ISBN:13: 978-1-4641-0981-2.

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Voet, D.J., Voet, J.G. and Pratt, C.W., (2008) Principles of Biochemistry 3rd ed., John Wiley & Sons, Inc. (New York), ISBN:13: 978



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DEPARTMENT OF BIOCHEMISTRY

LESSON PLAN

STAFF NAME: Mr. A. RAMAKRISHNAN

SUBJECT NAME: MEMBRANE BIOLOGY & BIOENERGETICS

SUB.CODE: 18BCU103

SEMESTER: I

CLASS: I B.Sc., BIOCHEMISTRY

S. No.	Duration of Period	Topics to be Covered	Books referred with Page No.	Web page referred
UNIT I				
1	1	Composition of biomembranes- prokaryotic, eukaryotic, neuronal and sub-cellular membranes.	R1: 409-411	
2	1	Study of membrane proteins. Fluid mosaic model with experimental proof.	R2: 371-372	
3	1	Polymorphic structures of amphiphilic molecules in aqueous solutions- micelles and bilayers.	R3: 423-428 R1: 412-415	
4	1	Monolayer, planer bilayer and liposomes as model membrane systems	R3: 423-426	
5	1	CMC, critical packing parameter. Membrane asymmetry. Macro and micro domains in membranes.	R3: 426-427	
6	1	Membrane skeleton, lipid rafts, caveolae and tight junctions. RBC membrane architecture	R3: 429-430	
7	1	Lateral, transverse and rotational motion of lipids and proteins.	R1: 416-420	
8	1	Techniques used to study membrane dynamics - FRAP, TNBS labeling etc.	R2: 381-385	
9	1	Transition studies of lipid bilayer, transition temperature. Membrane fluidity, factors affecting	R2: 380-381	

		membrane fluidity.		
Total No. of Hours planned for Unit I : 9 hours				
UNIT II				
1	1	Thermodynamics of transport. Simple diffusion and facilitated diffusion.	R3: 433-435	
2	1	Passive transport- glucose transporter, anion transporter and porins.	R1: 441-446	
3	1	Primary active transporters- P type ATPases,	R1: 447-448	
4	1	Primary active transporters- V type ATPases, F type ATPases.		
5	1	Secondary active transporters- lactose permease, Na ⁺ -glucose symporter. ABC family of transporters- MDR, CFTR. Group translocation.	R1: 452-456	
6	1	ABC family of transporters- MDR, CFTR. Group translocation.	R1: 452-456	
7	1	Ion channels- voltage-gated ion channels (Na ⁺ /K ⁺ voltage-gated channel), ligand-gated ion channels (acetyl choline receptor)	R2: 410-416	
8	1	I Aquaporins, bacteriorhodopsin. Ionophores - valinomycin, gramicidin.	R2: 406-408	
9	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit II : 9 hours				
UNIT III				
1	1	Types of vesicle transport and their function- clathrin,	R1: 592-598	
2	1	COP I and COP II coated vesicles		
3	1	Molecular mechanism of vesicular transport.	R1: 606-612	
4	1	Membrane fusion. Receptor mediated endocytosis of transferrin.		
5	1	Laws of thermodynamics, state functions, equilibrium constant, coupled reactions	R3: 88-89	
6	1	Energy charge, ATP cycle, phosphorylation potential, phosphoryl group transfers	R3: 89-93	
7	1	Chemical basis of high standard energy of hydrolysis of ATP, other phosphorylated compounds and thioesters		
8	1	Redox reactions, standard redox potentials and Nernst equation. Universal electron carriers	R1: 59-60 R3: 94-95	
9	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit III : 9 hours				

UNIT IV				
1	1	Mitochondria. Electron transport chain- its organization and function	R2: 691-701	
2	1	Inhibitors of ETC and uncouplers.		
3	1	Peter Mitchell's chemiosmotic hypothesis. Proton motive force.	R2: 701-704	
4	1	Fo F1 ATP synthase, structure and mechanism of ATP synthesis		
5	1	Metabolite transporters in mitochondria	R2: 708-714	
6	1	Regulation of oxidative phosphorylation. ROS production and antioxidant mechanisms	R2: 716-718	
7	1	Thermogenesis	R3: 228-229	
8	1	Alternative respiratory pathways in plants		
9	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit IV : 9 hours				
UNIT V				
1	1	General features of photophosphorylation, historical background, Hills reaction, photosynthetic pigments	R2: 723-724	
2	1	Light harvesting systems of plants and microbes and resonance energy transfer	R2: 725-730	
3	1	Bacterial photophosphorylation in purple bacteria, Green sulfur bacteria and <i>Halobacterium salinarum</i>	R2: 730-736	
4	1	Photophosphorylation in plants - structure of chloroplast	R1: 513-519	
5	1	Molecular architecture of Photosystem I and Photosystem II	R1: 519-524	
6	1	Z-scheme of photosynthetic electron flow		
7	1	Oxygen evolving complex and action of herbicides. Cyclic photophosphorylation and its significance	R2: 738-742	
8	1	Photo inhibition. Evolution of oxygenic photosynthesis	R2: 742-744	
9	1	Revision and Possible QP discussion		
Total No. of Hours planned for Unit V : 9 hours				
Previous year ESE Question Paper Discussion				
1	1	Previous year ESE question paper discussion		
2	1	Objective questions discussion		
3	1	Revision		
Total Hours Planned: 45 + 3 : 48				

REFERENCES

- R1:** Lodish, H., Berk, A., Kaiser, C.A., & Krieger, M., (2012). *Molecular Cell Biology*, 7th edition. W.H. Freeman & Company, London.
- R2:** Nelson, D.L. and Cox, M.M., W.H. Freeman., Lehninger: *Principles of Biochemistry* (2013) 6th ed., and Company (New York), ISBN:13: 978-1-4641-0962-1 / ISBN:10:1-4641-0962-1.
- R3:** Murray, R.K., Bender, D.A., Botham, K.M., & Kennelly, P.J., (2012).Harper's illustrated Biochemistry, 29th edition. McGraw-Hill Medical. London.

UNIT-I

SYLLABUS

Biomembranes, membrane structures and membrane dynamics: Composition of biomembranes-prokaryotic, eukaryotic, neuronal and sub-cellular membranes. Study of membrane proteins. Fluid mosaic model with experimental proof. Monolayer, planar bilayer and liposomes as model membrane systems. Polymorphic structures of amphiphilic molecules in aqueous solutions-micelles and bilayers. CMC, critical packing parameter. Membrane asymmetry. Macro and micro domains in membranes. Membrane skeleton, lipid rafts, caveolae and tight junctions. RBC membrane architecture. Lateral, transverse and rotational motion of lipids and proteins. Techniques used to study membrane dynamics - FRAP, TNBS labeling etc. Transition studies of lipid bilayer, transition temperature. Membrane fluidity, factors affecting membrane fluidity.

Biomembranes, membrane structures and membrane dynamics

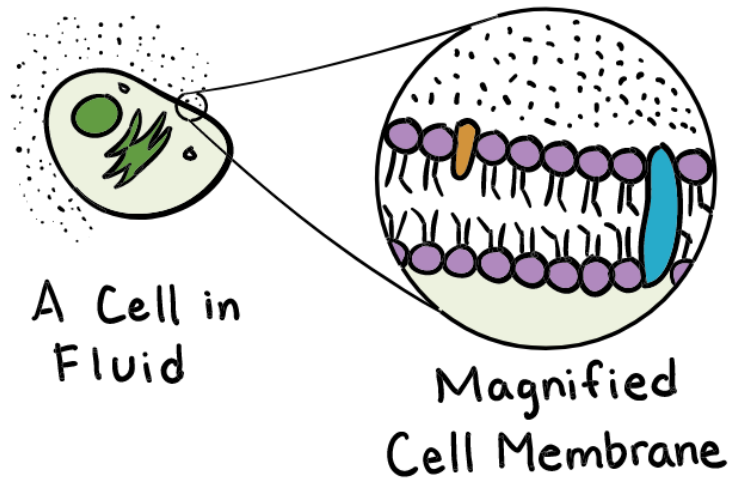
Composition of biomembranes

All cells are surrounded by a layer of membrane that separates their internal environment (cytoplasm) from the external environment (exoplasm or extracellular matrix). Additionally, the organelles in cells are compartmentalized with the help of bio membranes, with similar chemical composition as that of the plasma membrane.

The membrane-enclosed organelles in the cytoplasm of cells (apart from the cell itself and the nucleus) are endoplasmic reticulum, Golgi apparatus, lysosome, vacuole, chloroplasts and mitochondria. So it is very important for us to understand the structure and chemical composition of the biomembranes.

It may seem like the human body is made up of a chaotic mix of random parts, but that's not the case. The liquid nutrients, cell machinery, and blueprint information that make up the human body are tucked away inside individual cells, surrounded by a double layer of lipids.

The purpose of the cell membrane is to hold the different components of the cell together and to protect it from the environment outside the cell. The cell membrane also regulates what enters and exits the cell so that it doesn't lose too many nutrients, or take in too many ions. It also does a pretty good job of keeping harmful things out.

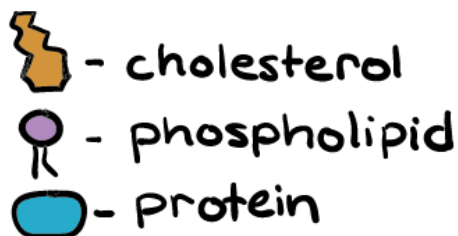
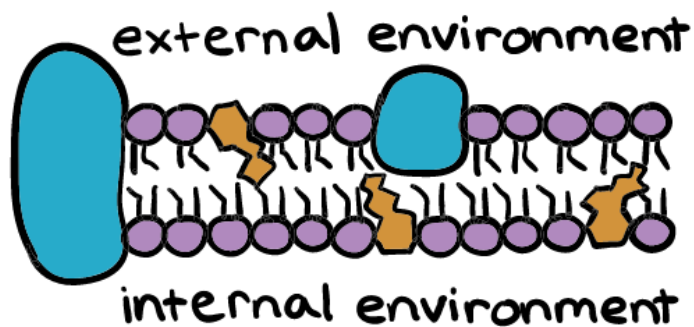


What's it made up of?

The cell membrane is primarily made up of three things:

1. Phospholipids
2. Cholesterol
3. Proteins

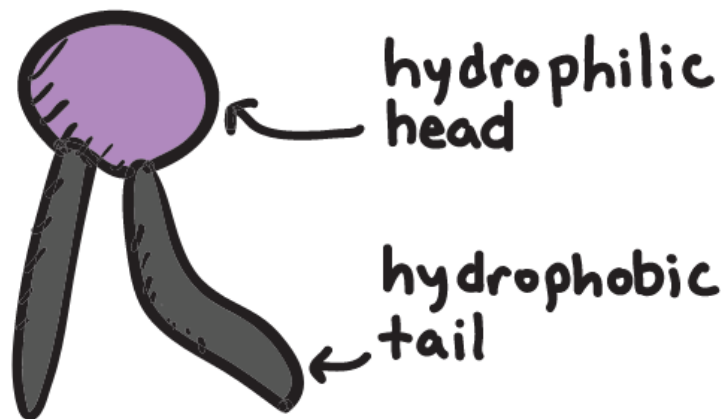
Phospholipid Bilayer



1) Phospholipids

- There are two important parts of a phospholipid: the head and the two tails.
- The head is a phosphate molecule that is attracted to water (*hydrophilic*).
- The two tails are made up of fatty acids (chains of carbon atoms) that aren't compatible with, or repel, water (*hydrophobic*).
- The cell membrane is exposed to water mixed with electrolytes and other materials on the outside and the inside of the cell.
- When cellular membranes form, phospholipids assemble into two layers because of these hydrophilic and hydrophobic properties.
- The phosphate heads in each layer face the aqueous or watery environment on either side, and the tails hide away from the water between the layers of heads, because they are hydrophobic. Biologists call this neat assembling characteristic "self-assembly".

phospholipid



2) Cholesterol

Cholesterol is a type of steroid which is helpful in regulating molecules entering and exiting the cell. We'll talk about this in more depth later, but for now remember it's part of the cell membrane.

3) Proteins

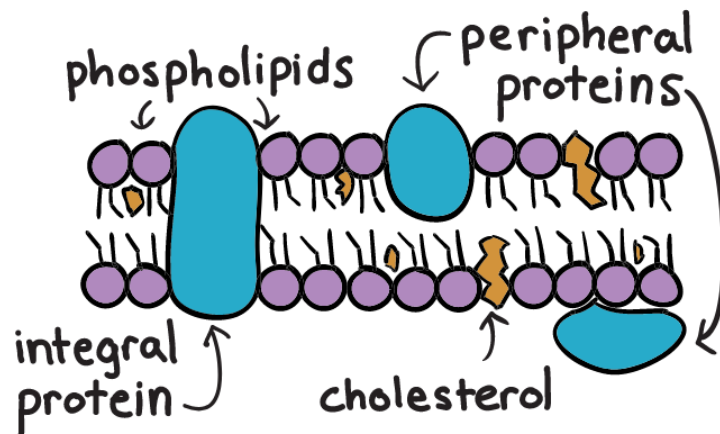
The cell is made up of two different types, or "classes", of proteins.

- Integral proteins* are nestled into the phospholipid bilayer and stick out on either end. Integral proteins are helpful for transporting larger molecules, like glucose, across the cell membrane. They have regions, called "*polar*" and "*nonpolar*" regions, that correspond with the polarity of the phospholipid bilayer.

Polar and nonpolar refer to the concentration of electrons on a molecule. Polar means the electrons are not evenly distributed, making one side of the molecule more positively charged or negatively

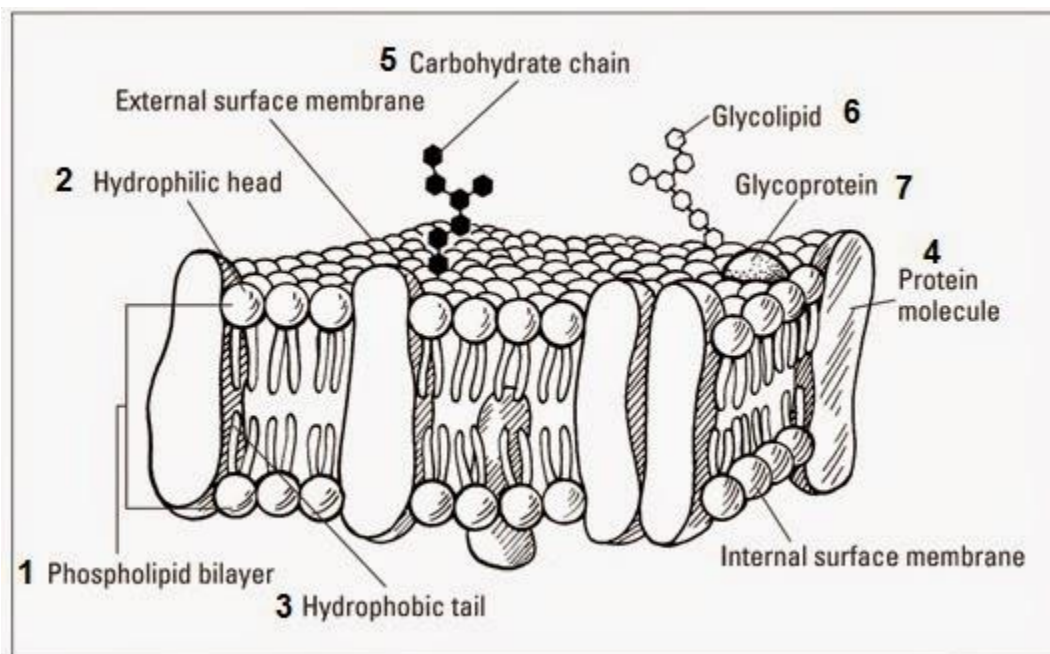
charged than another side. Nonpolar means the electrons are evenly distributed, so the molecule is evenly charged across the surface.

- ii) The other class of protein is called *peripheral proteins*, which don't extend across the membrane. They can be attached to the ends of integral proteins, or not, and help with transport or communication.



The Fluid Mosaic Model of Biological Membranes

The fluid mosaic model of lipid bilayer membranes, codified by Singer and Nicolson in 1972, describes the essential features of the biological membrane. It is a two-dimensional fluid, or liquid crystal, in which the hydrophobic integral components such as lipids and membrane proteins are constrained within the plane of the membrane, but are free to diffuse laterally. In the image shown here, two integral membrane proteins are imbedded in the membrane.



Monolayer, planer bilayer and liposomes as model membrane systems

A monolayer is a single, closely packed layer of atoms, molecules or cells.

In some cases it is referred to as a self-assembled monolayer.

Outer membrane the high surface tension of water in nature

E.g of monolayer Species

1. Bacteriorhodospin
2. Gramicidin
3. Rhodospin
4. Micelles

Planer bilayer

The lipid is a thin polar membrane

This is made of two layers of lipid molecules

The barrier around all cells

Types of bilayer membranes

1. Black lipid membrane
2. Supported lipid bilayers
3. Tethered bilayer lipid membranes

1. Black lipid membrane

Problems associated with painted bilayers are residual solvent and limited life time.

Some researchers believe that pockets of solvent trapped between the two bilayers leaflets can disrupt normal protein function.

2. Supported lipid bilayers

Advantage

This is planer structure sitting on a solid support

This upper face of the bilayer is exposed to free solution

High flow rates or vibration

Disadvantage

Unwanted interaction with the substrate

Interaction with the polymer/lipid

3. Tethered bilayer lipid membranes

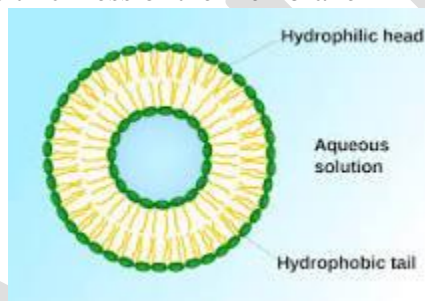
Intra membrane mobility of supported lipid bilayers

Half membrane spanning tether lipids with benzyl disulphide

Synthetic archaea analogue full membrane spanning lipids with phytanoly chains to stabilize the structure.

Liposomes

A liposome is a spherical vesicle having at least one lipid bilayer. Liposomes are most often composed of phospholipids, especially phosphatidylcholine, but may also include other lipids, such as egg phosphatidylethanolamine, so long as they are compatible with lipid bilayer structure. Liposome size 20nm, Liposome thickness of the membrane 4nm.

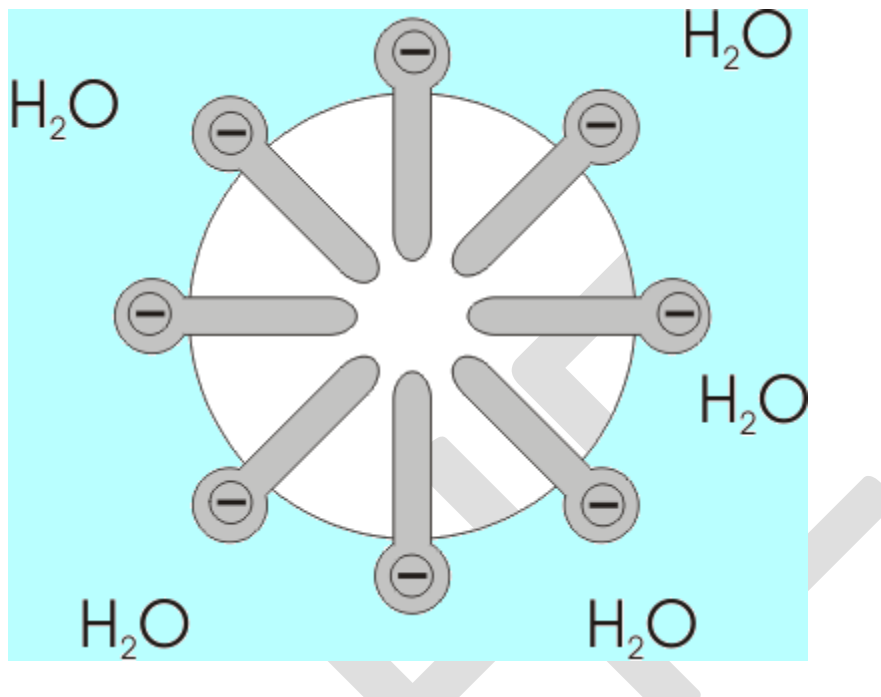


Polymorphic structures of amphiphilic molecules in aqueous solutions- micelles and bilayers.

Micelles

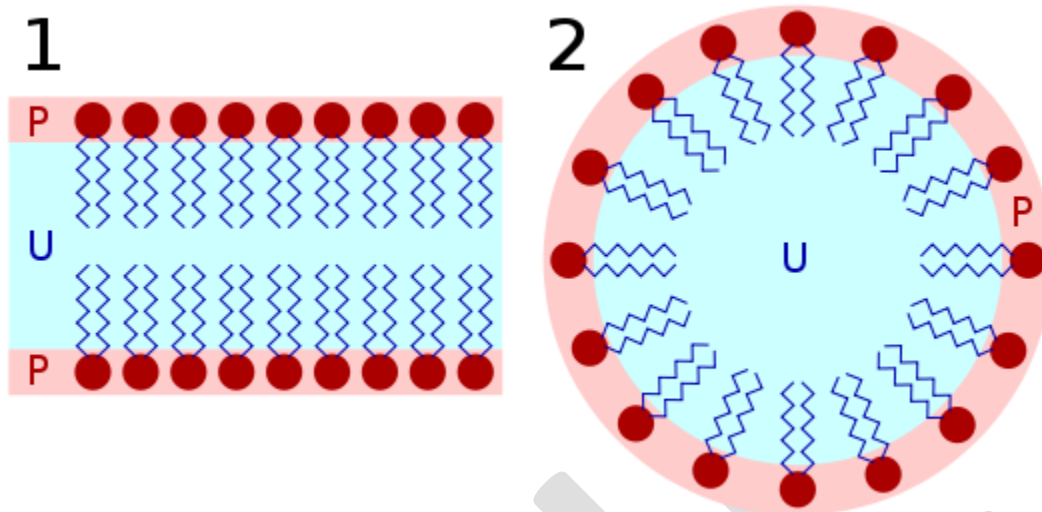
Micelles are lipid molecules that arrange themselves in a spherical form in aqueous solutions. The formation of a micelle is a response to the amphipathic nature of fatty acids, meaning that they contain both hydrophilic regions (polar head groups) as well as hydrophobic regions (the long hydrophobic chain). Micelles contain polar head groups that usually form the outside as the surface of micelles. They face to the water because they are polar. The hydrophobic tails are inside and away from the water since they are nonpolar. Fatty acids from micelles usually have a single hydrocarbon chain as opposed to two hydrocarbon tails. This allows them to conform into a spherical shape for lesser steric hindrance within a fatty acid. Fatty acids from Glycolipids and phospholipids, on the other hand, have two hydrophobic chains that are too bulky to fit into the spherical shape as micelles do. Micelles form spontaneously in water, as stated above this spontaneous arrangement is due to the amphipathic nature of the molecule. The driving force for this arrangement is the hydrophobic interactions the molecules experience. When the hydrophobic tails are not sequestered from water this results in the water forming an organized cage around the hydrophobic tail, and this entropy is unfavourable. However, when the lipids form micelles the hydrophobic tails interact with each other rather than with water. Separating the water from the hydrophobic tails during micelle formation increases the disorder of the system, and this increase

in entropy is favorable.



Bilayers

The preferred structure of lipids in aqueous solutions are usually a bilayer sheet of lipids rather than spherical micelles. This is because the two fatty acid chains are too big and bulky to fit into the interior of a micelle. Therefore, micelles usually have one hydrocarbon chain instead of two. Lipid bilayers" form rapidly and spontaneously in an aqueous media and are stabilized by hydrophobic interactions, Van der Waals attractive forces, and electrostatic interactions. The function of the lipid bilayer is to form a barrier between the two sides of the membrane. Due to the fact that the lipid bilayer consists of hydrophobic fatty acid chains, ions and most polar molecules have trouble passing through the bilayer. The one exception to this rule is water because water has a high concentration, small size, and a lack of a complete charge. In order for a molecule to pass through the lipid bilayer it must move from an aqueous environment to a hydrophobic environment and then back into an aqueous environment. Therefore the permeability of small molecules is related to the solubility of said molecule in a nonpolar solvent versus the solubility of the molecule in water.

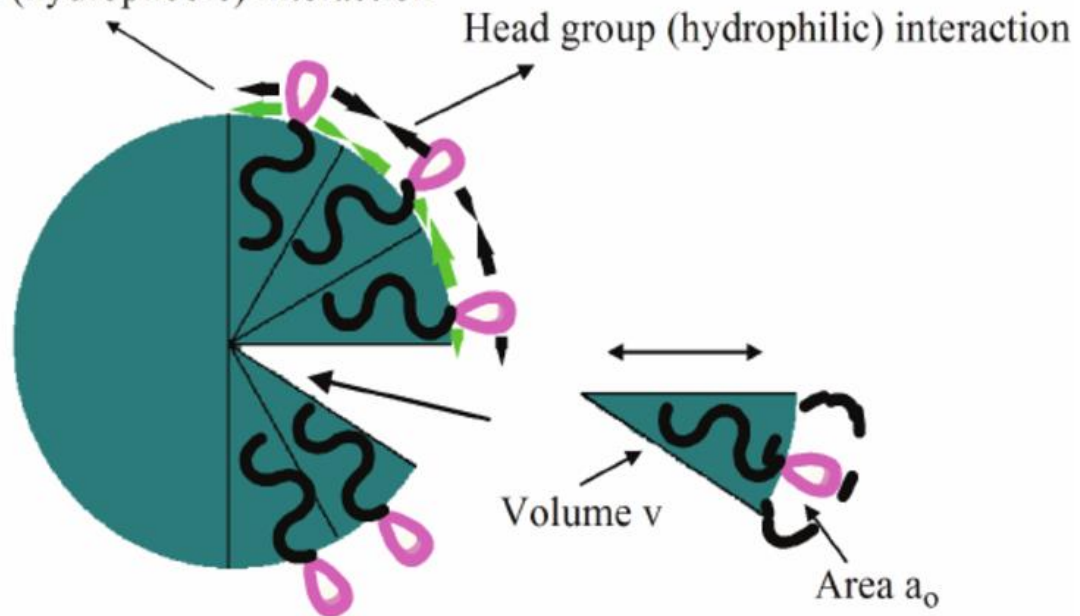


CMC, critical packing parameter

The surfactant's critical micelle concentration (CMC) plays a factor in Gibbs free energy of micellization. The exact concentration of the surfactants that yield the aggregates being thermodynamically soluble is the CMC. The Krafft temperature determines the solubility of the surfactants which in turn is the temperature that CMC is achieved. There are many parameters that affect the CMC. The interaction between the hydrophilic heads and the hydrophobic tails play a part, as well as the concentration of salt within the solution and surfactants.

The critical packing parameter relates the head group area, the length and the volume of the carbon chain into dimensionless number. The concept of HLB (Hydrophilic-Lipophilic Balance) was introduced by Griffin in 1949. As the name suggests, HLB is an empirical balance based on the relative percentage of the hydrophilic to the lipophilic moieties in the surfactant. Later, he (Griffin, 1954) defined an empirical equation that can be used to determine the HLB based on chemical composition. Davies et al. has offered a more general empirical equation (Davies et al., 1959) by assigning a number to the different hydrophilic and lipophilic chemical groups in a surfactant. The HLB number was calculated by the expression.

Interfacial (hydrophobic) interaction



Membrane asymmetry

The components of biological membranes are asymmetrically distributed between the membrane surfaces. Proteins are absolutely asymmetrical in that every copy of a polypeptide chain has the same orientation in the membrane, and lipids are nonabsolutely asymmetrical in that almost every type of lipid is present on both sides of the bilayer, but in different and highly variable amounts. Asymmetry is maintained by lack of transmembrane diffusion. Two types of membrane proteins, called ectoproteins and endoproteins, are distinguished. Biosynthetic pathways for both types of proteins and for membrane lipids are inferred from their topography and distribution in the formed cells. Note added in proof. A cell-free system has now been developed which permits the mechanisms of membrane protein assembly to be studied. The membrane glycoprotein of vesicular stomatitis virus has been synthesized by wheat germ ribosomes in the presence of rough endoplasmic reticulum from pancreas. The resulting polypeptide is incorporated into the membrane, spans the lipid bilayer asymmetrically, and is glycosylated. The amino terminal portion of this transmembrane protein is found inside the endoplasmic reticulum vesicle, while the carboxyl terminal portion is exposed on the outer surface of the vesicle. Furthermore, addition of the glycoprotein to membranes after protein synthesis does not result in incorporation of the

protein into the membrane in the manner described above. Consequently, protein synthesis and incorporation into the membrane must be closely coupled. Indeed, using techniques to synchronize the growth of nascent polypeptides, it has been shown that no more than one-fourth of the glycoprotein chain can be made in the absence of membranes and still cross the lipid bilayer when chains are subsequently completed in the presence of membranes. These findings demonstrate directly that the extracytoplasmic portion of an ectoprotein can cross the membrane only during biosynthesis, and not after.

Macro domain

The Macro domain is a module of about 180 amino acids which can bind ADP-ribose, an NAD metabolite, or related ligands. Binding to ADP-ribose can be either covalent or non-covalent in certain cases it is believed to bind non-covalently. While in other cases (such as Aprataxin) it appears to bind both non-covalently through a zinc finger motif, and covalently through a separate region of the protein.

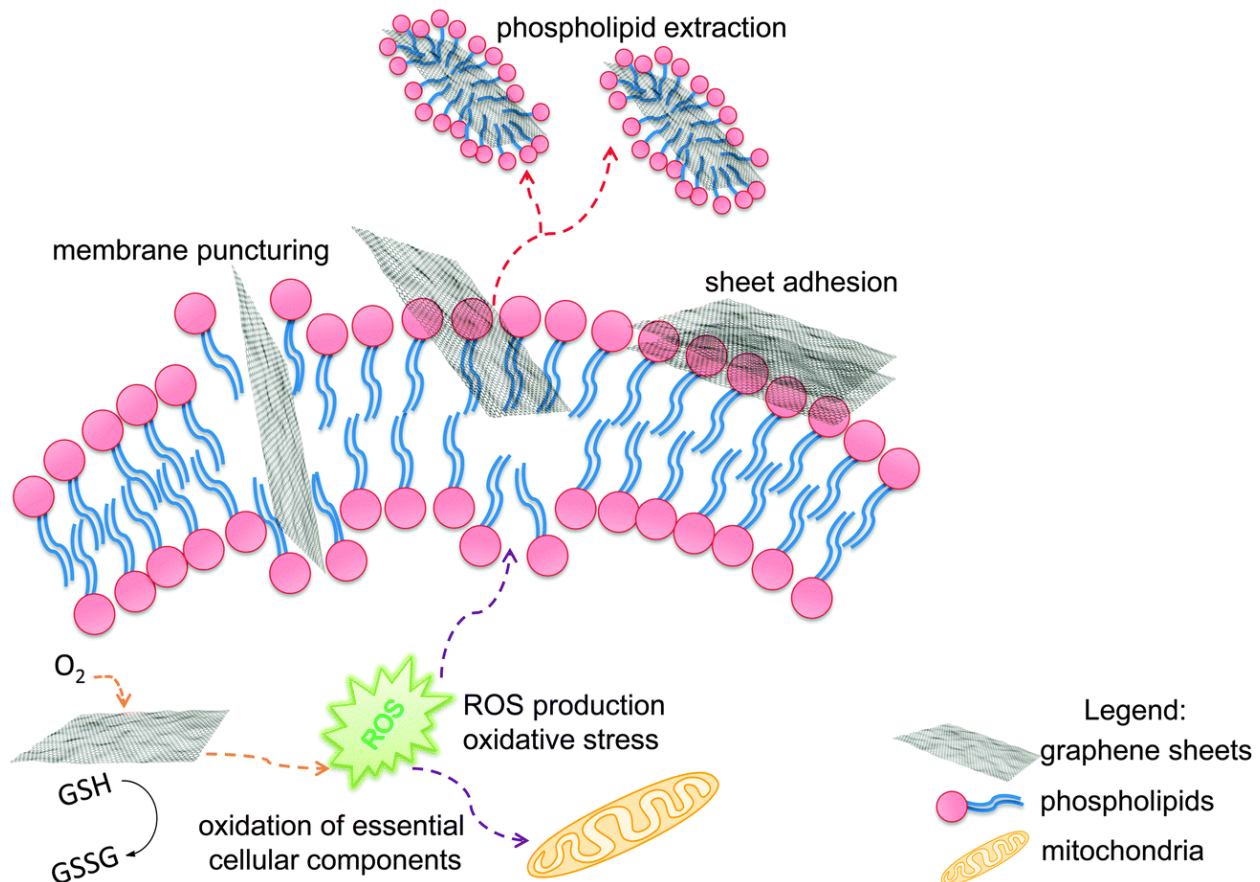
Micro domain

The plasma membrane consists of a mosaic of functional microdomains facilitating a variety of physiological processes associated with the cell surface. In most cells, the majority of the cell surface is morphologically featureless, leading to difficulties in characterizing its organization and microdomain composition. The reliance on indirect and perturbing techniques has led to vigorous debate concerning the nature and even existence of some microdomains. Recently, increasing technical sophistication has been applied to study cell surface compartmentalization providing evidence for small, short-lived clusters that may be much less than 50 nm in diameter. Lipid rafts and caveolae are cholesterol-dependent, highly ordered microdomains that have received most attention in recent years, yet their precise roles in regulating functions such as cell signalling remain to be determined. Endocytosis of lipid rafts/caveolae follows a clathrin-independent route to both early endosomes and non-classical caveosomes. The observation that a variety of cellular pathogens localize to and internalize with these microdomains provides an additional incentive to characterize the organization, dynamics and functions of these domains.

Membrane skeleton, lipid rafts, caveolae and tight junctions

Membrane (lipid) rafts and caveolae, a subset of rafts, are cellular domains that concentrate plasma membrane proteins and lipids involved in the regulation of cell function. In addition to providing signaling platforms for G-protein-coupled receptors and certain tyrosine kinase receptors, rafts/caveolae can influence redox signaling. Molecular characteristics of and methods to study rafts/caveolae, determinants that contribute to the localization of molecules in these entities, an overview of signaling molecules that show such localization, and the contribution of rafts/caveolae to redox signaling. Of particular note is the evidence that endothelial nitric oxide synthase (eNOS), NADPH oxygenase, and heme oxygenase, along with other less well-studied redox systems, localize in rafts and caveolae.

RBC membrane architecture



Lateral, transverse and rotational motion of lipids and proteins

Lateral diffusion refers to the lateral movement of lipids and proteins found in the membrane. Membrane lipids and proteins are generally free to move laterally if they are not restricted by certain interactions. Lateral diffusion is a fairly quick and spontaneous process.

Techniques used to study membrane dynamics

- ✓ Fluorescence recovery after photobleaching (FRAP) is a method to qualitatively and quantitatively study biomolecule dynamics in living cells.
- ✓ FRAP is based on irreversibly bleaching a pool of fluorescent probes with high intensity light and monitoring the recovery in fluorescence due to movement of surrounding intact probes into the bleached spot.
- ✓ FRAP experiments are often conducted on confocal microscopes. To derive quantitative results from such experiments, several parameters and controls need to be considered and utilised in the analysis.
- ✓ There are several FRAP-related methods that have been developed for specific applications and biological questions.

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COURSE NAME: MEMBRANE BIOLOGY AND BIOENERGETICS

COURSE CODE: 18BCU103 UNIT: I BATCH-2018-2021

- ✓ FRAP is a versatile and popular method in modern biomedical research. Its application is broad and is increasingly applied in pharmacological, therapeutic and diagnostic areas.

Factors affecting fluidity of membranes:

- ✓ As temp decreases, membrane fluidity decreases, because phospholipids settle into a closely packed arrangement and membrane solidifies. This is called phase transition.
- ✓ Length of fatty acid. The longer the hydrocarbon chains, the higher the melting point. Degree of saturation of fatty acids; unsaturated lipids have kinks that will prevent hydrocarbon chain from packing closely together, hence enhances fluidity.
- ✓ Amount of cholesterol. Cholesterol increases stability and maintains fluidity of membrane.

POSSIBLE QUESTIONS

UNIT-I

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Define CMC.
2. What is membrane fluidity?
3. Name the major components of biological membranes.
4. Expand FRAP and write its application.
5. What are lipid rafts?
6. Name the techniques used to study membrane dynamics.
7. What is lateral motion of lipids and proteins?
8. What is transverse motion of lipids and proteins?
9. What is rotational motion of lipids and proteins?
10. Expand FRAP and TNBS.

PART-C (8 MARKS)

1. Describe the composition of cell membrane
2. What are the main proteins of RBC membrane? Highlight their functions.
3. Elaborate the significance of liposomes as model system
4. What is membrane fluidity? Explain various factors regulating membrane fluidity.
5. Describe fluid mosaic model with neat diagram.
6. Write a note on the different techniques used to study membrane dynamics.
7. Write a note on the macro and micro domains in membranes.
8. Write a note on the different motion of lipids and proteins in membranes.

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DEPARTMENT OF BIOCHEMISTRY
I B.Sc BIOCHEMISTRY
BATCH: 2018 - 2021

18BCU103 - Membrane Biology and Bioenergetics

PART A (20 X 1 = 20 MARKS) - Online MCQ questions

Unit 1

S.No	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	The membranes of which domains are	Archaea and Bacteria	Bacteria and Eukarya	Eukarya and Archaea	membranes of all three	Bacteria and Eukarya
2	All membranes of free-living organisms have	Bacteria	Fungi	Archaea	Protozoa	archaea
3	Which method is used and best suited to	FRAP	Northern Blotting	Southern blotting	RNA Analysis	FRAP
4	Molecules present in the membrane are	Static	Not Static	Submerged	Stationary	Static
5	The area of membrane where active	lipid rafts	carbohydrate rafts	protein rafts	nucleotide rafts	lipid rafts
6	The temperature at which the membrane	Temperature coefficient	Transition Temperature	Thermal Coefficient	Free Energy	Transition Temperature
7	G- Proteins are	Monomeric	dimeric	trimeric	tetrameric	trimeric
8	Intrinsic proteins are	easily isolated	isolated by changing pH	isolated by changing	isolated using detergents	isolated using detergents
9	GPCR is an example for	Intrinsic proteins	Extrinsic proteins	membrane lipid	membrane carbohydrate	Intrinsic proteins
10	Transition temperature of lipid bilayers of cell	Saturated fatty acids	Cholesterol	Unsaturated fatty acids	Hydrocarbon	Saturated fatty acids
11	What is the outer boundary of the cell?	cell wall	plasma membrane	nuclear membrane	endoplasmic reticulum	plasma membrane
12	Which of the following describes the	phospholipid bilayer with	phospholipid trilayer with	triglyceride bilayer with	triglyceride monolayer	phospholipid bilayer with
13	Which of the following statements is NOT	Protein molecules may be	Protein molecules are	Phospholipids form a	Phospholipids have a fluid	Protein molecules are
14	Which of the following statements is NOT	Each phospholipid molecule	Each phospholipid	The phospholipid heads	The phospholipid tails are	Each phospholipid molecule
15	Which of the following molecules would NOT	phospholipids	glycolipids	cholesterols	nucleic acids	nucleic acids
16	Which molecule in animal plasma membranes	proteins	phospholipids	glycolipids	cholesterol	cholesterol
17	Which type of protein in the plasma	carrier protein	channel protein	cell -recognition protein	receptor protein	cell -recognition protein
18	If a particular protein was identified in the	carrier	channel	cell -recognition	receptor	cell -recognition
19	Which statement best describes the plasma	It is freely permeable to all	It is selectively permeable	It is nonpermeable to all	it does not allow water to	It is selectively permeable to
20	Which of the following is a function of	compartmentalization	selectively permeable	mediates intercellular	Compartmentalization,	Compartmentalization,
21	What evidence convinced Overton that	He could see the lipids in	Membranes were	He found that more lipid-	Membranes dissolved in	He found that more lipid-
22	How did the Singer and Nicolson FluidMosaic	They proposed that the	They proposed that	They proposed that	2 and 3	2 and 3
23	_____ are fluid-filled, membrane-bound	Liposomes	Micelles	Syringes	Lysosomes	Liposomes
24	Membrane-associated carbohydrates exhibit a	Membrane-associated	Membrane-associated	Internal cellular	2 and 3	2 and 3
25	What role are glycolipids like gangliosides	They are thought to	They are thought to serve	They help to destroy	a and b	They are thought to serve as

26	The carbohydrates on the red blood cell	N-acetylgalactosamine	galactose	maltose	b and c	b and c
27	According to widely accepted 'fluid mosaic	Proteins in cell membranes	Proteins can remain	Proteins can also	Many proteins remain	Proteins can also undergo
28	Cell membrane contains	lipid monolayer	bilayer	trilayer	tetralayer	bilayer
29	Photobleaching is involved in	FRAP	TNBS	WB	PCR	FRAP
30	ABO antigens are basically	Carbohydrates	Proteins	Lipids	Adjuvants	Carbohydrates
31	Rh factor is basically a	Carbohydrates	Proteins	Lipids	Adjuvants	Proteins
32	CMC in membrane biology refers to	Critical micelle	Christian medical college	cell membrane crystal	cell membrane	Critical micelle
33	Adenylate cyclase is a	nuclear protein	mitochondrial protein	membrane protein	lysosomal protein	membrane protein
34	Phospholipid constitute -----% of total lipids in	20	75	5	90	75
35	Glycolipid constitute -----% of total lipids in	20	75	5	90	5
36	Cholesterol constitute -----% of total lipids in	20	75	5	90	20
37	Amphiphilic refers to	hydrophobicity alone	hydrophilicity alone	hydrophobicity and	neither hydrophilic nor	hydrophobicity and
38	liposomes are basically	cell membranes	cytoplasm	lipsticks	vitamins	cell membranes
39	The term aqueous refers to	Water	Acid	Base	Mild alkali	Water
40	pH 7 is	Acidic	Basic	Not neutral	Neutral	Neutral
41	Polyethylene glycol is added to liposomes to	protect from immune	protect from nervous	protect from circulatory	protect from skeletal	protect from immune
42	Antibody is added to liposomes to	improve circulation	improve targeted delivery	increase drug half life	antibody is absent in	improve targeted delivery of
43	TNBS labeling is done to study	cytosolic proteins	membrane proteins	Water molecule	TNBS labeling is not	membrane proteins
44	Band 3 protein is present in	hepatocyte	glial cells	RBC	Osteoclasts	RBC
45	Glycophorin is present abundantly in	hepatocyte	glial cells	RBC	Osteoclasts	RBC
46	CD4 is present in	T cytotoxic cells	B Cells	Antibody	T helper cells	T helper cells
47	CD8 is present in	T cytotoxic cells	B Cells	Antibody	T helper cells	T cytotoxic cells
48	Blood group A contains an extra	glucose	fructose	N-acetylgalactosamine	galactose	N-acetylgalactosamine
49	Blood group B contains an extra	glucose	fructose	N-acetylgalactosamine	galactose	galactose
50	Persons with AB positive blood group can	donate blood to all	donate to AB negative	accept blood from all	can donate to O positive	accept blood from all
51	Electron transport occurs in the membrane of	Mitochondria	Endoplasmic Reticulum	Lysosome	Nucleus	Mitochondria
52	Electron transport occurs in	Mitochondrial matrix	Outer membrane of	inner membrane of	inner membrane space of	inner membrane space of
53	Which organelle is referred as power house of	Mitochondria	Endoplasmic Reticulum	Lysosome	Nucleus	Mitochondria
54	Glycosylation of proteins occurs at	Mitochondria	Endoplasmic Reticulum	Golgi bodies	Nucleus	Golgi bodies
55	Protein biosynthesis takes place at	cell membranes	Rough endoplasmic	Smooth endoplasmic	Lysosomes	Rough endoplasmic
56	Steroidogenesis occurs in	cell membranes	Rough endoplasmic	Smooth endoplasmic	Lysosomes	Smooth endoplasmic
57	Carbohydrates in RBC membrane is present	on extracellular side	intracellular side	embedded in the	not present	on extracellular side
58	Monocytes present in tissues are called	T lymphocytes	B lymphocytes	Macrophages	they are not present in	Macrophages
59	Macrophages present in CNS are	glial cells	kupfer cells	osteoclasts	osteoblasts	glial cells

60	Macrophages present in bone are	glial cells	kupfer cells	osteoclasts	osteoblasts	osteoclasts
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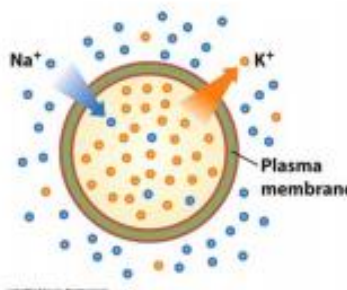
UNIT-II SYLLABUS

Membrane transports: Thermodynamics of transport. Simple diffusion and facilitated diffusion. Passive transport- glucose transporter, anion transporter and porins. Primary active transporters- P type ATPases, V type ATPases, F type ATPases. Secondary active transporters- lactose permease, Na⁺-glucose symporter. ABC family of transporters- MDR, CFTR. Group translocation. Ion channels- voltage-gated ion channels (Na⁺/K⁺ voltage-gated channel), ligand-gated ion channels (acetyl choline receptor), aquaporins, bacteriorhodopsin. Ionophores - valinomycin, gramicidin.

Membrane Transports

Thermodynamics of transport

- Membrane potential— volts
- For +1 ion, potential = $0.058V(\log \frac{ion_{in}}{ion_{out}})$
- If potential is negative, the inside of cell is more negative

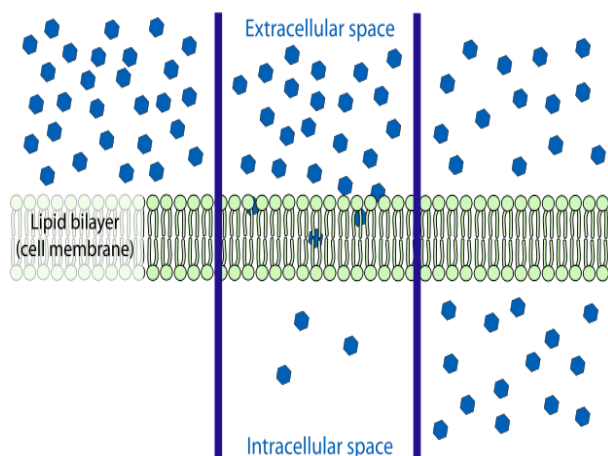


$$\Delta\psi = \frac{RT}{ZF} \ln \frac{[ion]_{in}}{[ion]_{out}}$$

Diffusion Simple diffusion Definition

Simple Diffusion: Simple diffusion is an unassisted type of diffusion in which a particle moves from higher to a lower concentration.

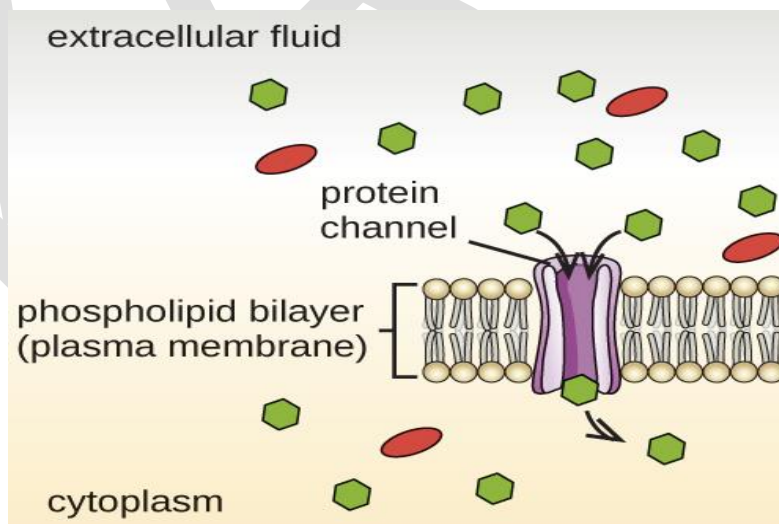
The directional movement through the concentration gradient is passive. Once the molecules become evenly distributed, the molecules on the either sides of the cell membrane achieve an equilibrium where no net movement of molecules is observed. Generally, small non-polar molecules like oxygen, carbon dioxide, and ethanol freely diffuse across the cell membrane. The rate of diffusion depends on the temperature, molecular size, and the steepness of concentration gradient. Temperature affects the kinetic energy of particles in a solution. Large particles are subjected to a higher resistance within a solution when compared to smaller particles. Moreover, when the concentration gradient is high, more molecules will pass through the membrane. Simple diffusion across the cell membrane is shown in figure.



TIME

Facilitated Diffusion

Facilitated diffusion is the transport of substances across a biological membrane through a concentration gradient by means of a carrier molecule. During facilitated diffusion, large ions and polar molecules are dissolved in water and are specifically and passively transported across the cell membrane. Polar ions diffuse through transmembrane channels proteins and large molecules diffuse through transmembrane carrier proteins. Channel proteins make hydrophobic tunnels across the membrane, allowing the selected hydrophobic molecules to pass through the membrane. Some channel proteins are 'opened' at all the time and some like ion channel proteins are 'gated'. Carrier proteins like permeases change their conformation as molecules like glucose or amino acids are transported through them. Aquaporins are the other type of transport proteins that allow water to cross the membrane so quickly. Facilitated diffusion through a channel protein is shown in figure below.



facilitated diffusion

Similarities between Simple Diffusion and Facilitated Diffusion

- Both simple and facilitated diffusion occur down the concentration gradient from a high concentration to a low concentration of molecules.
- Both types do not require energy for the transportation of molecules.
- The net movement of molecules on either side of the cell membrane is zero at the equilibrated state.

Differences between Simple Diffusion and Facilitated Diffusion

Occurrence

Simple Diffusion: Simple diffusion occurs through the phospholipid bilayer.

Facilitated Diffusion: Facilitated diffusion occurs through transmembrane proteins.

Transported Molecules

Simple Diffusion: Simple diffusion transports small, non-polar particles.

Facilitated Diffusion: Facilitated diffusion transports large or polar particles.

Facilitator Molecules

Simple Diffusion: Simple diffusion occurs directly through the cell membrane.

Facilitated Diffusion: Facilitated diffusion occurs through specific facilitator molecules called transmembrane integral proteins.

Rate of Diffusion

Simple Diffusion: The rate of simple diffusion is directly proportional to the concentration gradient across the membrane as well as the membrane permeability of the solute molecule.

Facilitated Diffusion: The rate of facilitated diffusion depends on the kinetics of carrier-mediated transport.

At Low Concentration Gradients

Simple Diffusion: The rate of the simple diffusion is low at low solute concentrations.

Facilitated Diffusion: The rate of facilitated diffusion is high at low solute concentrations compared to that of simple diffusion.

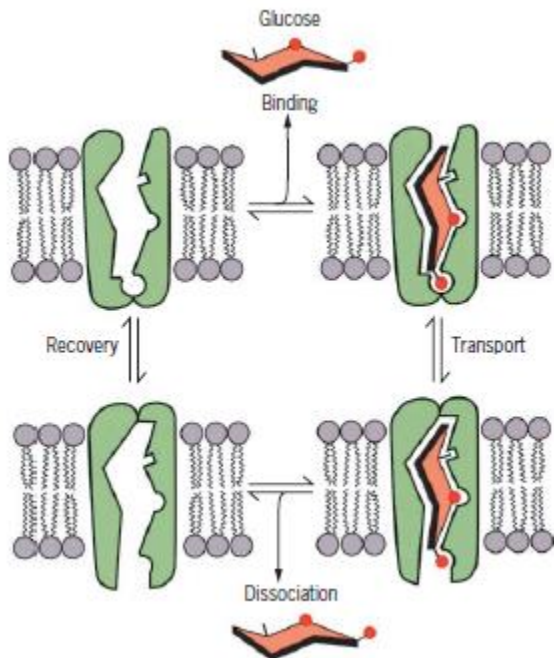
Examples

Simple Diffusion: Diffusion of gases across the respiratory membrane and diffusion of molecules from the blood to the cells through the interstitial fluid are examples of simple diffusion.

Facilitated Diffusion: The counter-transport of chloride/bicarbonate in renal tubular cells and the cotransport of sodium with sugars like glucose, galactose, and fructose and amino acids are examples of facilitated diffusion.

Glucose transporter

Glucose is the body's primary source of direct energy, and most mammalian cells contain a membrane protein that facilitates the diffusion of glucose from the bloodstream into the cell. A gradient favoring the continued diffusion of glucose into the cell is maintained by phosphorylating the sugar after it enters the cytoplasm, thus lowering the intracellular glucose concentration. Humans have at least five related proteins (isoforms) that act as facilitative glucose transporters. These isoforms, termed GLUT1 to GLUT5, are distinguished by the tissues in which they are located, as well as their kinetic and regulatory characteristics.



Insulin is a hormone produced by endocrine cells of the pancreas and plays a key role in maintaining proper blood sugar levels. An increase in blood glucose levels triggers the secretion of insulin, which stimulates the uptake of glucose into various target cells, most notably skeletal muscle and fat cells (adipocytes). Insulin-responsive cells share a common isoform of the facilitative glucose transporter, specifically GLUT4. When insulin levels are low, these cells contain relatively few glucose transporters on their plasma membrane. Instead, the transporters are present within the membranes of cytoplasmic vesicles. Rising insulin levels act on target cells to stimulate the fusion of the cytoplasmic vesicles to the plasma membrane, which moves transporters to the cell surface where they can bring glucose into the cell.

The Chloride-Bicarbonate Exchanger Catalyzes Electroneutral Cotransport of Anions across the Plasma Membrane

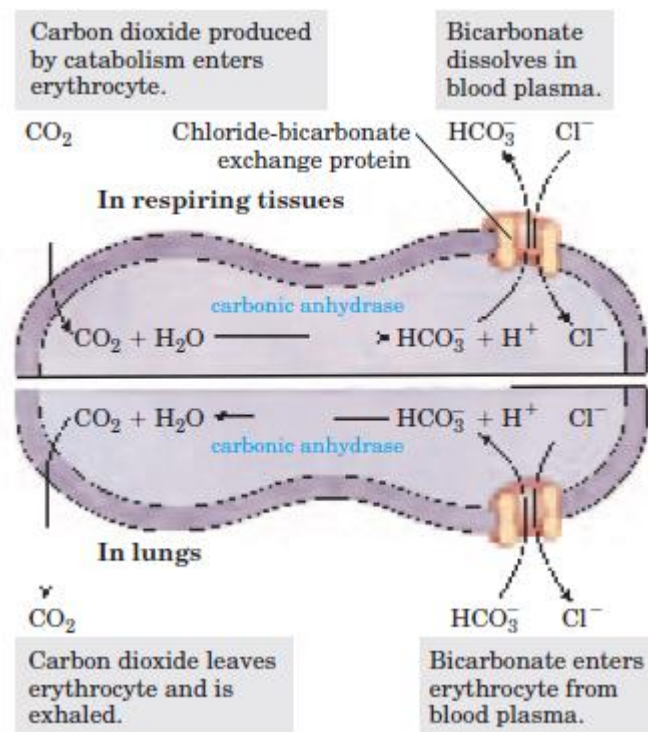
The erythrocyte contains another facilitated diffusion system, an anion exchanger that is essential in CO₂ transport to the lungs from tissues such as skeletal muscle and liver. Waste CO₂ released from respiring tissues into the blood plasma enters the erythrocyte, where it is converted to bicarbonate (HCO₃⁻) by the enzyme carbonic anhydrase. The HCO₃⁻ reenters the blood plasma for transport to the lungs.

KARPAGAM ACADEMY OF HIGHER EDUCATION

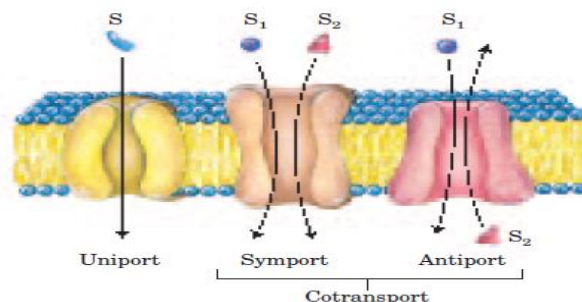
CLASS: I B.SC., BIOCHEMISTRY

COURSE NAME: MEMBRANE BIOLOGY AND BIOENERGETICS

COURSE CODE: 18BCU103 UNIT: II BATCH-2018-2021



Because HCO_3^- is much more soluble in blood plasma than is CO_2 , this roundabout route increases the capacity of the blood to carry carbon dioxide from the tissues to the lungs. In the lungs, HCO_3^- reenters the erythrocyte and is converted to CO_2 , which is eventually released into the lung space and exhaled. To be effective, this shuttle requires very rapid movement of HCO_3^- across the erythrocyte membrane. The chloride-bicarbonate exchanger, also called the anion exchange (AE) protein, increases the permeability of the erythrocyte membrane to HCO_3^- more than a millionfold. Like the glucose transporter, it is an integral protein that probably spans the membrane at least 12 times. This protein mediates the simultaneous movement of two anions: for each HCO_3^- ion that moves in one direction, one Cl^- ion moves in the opposite direction (Fig. 11–33), with no net transfer of charge; the exchange is electroneutral. The coupling of Cl^- and HCO_3^- movements is obligatory; in the absence of chloride, bicarbonate transport stops. In this respect, the anion exchanger is typical of all systems, called cotransport systems, that simultaneously carry two solutes across a membrane. When, as in this case, the two substrates move in opposite directions, the process is antiport.

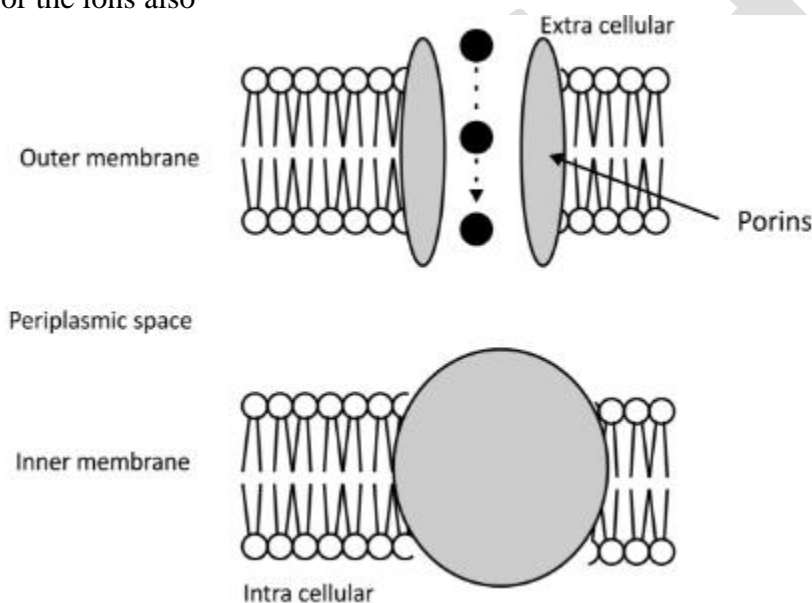


In symport, two substrates are moved simultaneously in the same direction. As we noted earlier, transporters that carry only one substrate, such as the erythrocyte glucose transporter, are uniport systems. The human genome has genes for three closely related chloride-bicarbonate exchangers, all with the same predicted transmembrane topology. Erythrocytes contain the AE1 transporter, AE2 is prominent in liver, and AE3 is present in plasma membranes of the brain, heart, and retina. Similar anion exchangers are also found in plants and microorganisms.

Porins:

A typical porin is a trimer of β barrel trans membrane proteins that act as a specific pore or channel large enough to allow passive diffusion. These are partially blocked by a loop, called eyelet. Which projects into the cavity to determine the size of molecule to be transverse the channel, with an asymmetric arrangement of glutamate and seven Aspartate residues (in contrast to one histidine, two lysine and three arginine residues) is partially compensated for by two bound calcium atoms.

These are found in mitochondria, chloplast and in the external membrane of gram negative bacteria and some member of bacterial group, mycolata. Porins are especially useful in the regulation of diffusion regarding small molecular weighted metabolites as sugars amino acids or the ions also



Voltage dependent anion channels (VDCA) are example of porin ion selective channels between mitochondria and cytoplasm, it is a major protein of outer mitochondrial membrane of eukaryotes. The channel acquire its open conformation at low or zero membrane potential and closed conformation at 30-40 mV. These channels communicate with all metabolic enzymes and get involved in ransport of ATP, ADP, pyruvate, malate, and other relevant metabolites.

VDAC also regulates the Ca^{2+} transport of mitochondria and as permeability transition pore (PTP), allowing the release of cyt C, which is an essential factor during apoptosis. The significant role of VDAC in apoptosis, suggesting it is potent target for chemotherapy.

Primary active transporters

The primary active transport that functions with the active transport of sodium and

potassium allows secondary active transport to occur. The secondary transport method is still considered active because it depends on the use of energy as does primary transport.

The ATPase Family

ATPases are membrane-bound ion channels (actually transporters, as they are not true ion channels) that couple ion movement through a membrane with the synthesis or hydrolysis of a nucleotide, usually ATP. Different forms of membrane-associated ATPases have evolved over time to meet specific demands of cells. These ATPases have been classified as F-, V-, A-, P- and E-ATPases based on functional differences. They all catalyse the reaction of ATP synthesis and/or hydrolysis. The driving force for the synthesis of ATP is the H^+ gradient, while during ATP hydrolysis the energy from breaking the ATP phosphodiester bond is the driving force for creating an ion gradient. Structurally these ATPases can differ: F-, V- and A-ATPases are multi-subunit complexes with a similar architecture and possibly catalytic mechanism, transporting ions using rotary motors. The P-ATPases are quite distinct in their subunit composition and in the ions they transport, and do not appear to use a rotary motor.

F-ATPases

The F-ATPases (for 'phosphorylation Factor', and also known as H^+ -transporting ATPases or F(0)F(1)-ATPases) are the prime enzymes used for ATP synthesis, and are remarkably conserved throughout evolution. They are found in the plasma membranes of bacteria, in the thylakoid membranes of chloroplasts, and in the inner membranes of mitochondria. These membrane proteins can synthesize ATP using a H^+ gradient, and work in the reverse to create a H^+ gradient using the energy gained from the hydrolysis of ATP. In certain bacteria, Na^+ -transporting F-ATPases have also been found.

V-ATPases

V-ATPases (for 'Vacuole') are found in the eukaryotic endomembrane system (vacuoles, Golgi apparatus, endosomes, lysosomes, clathrin-coated vesicles {transport external substances inside the cell}, and plant tonoplasts), and in the plasma membrane of prokaryotes and certain specialised eukaryotic cells. V-ATPases hydrolyse ATP to drive a proton pump, but cannot work in reverse to synthesize ATP. V-ATPases are involved in a variety of vital intra- and inter-cellular processes such as receptor mediated endocytosis, protein trafficking, active transport of metabolites, homeostasis and neurotransmitter release

A-ATPases

A-ATPases (for 'Archaea') are found exclusively in Archaea and have a similar function to F-ATPases (reversible ATPases), even though structurally they are closer to V-ATPases. A-ATPases may have arisen as an adaptation to the different cellular needs and the more extreme environmental conditions faced by Archaeal species.

P-ATPases

P-ATPases (also known as E1-E2 ATPases) are found in bacteria and in a number of eukaryotic plasma membranes and organelles. P-ATPases function to transport a variety of different compounds, including ions and phospholipids, across a membrane using ATP hydrolysis for energy. There are many different classes of P-ATPases, each of which transports a specific type of ion: H^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ag^+ and Ag^{2+} , Zn^{2+} , Co^{2+} , Pb^{2+} , Ni^{2+} , Cd^{2+} , Cu^+ and Cu^{2+} . For example, gastric P-ATPase is a H^+/K^+ pump responsible for acid secretion in the stomach, transporting H^+ from the cytoplasm of stomach parietal cells to create a large pH gradient in

exchange for getting K^+ ions inside the cell, using ATP hydrolysis as the energy source. P-ATPases can be composed of one or two polypeptides (fewer than the other ATPases), and can assume two conformations called E1 and E2.

E-ATPases

E-ATPases (for 'Extracellular') are membrane-bound cell surface enzymes that have broad substrate specificity, hydrolysing other NTPs besides ATP, as well as NDPs – although their most likely substrates are ATP, ADP and UTP, as well as extracellular ATP. There are at least three classes of E-ATPases: ecto-ATPases, CD39s, and ecto-ATP/Dases. An example is ecto-ATPase from the smooth muscle membranes of chickens, which is thought to exhibit a range of activities determined by the oligomerisation of the enzyme, which in turn is affected by different membrane events.

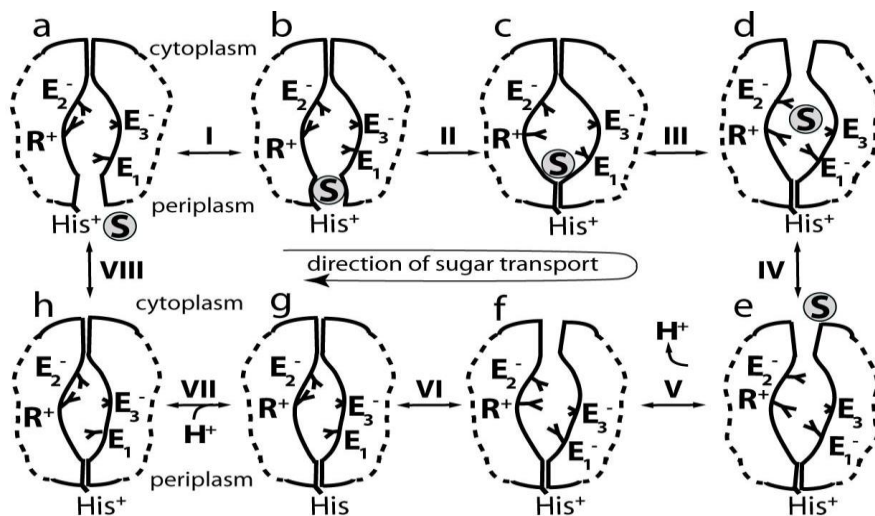
Secondary active transporters

Unlike in primary active transport, in secondary active transport, ATP is not directly coupled to the molecule of interest. Instead, another molecule is moved up its concentration gradient, which generates an electrochemical gradient. The molecule of interest is then transported down the electrochemical gradient. While this process still consumes ATP to generate that gradient, the energy is not directly used to move the molecule across the membrane, hence it is known as secondary active transport. Both antiporters and symporters are used in secondary active transport. Co-transporters can be classified as symporters and antiporters depending on whether the substances move in the same or opposite directions across the cell membrane.

Lactose permease

Lactose permease (LacY) is an integral protein that facilitate the passage of lactose, one of the essential nutrients for all life forms, across the otherwise impermeable phospholipid bilayers that surround all cells and organelles. The active transport uses the energy of the electrochemical proton gradient, i.e. one H^+ is transported in with each sugar (co-transport). The proteins play a critical role in transmembrane traffic, and, therefore, are critical for a healthy metabolism of a wide range of living organism, including human being. Malfunction of these transporters is associated with various pathophysiological conditions, such as diabetes and depression. The crystal structure of LacY of *E. coli* exhibits 12 transmembrane helices. Two halves of the protein (N-domain and C-domain, respectively) form a hydrophilic cavity opening to the cytoplasm, where the substrate is bound in its binding pocket. The periplasmic side of the protein is closed and the substrate is ready to diffuse into the cell through the opening of the cavity.

The crystal structure of LacY represents the inward open state of the protein, in which the substrate is accessible only from the cytoplasmic side. Apparently, the accessibility of the substrate from the periplasm is necessary for the import of lactose from outside of the cell. One of the most important unknowns in the mechanism of sugar transport in LacY is the nature of protein conformational changes that switch substrate accessibility from the cytoplasmic part to the periplasmic one.



Substrate transport in LacY.

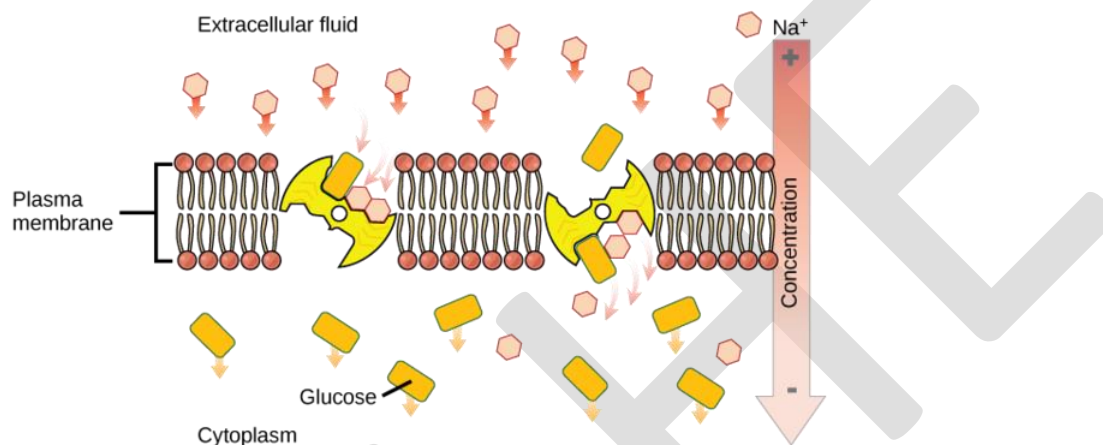
Beginning from a putative outward open conformation, in which the substrate is accessible from the periplasm, the right figure provides the schematic representation of a possible lactose/ H^+ co-transport mechanism. Intermediates d, e, and f correspond to inward open conformation as seen in the crystal structure; intermediates a and b correspond to the outward open conformation that has not yet been resolved structurally by observation. A histidine (His in the right Figure) in the periplasmic side of LacY is postulated as a possible proton acceptor. It is protonated in the ground state (intermediate a) of LacY considering that the periplasm is rich in protons. The putative transport process shown is composed of eight steps:

- I. Lactose binds to ground state LacY, in which a Glutamate E_1 is protonated.
- II. The periplasmic side of LacY closes, induced by the movement of the lactose to the binding pocket.
- III. A proton is transferred from E_1 to Glutamate E_3 , and Arginine R^+ in the N-domain and unprotonated E_1 forms a salt bridge which induces the inward open conformation d. Meanwhile lactose moves toward the cytoplasmic side.
- IV. Lactose is released to the cytoplasm.
- V. The proton on E_3 is released to the cytoplasm;
- VI. A histidine in the periplasmic half (His) transfers its proton to E_1 and LacY closes at the cytoplasm.
- VII. His becomes protonated.
- VIII. LacY opens at the periplasm

Na^+ /glucose cotransporter

Secondary active transport brings sodium ions, and possibly other compounds, into the

cell. As sodium ion concentrations build outside the plasma membrane because of the action of the primary active transport process, an electrochemical gradient is created. If a channel protein exists and is open, the sodium ions will be pulled through the membrane. This movement is used to transport other substances that can attach themselves to the transport protein through the membrane. Many amino acids, as well as glucose, enter a cell this way. This secondary process is also used to store high-energy hydrogen ions in the mitochondria of plant and animal cells for the production of ATP. The potential energy that accumulates in the stored hydrogen ions is translated into kinetic energy as the ions surge through the channel protein ATP synthase, and that energy is used to convert ADP into ATP.



ABC family of transporters

MDR

Discovery of the first eukaryotic ABC protein to be recognized came from studies on tumor cells and cultured cells that exhibited resistance to several drugs with unrelated chemical structures. Such cells eventually were shown to express elevated levels of a multidrug-resistance (MDR) transport protein known as MDR1. This protein uses the energy derived from ATP hydrolysis to export a large variety of drugs from the cytosol to the extracellular medium. The Mdr1 gene is frequently amplified in multidrug-resistant cells, resulting in a large overproduction of the MDR1 protein.

Most drugs transported by MDR1 are small hydrophobic molecules that diffuse from the medium across the plasma membrane, unaided by transport proteins, into the cell cytosol, where they block various cellular functions. Two such drugs are colchicine and vinblastine, which block assembly of microtubules. ATP-powered export of such drugs by MDR1 reduces their concentration in the cytosol. As a result, a much higher extracellular drug concentration is required to kill cells that express MDR1 than those that do not. That MDR1 is an ATP-powered small-molecule pump has been demonstrated with liposomes containing the purified protein. The ATPase activity of these liposomes is enhanced by different drugs in a dose-dependent manner corresponding to their ability to be transported by MDR1.

About 50 different mammalian ABC transport proteins are now recognized. These are expressed in abundance in the liver, intestines, and kidney—sites where natural toxic and waste products are removed from the body. Substrates for these ABC proteins include sugars, amino

acids, cholesterol, peptides, proteins, toxins, and xenobiotics. Thus the normal function of MDR1 most likely is to transport various natural and metabolic toxins into the bile, intestinal lumen, or forming urine. During the course of its evolution, MDR1 appears to have acquired the ability to transport drugs whose structures are similar to those of these endogenous toxins. Tumors derived from MDR-expressing cell types, such as hepatomas (liver cancers), frequently are resistant to virtually all chemotherapeutic agents and thus difficult to treat, presumably because the tumors exhibit increased expression of the MDR1 or the related MDR2.

CFTR

Cystic fibrosis (CF) is a serious and relatively common hereditary disease of humans. About 5% of white Americans are carriers, having one defective and one normal copy of the gene. Only individuals with two defective copies show the severe symptoms of the disease: obstruction of the gastrointestinal and respiratory tracts, commonly leading to bacterial infection of the airways and death due to respiratory insufficiency before the age of 30. In CF, the thin layer of mucus that normally coats the internal surfaces of the lungs is abnormally thick, obstructing air flow and providing a haven for pathogenic bacteria, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The defective gene in CF patients was discovered in 1989. It encodes a membrane protein called cystic fibrosis transmembrane conductance regulator, or CFTR. Hydropathy analysis predicted that CFTR has 12 transmembrane helices and is structurally related to the multidrug (MDR1) transporters of drug-resistant tumors. The normal CFTR protein proved to be an ion channel specific for Cl^- ions. The Cl^- channel activity increases greatly when phosphoryl groups are transferred from ATP to several side chains of the protein, catalyzed by cAMP-dependent protein kinase. The mutation responsible for CF in 70% of cases results in deletion of a Phe residue at position 508, with the effect that the mutant protein is not correctly folded and inserted in the plasma membrane. Other mutations yield a protein that is inserted properly but cannot be activated by phosphorylation. In each case, the fundamental problem is a nonfunctional Cl^- channel in the epithelial cells that line the airways, the digestive tract, and exocrine glands (pancreas, sweat glands, bile ducts, and vas deferens).

Normally, epithelial cells that line the inner surface of the lungs secrete a substance that traps and kills bacteria, and the cilia on the epithelial cells constantly sweep away the resulting debris. When CFTR is defective or missing, this process is less efficient, and frequent infections by bacteria such as *S. aureus* and *P. aeruginosa* progressively damage the lungs and reduce respiratory efficiency.

Group Translocation

It is an energy-dependent transport mechanism in which transported molecule is chemically modified during passage across the cell membrane. The best-known group translocation system is phosphoenolpyruvate (PEP): sugar phosphotransferase system (PTS). PTS is present in obligate anaerobes like *Clostridium*, *Fusobacterium* and in many facultative anaerobes including *Escherichia*, *Staphylococcus*, *Vibrio* and *Salmonella*. It transports a variety of sugars such as glucose, mannose, cellobiose and fructose. It is rare in obligate aerobes. Saul Roseman discovered PTS in 1964.

PTS is quite complex. Its composition and function (type of sugar transported) differ from organism to organism. Broadly, it has two sets of proteins: Soluble proteins and membrane-

associated proteins.

Soluble Proteins- they are nonspecific components of PTS as their main function is to transfer phosphate group from PEP to Enzyme II. Thus, they are involved in-group translocation of a variety of molecules. Such proteins are usually constitutive and include:

- Histidine-containing protein (HPr): low molecular weight, heat-stable small cytoplasmic protein.
- Enzyme (Enz) I: cytoplasmic protein

Membrane-associated Proteins- these proteins are together known as Enzyme II, although more than one enzyme are involved. They carry out phosphorylation as well as translocation of sugars. They are usually inducible and sugar specific.

- Enzyme II (E II): a cytoplasmic protein.^{aa}
 - Enzyme II_b (E II_b): a peripheral protein located on the inner membrane surface.
 - Enzyme II_c (E II_c): an integral membrane protein, serves as carrier protein.

During translocation by PTS, solutes get phosphorylated using phosphoenolpyruvate (PEP) as the phosphate donor. For example, PTS catalyzed general reaction for glucose is:

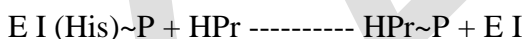


The mechanism of glucose uptake by PTS has been studied thoroughly in E.coli. It includes following steps (Figure 17):

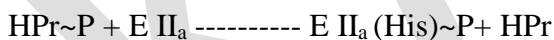
- At first, PEP transfers its high-energy phosphate group (P) to a histidine residue on Enzyme I.



- Enzyme I, in turn, transfers this phosphate to HPr.



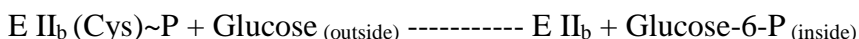
- Further, phosphate is transferred from HPr to EII._a



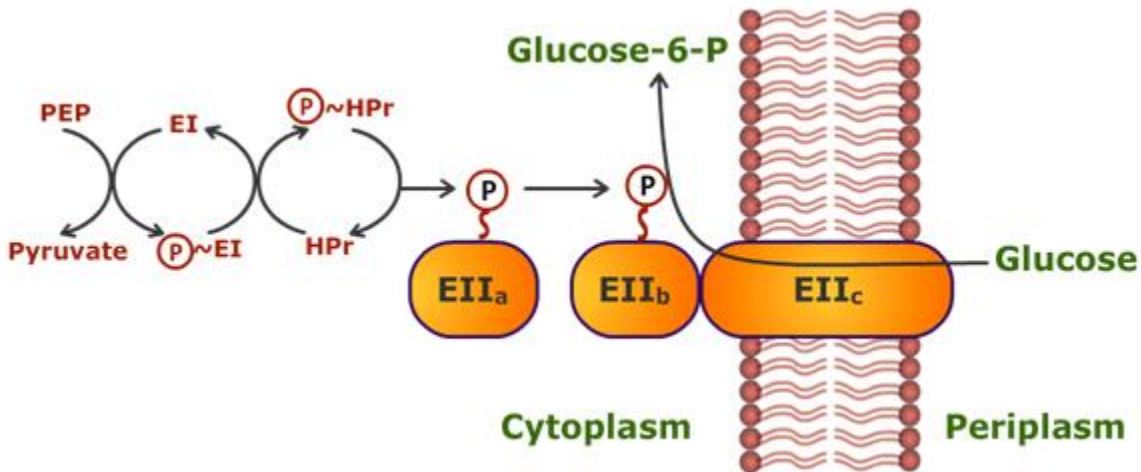
- EII is specific for glucose and transfers phosphate to the cysteine residue of Enzyme II._{ab}



- Lastly, Enzyme II phosphorylates glucose forming glucose-6-phosphate as it crosses the plasma membrane through Enzyme II._{bc}



The advantage of converting glucose into glucose-6-phosphate is that it does not affect the concentration gradient of glucose. Also, it will not leak out of the cell as the cell membrane is impermeable to glucose-6-phosphate.



Diagrammatic Representation of Glucose Uptake by the Phosphotransferase System (PTS) in Enteric Bacteria (e.g. E.coli).

Ion channels

Voltage-gated ion channels

The sodium-potassium pump cycle

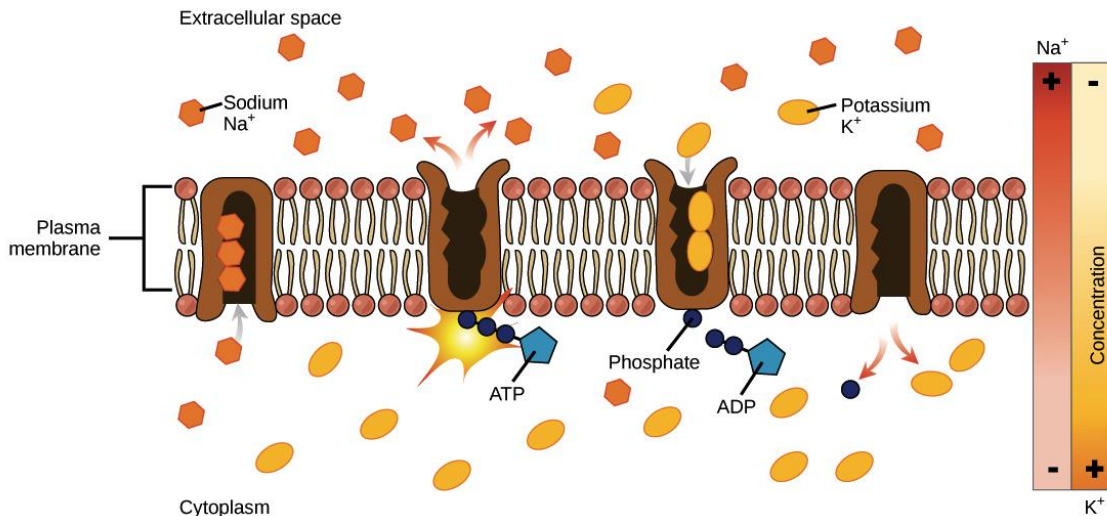


Figure showing the transport cycle of the sodium-potassium pump.

Image credit: OpenStax Biology. Image modified from original work by Mariana Ruiz Villareal.

The sodium-potassium pump transports sodium out of and potassium into the cell in a repeating cycle of conformational (shape) changes. In each cycle, three sodium ions exit the cell, while two potassium ions enter. This process takes place in the following steps:

1. To begin, the pump is open to the inside of the cell. In this form, the pump really likes to bind (has a high affinity for) sodium ions, and will take up three of them.

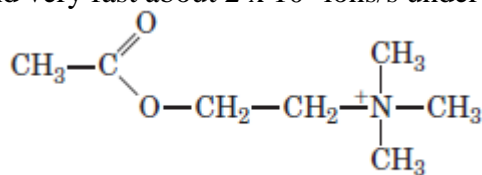
- When the sodium ions bind, they trigger the pump to hydrolyze (break down) ATP. One phosphate group from ATP is attached to the pump, which is then said to be phosphorylated. ADP is released as a by-product.
- Phosphorylation makes the pump change shape, re-orienting itself so it opens towards the extracellular space. In this conformation, the pump no longer likes to bind to sodium ions (has a low affinity for them), so the three sodium ions are released outside the cell.
- In its outward-facing form, the pump switches allegiances and now really likes to bind to (has a high affinity for) potassium ions. It will bind two of them, and this triggers removal of the phosphate group attached to the pump in step 2.
- With the phosphate group gone, the pump will change back to its original form, opening towards the interior of the cell.
- In its inward-facing shape, the pump loses its interest in (has a low affinity for) potassium ions, so the two potassium ions will be released into the cytoplasm. The pump is now back to where it was in step 1, and the cycle can begin again.

This may seem like a complicated cycle, but it just involves the protein going back and forth between two forms: an inward-facing form with high affinity for sodium (and low affinity for potassium) and an outward-facing form with high affinity for potassium (and low affinity for sodium). The protein can be toggled back and forth between these forms by the addition or removal of a phosphate group, which is in turn controlled by the binding of the ions to be transported.

Ligand-gated ion channels

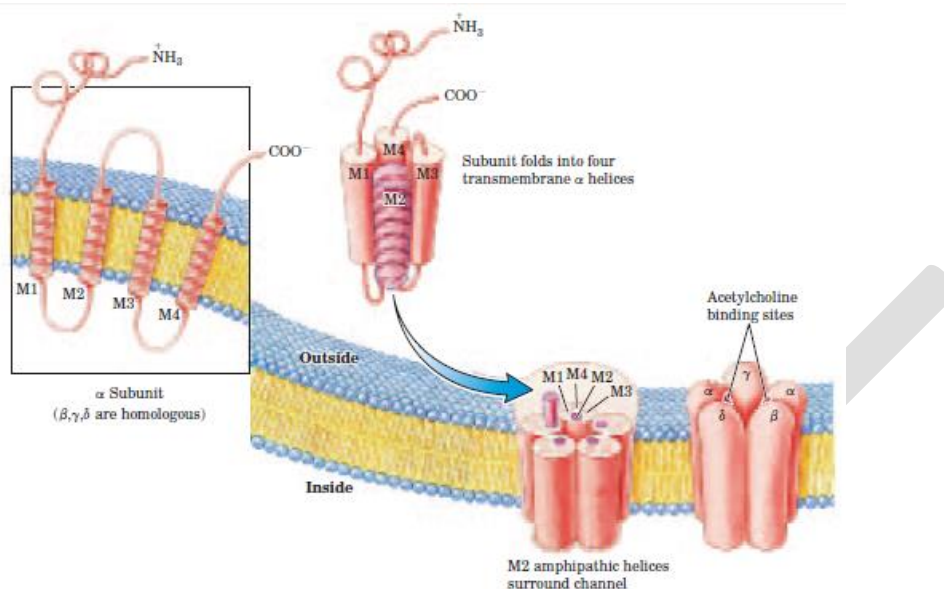
Acetylcholine receptor

Acetylcholine is the neurotransmitter at synapses between motor neurons and muscle cells, often called neuromuscular junctions. The nicotinic acetylcholine receptor, essential in the passage of an electrical signal from a motor neuron to a muscle fiber at the neuromuscular junction (signaling the muscle to contract). (Nicotinic receptors were originally distinguished from muscarinic receptors by the sensitivity of the former to nicotine, the latter to the mushroom alkaloid muscarine. They are structurally and functionally different.) Acetylcholine released by the motor neuron diffuses a few micrometers to the plasma membrane of a myocyte, where it binds to the acetylcholine receptor. This forces a conformational change in the receptor, causing its ion channel to open. The resulting inward movement of positive charges depolarizes the plasma membrane, triggering contraction. The acetylcholine receptor allows Na^+ , Ca^{2+} , and K^+ to pass through with equal ease, but other cations and all anions are unable to pass. Movement of Na^+ through an acetylcholine receptor ion channel is unsaturable (its rate is linear with respect to extracellular $[\text{Na}^+]$) and very fast about 2×10^7 ions/s under physiological conditions.

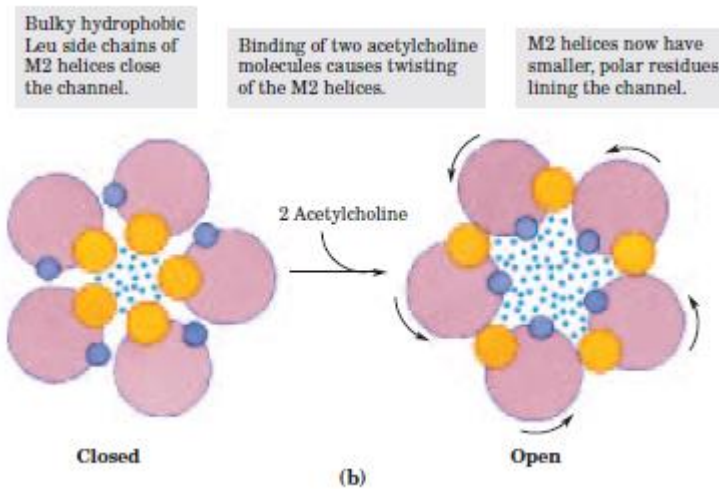


Acetylcholine

This receptor channel is typical of many other ion channels that produce or respond to electrical signals: it has a “gate” that opens in response to stimulation by a signal molecule (in this case acetylcholine) and an intrinsic timing mechanism that closes the gate after a split second. Thus the acetylcholine signal is transient an essential feature of electrical signal conduction. We understand the structural changes underlying gating in the acetylcholine receptor, but not the exact mechanism of “desensitization” of closing the gate even in the continued presence of acetylcholine.



The nicotinic acetylcholine receptor has five subunits: single copies of subunits β , γ and δ and two identical α subunits each with an acetylcholine-binding site. All five subunits are related in sequence and tertiary structure, each having four transmembrane helical segments (M1 to M4). The five subunits surround a central pore, which is lined with their M2 helices. The pore is about 20 Å wide in the parts of the channel that protrude on the cytoplasmic and extracellular surfaces, but narrows as it passes through the lipid bilayer.



Near the center of the bilayer is a ring of bulky hydrophobic side chains of Leu residues in the M2 helices, positioned so close together that they prevent ions from passing through the channel. Allosteric conformational changes induced by acetylcholine binding to the two α subunits include a slight twisting of the M2 helices, which draws these hydrophobic side chains away from the center of the channel, opening it to the passage of ions.

Based on similarities between the amino acid sequences of other ligand-gated ion channels and the acetylcholine receptor, the receptor channels that respond to the extracellular signals γ -aminobutyric acid (GABA), glycine, and serotonin have been classified in the acetylcholine receptor superfamily, and probably share three-dimensional structure and gating mechanisms. The GABA_A and glycine receptors are anion channels specific for Cl^- or HCO_3^- , whereas the serotonin receptor, like the acetylcholine receptor, is cation-specific. The subunits of each of these channels, like those of the acetylcholine receptor, have four transmembrane helical segments and form oligomeric channels.

Aquaporins: Water Channels

Water crosses cell membranes by two routes: by diffusion through the lipid bilayer and through water channels called aquaporins. Functional characterization of the first aquaporin was reported in 1992, but water channels were suspected to exist well before that time, because the osmotic permeability of some types of epithelial cells was much too large to be accounted for by simple diffusion through the plasma membrane.

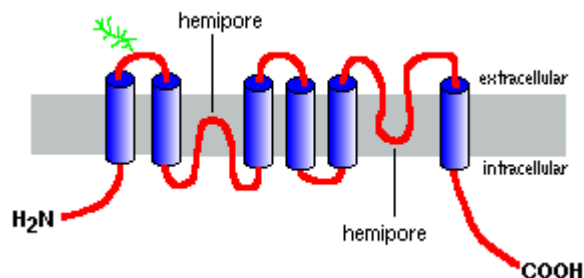
A single human aquaporin-1 channel facilitates water transport at a rate of roughly 3 billion water molecules per second. Such transport appears to be bidirectional, in accordance with the prevailing osmotic gradient.

The classical aquaporins transport solute-free water across cell membranes; they appear to be exclusive water channels and do not permeate membranes to ions or other small molecules. Some aquaporins - known as aquaglyceroporins - transport water plus glycerol and a few other small molecules.

The Aquaporin Family

More than 10 different mammalian aquaporins have been identified to date, and additional

members are suspected to exist. Closely related water channel proteins have been isolated from plants, insects and bacteria. Aquaporin-1 from human red blood cells was the first to be discovered and is probably the best studied.



Hydrophobicity plots of their amino acid sequences predict that the aquaporins have six membrane-spanning segments, as depicted in the model of aquaporin-1 to the right.

Based on studies with aquaporin-1, it appears that aquaporins exist in the plasma membrane as homotetramers. Each aquaporin monomer contains two hemi-pores, which fold together to form a water channel.

Different aquaporins have different patterns of glycosylation. In the case of aquaporin-1, the peptide backbone is roughly 28 kDa and the glycosylated forms range from 40 to 60 kDa in mass. Most aquaporins have a protein kinase A phosphorylation motif in one of the cytoplasmic loops, and differential phosphorylation is suspected to impart a regulatory function to the molecule.

Bacteriorhodopsin

The retinal protein bacteriorhodopsin is the major photosynthetic protein of the archaeon *Halobacterium salinarum*. It converts the energy of "green" light (500-650 nm, max 568 nm) into an electrochemical proton gradient, which in turn is used for ATP production by ATP synthase. It functions as a light-driven proton pump, transporting protons out of the cell, and exemplifies vectorial catalysis.

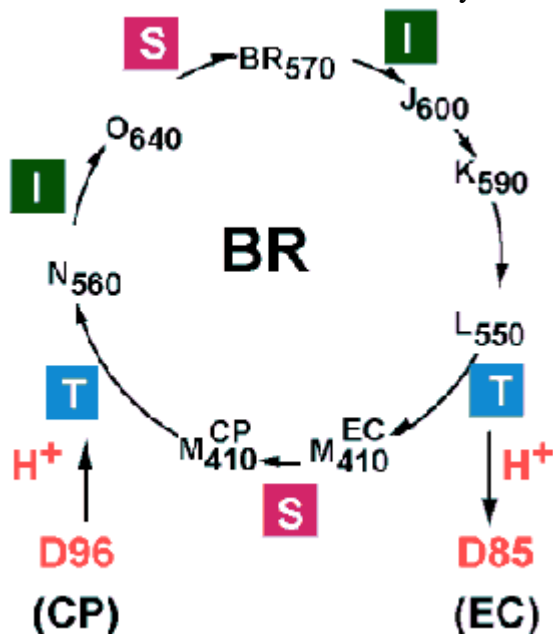
Bacteriorhodopsin is the focus of much interest and has become a paradigm for membrane proteins in general and transporters in particular. Its structure and function have been analyzed in great detail using a multitude of different experimental techniques and has become the best-understood example of vectorial catalysis.

The catalytic cycle of bacteriorhodopsin

Absorption of a photon by bacteriorhodopsin initiates a catalytic cycle that leads to transport of a proton out of the cell. Several intermediates in the photocycle have been identified by spectroscopic techniques (Fig.3). By application of a multitude of biophysical techniques, the exact nature of the changes in each step of the cycle has been determined and has been related to transport function.

The cycle can be formally described in terms of six steps of isomerization (I), ion transport (T), and accessibility change (switch S). Retinal first photo-isomerizes from an all-trans to a 13-cis configuration followed by a proton transfer from the Schiff base to the proton acceptor Asp-85. To allow vectoriality, reprotonation of the Schiff base from Asp-85 must be excluded. Thus, its accessibility is switched from extracellular to intracellular. The Schiff base is then reprotonated from Asp-96 in the cytoplasmic channel. After reprotonation of Asp-96 from the cytoplasmic surface, retinal re-isomerizes thermally and the accessibility of the Schiff base switches back to

extracellular to reestablish the initial state. These steps represent the minimal number of steps needed to account for vectorial catalysis in wild-type bacteriorhodopsin.



The catalytic cycle step by step

Dynamic structural changes occurring in chromophore and protein during the light-induced reaction cycle can be detected either directly by time-resolved spectroscopic techniques (ultrafast laser spectroscopy, flash photolysis, ESR spectroscopy, FTIR spectroscopy) or by trapping intermediate states, determining their structures by static methods (NMR spectroscopy, electron microscopy, neutron scattering) and comparing it with the ground state.

- **primary reaction: the photoisomerization of retinal from all- trans to 13- cis** In a stereoselective process, all- trans retinal is photoisomerized to 13- cis retinal. This process has been time-resolved to few femtoseconds. Within 500 fs, all- trans retinal isomerizes to 13- cis retinal, resulting in J600 which is converted to K590 within another 5 ps.
- **from the K590 to the L550 intermediate** The K590 intermediate is transformed to the L550 intermediate within 2 μ s. The hydrogen bonding interaction in the extracellular channel between the protonated Schiff base and Asp-85, which involves a water molecule, is strengthened.
- **first proton translocation step: from L550 to M410(EC)** The M state is reached from the L state within several microseconds. This transition involves transfer of a proton from the Schiff base to Asp-85 in the extracellular half-channel.
- **first accessibility switch reaction from extracellular to cytoplasmic: M410(EC) to M410(CP)** To allow vectorial proton transport, de- and reprotonation of the Schiff base must occur from different sides of the membrane. This accessibility switch occurs at the

level of the M intermediate: M410(EC) to M410(CP). Thus, the originally described "M" intermediate is in fact split into two or more different intermediates all having yellow color.

- **second proton transfer step: from M410(EC) to N560** Reprotonation of the Schiff base from Asp-96 in the cytoplasmic half-channel occurs during transformation from the M410(EC) to the N560 intermediate within milliseconds. Reprotonation of Asp-96 by a proton from the cytoplasm also occurs during the lifetime of the N560 intermediate. It should be noted that Asp-96 functions as a proton storage for reprotonation of the Schiff base. Therefore, the proton does not originate directly from the cytoplasm. This detail solves the puzzling phenomenon that the transport rate of this proton transporter is not pH-dependent (within limits).
- **thermoisomeration of retinal from 13- cis to all- trans : N560 to O640** The transition of the N560 to the O640 intermediate is the thermal 13- cis to all- trans isomerization of retinal in the environment of protonated Asp-96 and protonated Asp-85.
- **second accessibility switch reaction from cytoplasmic to extracellular: O640 to BR** Deprotonation of Asp-85 completes the catalytic cycle. Switching the accessibility of the Schiff base back from extracellular to intracellular occurs within ca 5 ms and results in restoration of the initial state.

Ionophores

Ionophores are lipid-soluble molecules that transport ions across lipid cell membranes. The subsequent disruption of cell membrane permeability results in antibacterial effects. Monensin is an ionophore antibiotic derived from *Streptomyces* that forms complexes with monovalent cations, including sodium and potassium. The complexes are transported in a nonpolar manner across the bacterial cell membrane. As such, it acts as a Na^+/H^+ antiporter. Monensin blocks intracellular protein transport, resulting in antibacterial and antimalarial effects. Monensin is used extensively in the beef and dairy industries in feed to prevent coccidiosis and improve feed efficiency. Monensin also increases the production of propionic acid and thus prevents bloat.

Valinomycin

Valinomycin displays a striking selectivity with respect to monovalent cation binding. It binds K^+ and Rb^+ tightly, but shows about a thousandfold lower affinity for Na^+ and Li^+ . The smaller ionic radii of Na^+ and Li^+ (compared to K^+ and Rb^+) may be responsible in part for the observed differences. However, another important difference between Na^+ and K^+ is shown in Table 10.5. The free energy of hydration for an ion is the stabilization achieved by hydrating that ion. The process of dehydration, a prerequisite to forming the ion-valinomycin complex, requires energy input. As shown in Table 10.5, considerably more energy is required to desolvate a Na^+ ion than to desolvate a K^+ ion. It is thus easier to form the K^+ -valinomycin complex than to form the corresponding Na^+ complex.

Gramicidin is a Channel-Forming Ionophore

In contrast to valinomycin, all protein-derived membrane transport systems appear to

function as channels, not mobile carriers. All of the proteins discussed in this chapter use multiple transmembrane segments to create channels in the membrane, through which species are transported. For this reason, it may be more relevant to consider the pore or channel ionophores. Gramicidin from *Bacillus Brevis* is a linear peptide of 15 residues and is a prototypical channel ionophore. Gramicidin contains alternating L- and D-residues, a formyl group at the N-terminus, and an ethanolamine at the C-terminus. The predominance of hydrophobic residues in the gramicidin structure facilitates its incorporation into lipid bilayers and membranes. Once incorporated in lipid bilayers, it permits the rapid diffusion of many different cations. Gramicidin possesses considerably less ionic specificity than does valinomycin but permits higher transport rates. A single gramicidin channel can transport as many as 10 million K^+ ions per second. Protons and all alkali cations can diffuse through gramicidin channels, but divalent cations such as Ca^{2+} block the channel.

Gramicidin forms two different helical structures. A double helical structure predominates in organic solvents, whereas a helical dimer is formed in lipid membranes. (An α -helix cannot be formed by gramicidin because it has both D- and L-amino acid residues.) The helical dimer is a head-to-head or amino terminus-to-amino terminus (N-to-N) dimer oriented perpendicular to the membrane surface, with the formyl groups at the bilayer center and the ethanolamine moieties at the membrane surface. The helix is unusual, with 6.3 residues per turn and a central hole approximately 0.4 nm in diameter. The hydrogen-bonding pattern in this structure, in which N—H groups alternately point up and down the axis of the helix to hydrogen-bond with carbonyl groups, is reminiscent of a β -sheet. For this reason, this structure has often been referred to as a β -helix.

POSSIBLE QUESTIONS

UNIT-I

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Define active transporters.
2. What is simple diffusion?
3. What is facilitated diffusion?
4. Define symporter.
5. What are porins?
6. Define Ionophores.
7. What are P type ATPases?
8. What are V type ATPases?
9. What are F type ATPases?
10. What is bacteriorhodopsin?
11. Expand CFTR and write its significance.
12. What is gramicidin?
13. Define diffusion.

PART-C (8 MARKS)

1. Differentiate simple diffusion and facilitated diffusion?
2. Elaborate primary active transporters with appropriate examples.
3. Explain ion channels mediated transport mechanisms.
4. Explain different modes of transport across membrane.
5. What is ABC family of transporters? Explain its significance in drug uptake.
6. What is symporter? Write a note on sodium glucose symporter.
7. How would you differentiate between primary and secondary active transporters.
8. Explain sodium potassium pump with relevant example.

9. Write a note on Na⁺ glucose symporter.
10. Differentiate diffusion and active transport with relevant examples.
11. Write a note on secondary active transporters.
12. How glucose is transported across membranes?

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18BCU103 - Membrane Biology and Bioenergetics

PART A (20 X 1 = 20 MARKS) - Online MCQ questions

Unit 2

S.No	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	All are inhibitors of oxidative phosphorylation	Carboxin	Oligomycin	Valinomycin	Atractyloside	Carboxin
2	Ionophores have following action except -	Inhibit ADP to ATP	Abolish proton gradient	Abolish pH gradient	Hydrophilic in character	Hydrophilic in character
3	Some antibiotics act as ionophores, which	interfere directly with	increase cell membrane	inhibit both transcription	inhibit electrophoretic	increase cell membrane
4	Sodium and potassium ions are transported	cell-recognition	channel	carrier	receptor	carrier
5	Which of the following is NOT an active	simple diffusion	active transport	endocytosis	exocytosis	simple diffusion
6	Pinocytosis is a type of _____.	endocytosis	exocytosis	active transport	simple diffusion	endocytosis
7	_____ is the net movement of any type of	Osmosis	Diffusion	Facilitated diffusion	Active transport	Diffusion
8	Lipid-soluble molecules and gases enter the	diffusion through the	osmosis through the	diffusion through the lipid	osmosis through the lipid	diffusion through the lipid
9	Which of the following conditions does NOT	carrier proteins bind to	transports molecules down	does not require ATP	transports molecules from	carrier proteins bind to
10	Which of the following processes uses a carrier	simple diffusion	osmosis	facilitated diffusion	active transport	active transport
11	Which of the following conditions does NOT	requires ATP	transports molecules from	requires a carrier protein	carrier proteins bind	transports molecules from a
12	Why are proteins involved in active transport	They use energy to move	They use energy to move a	They use energy to bind	They use energy to	They use energy to move a
13	Which process will transport sodium ions to	simple diffusion	facilitated diffusion	osmosis	active transport	active transport
14	The passage of salt (Na+Cl ⁻) across a plasma	Sodium ions are first	Chloride ions then diffuse	The negatively charged	Chloride ions are first	Chloride ions are first
15	Which of the following statements about the	In the Na ⁺ /K ⁺ ATPase	In the Na ⁺ /K ⁺ ATPase	Cardiac glycosides inhibit	In the Na ⁺ /K ⁺ ATPase	In the Na ⁺ /K ⁺ ATPase pump
16	Which of the following statements about ATP-	A number of genetic	ATP-binding cassette (ABC)	The multidrug resistance	ATP-binding cassette (ABC)	ATP-binding cassette (ABC)
17	Which of the following statements is not true	Ligand-gated ion channel	Neurotransmitters can act	Ligand-gated ion channels	Differences in membrane	Ligand-gated ion channel
18	A substance can only be accumulated against	facilitated diffusion	Passage through ion	Diffusion through a	Active transport	Active transport
19	The principal intracellular cation is:	Na	Cl	K	Ca	K
20	Which of the following is an example of	Cl ⁻ -HCO ³⁻ exchange	Na ⁺ - H exchange	Na ⁺ -Ca exchange	The Na ⁺ , K ATPase	The Na ⁺ , K ATPase
21	Type of transport which always involves a	passive transport	active transport	lateral diffusion	flip flop	active transport
22	ATP hydrolysis is coupled in	primary active transport	Secondary active transport	Tertiary active transport	Quaternary active	primary active transport
23	If two solute are transported in opposite	Uniport	Symport	Antiport	Airport	Antiport
24	If two solute are transported in same direction	Uniport	Symport	Antiport	Airport	Symport
25	LDL is transported by	endocytosis	exocytosis	pinocytosis	diffusion	endocytosis

26	Iron is transported by	endocytosis	exocytosis	pinocytosis	diffusion	endocytosis
27	Which of the following do not transport iron	ferritin	hemosiderin	transferrin	calmodulin	calmodulin
28	Water is transported by	glycophorin	aquaporin	uniporin	biporin	aquaporin
29	Aquaportin is regulated by	insulin	TRH	Vasopressin	TSH	Vasopressin
30	CFTR functions as	cation channel	anion channel	neutral channel	CFTR is not a ion channel	anion channel
31	CFTR transports	chloride	sodium	potassium	carbon	chloride
32	Deficiency of CFTR causes	Diabetes	Cystic fibrosis	ciliasis	it does not any disease	Cystic fibrosis
33	CFTR is related to	cholera	not related to cholera	cation transport	mercury ion transport	cholera
34	ABC transporters utilize the energy of	ATP	TTP	UTP	GTP	ATP
35	Bacterial ABC transporters are NOT essential in	cell viability	virulence	pathogenicity	for diffusion	for diffusion
36	Cyanide inhibits the ETC specifically	complex 1	complex 4	complex 2	complex 3	complex 4
37	Aquaportins are integral	membrane proteins	membrane lipids	membrane carbohydrates	NOT integral proteins	membrane proteins
38	Aquaporin is a	monomer	dimer	trimer	tetramer	tetramer
39	Aquaportin is mainly expressed in	liver	stomach	kidneys	pancreas	kidneys
40	Devic disease is due to autoimmune reaction	aquaporin 1	aquaporin 2	aquaporin 4	aquaporin 3	aquaporin 4
41	Diabetes insipidus is mainly due to mutations	aquaporin 1	aquaporin 2	aquaporin 4	aquaporin 3	aquaporin 2
42	lactose permeases are	membrane proteins	membrane lipids	membrane carbohydrates	NOT integral proteins	membrane proteins
43	lactose permeases can be classified as	Uniport	Symport	Antiport	Airport	Symport
44	lactose permease has how many	2	10	12	28	12
45	Lactose permease is encoded by which gene	lac Y	lac Z	lac A	lac B	lac Y
46	valinomycin is involved in the transport of	carbon	selenium	paladium	potassium	potassium
47	valinomycin is a	dodecadepsipeptide	decapeptide	nonapeptide	steroid	dodecadepsipeptide
48	Bacteriorhodopsin is a	proton pump	electron pump	neutron pump	not an ion pump	proton pump
49	Gramicidin contains	d-aminoacids	l-aminoacids	alternative d and l	aminopterin	alternative d and l aminoacids
50	Gramicidin is a/an	bactericidal	specific to virus	specific to protozoan	fungicide	bactericidal
51	Congenital myasthenic syndrome is due to	defective acetylcholine	defective arylhydrocarbon	acquired infections	no reasons	defective acetylcholine
52	seven transmembrane receptors are present in	nucleus	cytoplasm	cell membrane	mitochondria	cell membrane
53	Which channel predominantly functions to	Chloride channel	proton channel	potassium channel	calcium channel	proton channel
54	Sodium-dependent glucose cotransport is an	primary active transport	Secondary active transport	Tertiary active transport	diffusion	Secondary active transport
55	Simple diffusion requires	ATP	UTP	TTP	No energy	No energy
56	Porins are	alpha barrel proteins	beta barrel proteins	gamma barrel proteins	delta barrel proteins	beta barrel proteins
57	beta strands in porins lie	parallel	antiparallel	perpendicular	hyperbolic	antiparallel
58	Choline is a	quaternary ammonium	protein	lipid	aminoacid	quaternary ammonium salt
59	myasthenia gravis is due to	insulin	defective acetylcholine	pancreas	gut	defective acetylcholine

60	ABC transporters are	not membrane bound	bound on the periphery of	transmembrane proteins	not proteins	transmembrane proteins
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UNIT-III

SYLLABUS

Vesicular transport, membrane fusion and bioenergetics: Types of vesicle transport and their function- clathrin, COP I and COP II coated vesicles. Molecular mechanism of vesicular transport. Membrane fusion. Receptor mediated endocytosis of transferrin. Laws of thermodynamics, state functions, equilibrium constant, coupled reactions, energy charge, ATP cycle, phosphorylation potential, phosphoryl group transfers. Chemical basis of high standard energy of hydrolysis of ATP, other phosphorylated compounds and thioesters. Redox reactions, standard redox potentials and Nernst equation. Universal electron carriers.

Vesicular transport, membrane fusion and bioenergetics

Types of vesicle transport and their function

There are three different kinds of vesicles.

After budding from the trans-Golgi network, (1) the first type of vesicle immediately moves to and fuses with the plasma membrane, releasing its contents by exocytosis. In all cell types, at least some proteins are loaded into such vesicles and secreted continuously in this manner. Examples of proteins released by such constitutive (or continuous) secretion include collagen by fibroblasts, serum proteins by hepatocytes, and antibodies by activated B lymphocytes.

(2) The second type of vesicle to bud from the trans-Golgi network, known as secretory vesicles, are stored inside the cell until a signal for exocytosis causes release of their contents at the plasma membrane. Among the proteins released by such regulated secretion are peptide hormones (e.g., insulin, glucagon, ACTH) from various endocrine cells, precursors of digestive enzymes from pancreatic acinar cells, milk proteins from the mammary gland, and neurotransmitters from neurons.

(3) The third type of vesicle that buds from the trans-Golgi network is directed to the lysosome, an organelle responsible for the intracellular degradation of macromolecules, and to lysosome-like storage organelles in certain cells. Secretory proteins destined for lysosomes first are transported by vesicles from the trans-Golgi network to a compartment usually called the late endosome; proteins then are transferred to the lysosome by a mechanism that is not well understood but may involve direct fusion of the endosome with the lysosomal membrane.

- i. Soluble proteins delivered by this pathway include lysosomal digestive enzymes (e.g., proteases, glycosidases, and phosphatases) and membrane proteins (e.g., V-class proton pump) that pump H^+ from the cytosol into the acidic lumen of the endosome and lysosome.
- ii. As we will see, some of the specific protein-processing and –sorting events that take place within these organelles depend on their low luminal pH.
- iii. The endosome also functions in the endocytic pathway in which vesicles bud from the plasma membrane bringing membrane proteins and their bound ligands into the cell.

After being internalized by endocytosis, some proteins are transported to lysosomes, while others are recycled back to the cell surface.

- iv. Endocytosis is a way for cells to take up nutrients that are in macromolecular form—for example, cholesterol in the form of lipoprotein particles and iron complexed with the serum protein transferrin.
- v. Endocytosis also can function as a regulatory mechanism to decrease signaling activity by withdrawing receptors for a particular signaling molecule from the cell surface.

The Mechanism of Vesicular Transport

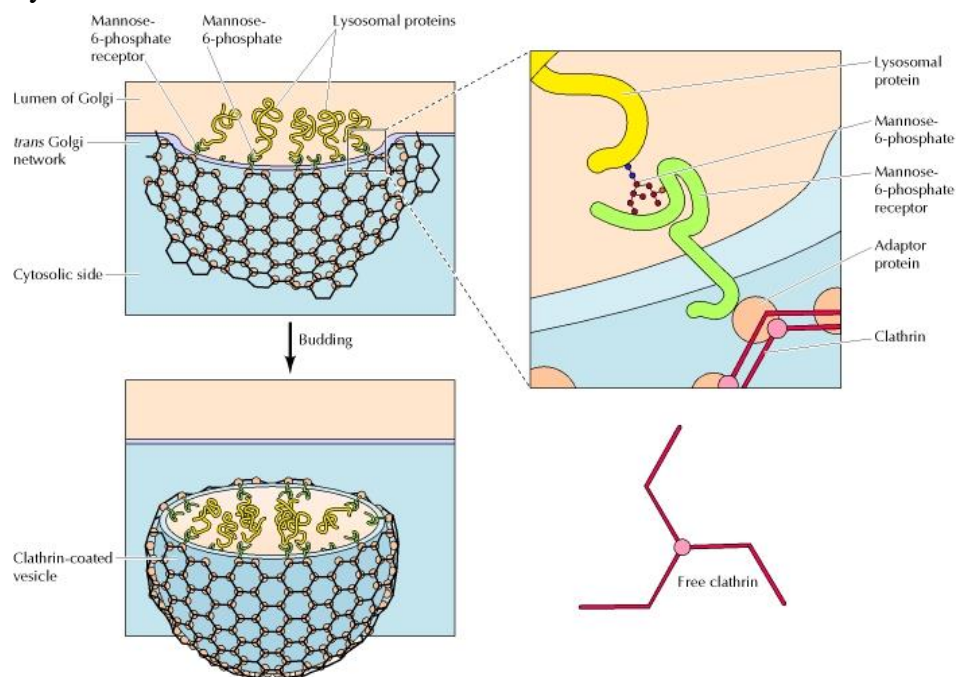
As is evident from the preceding sections of this chapter, transport vesicles play a central role in the traffic of molecules between different membrane-enclosed compartments of the secretory pathway. Vesicles are similarly involved in the transport of materials taken up at the cell surface. Vesicular transport is thus a major cellular activity, responsible for molecular traffic between a variety of specific membrane-enclosed compartments. The selectivity of such transport is therefore key to maintaining the functional organization of the cell. For example, lysosomal enzymes must be transported specifically from the Golgi apparatus to lysosomes—not to the plasma membrane or to the ER. Some of the signals that target proteins to specific organelles, such as lysosomes, were discussed earlier in this chapter. These proteins are transported within vesicles, so the specificity of transport is based on the selective packaging of the intended cargo into vesicles that recognize and fuse only with the appropriate target membrane. Because of the central importance of vesicular transport to the organization of eukaryotic cells, understanding the molecular mechanisms that control vesicle packaging, budding, and fusion is a major area of research in cell biology.

Coat Proteins and Vesicle Budding

The first step in vesicular transport is the formation of a vesicle by budding from the membrane. The cytoplasmic surfaces of transport vesicles are coated with proteins, and it appears to be the assembly of these protein coats that drives vesicle budding by distorting membrane conformation. Three kinds of coated vesicles, which appear to function in different types of vesicular transport, have been characterized. The first to be described were the **clathrin-coated vesicles**, which are responsible for the uptake of extracellular molecules from the plasma membrane by endocytosis as well as the transport of molecules from the *trans* Golgi network to lysosomes. Two other types of coated vesicles have been identified as budding from the ER and Golgi complex. These vesicles are called nonclathrin-coated or **COP-coated vesicles** (COP stands for coat protein). One class of these vesicles (COPII-coated vesicles) bud from the ER and carry their cargo forward along the secretory pathway, to the Golgi apparatus. In contrast, COPI-coated vesicles bud from the ER-Golgi intermediate compartment or the Golgi apparatus and function in the retrieval pathways that serve to retain resident proteins in the Golgi and ER. For example, COPI-coated vesicles transport resident ER proteins marked by the KDEL or KKXX retrieval signals back to the ER from the ER-Golgi intermediate compartment or the *cis* Golgi network.

The coats of clathrin-coated vesicles are composed of two types of protein complexes, clathrin and adaptor proteins, which assemble on the cytosolic side of membranes. Clathrin plays a structural role by assembling into a basketlike lattice structure that distorts the membrane and

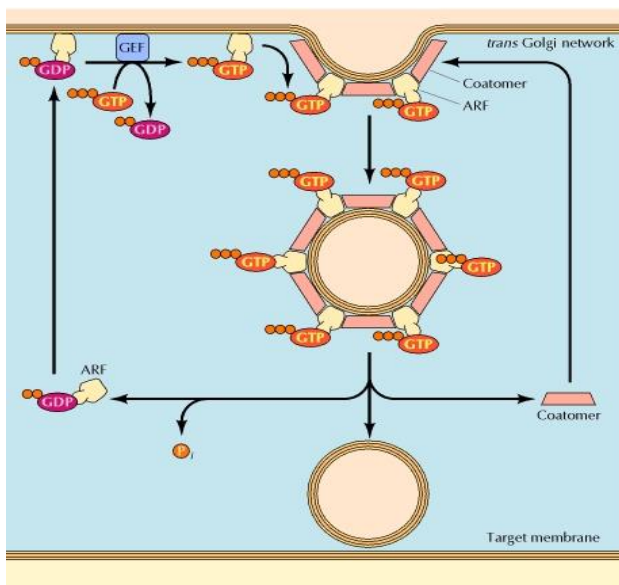
drives vesicle budding. The binding of clathrin to membranes is mediated by a second class of proteins, called adaptor proteins. Different adaptor proteins are responsible for the assembly of clathrin-coated vesicles at the plasma membrane and at the *trans* Golgi network, and it is the adaptor proteins that are involved in selecting the specific molecules to be incorporated into the vesicles. For example, the AP-1 adaptor protein involved in budding from the *trans* Golgi network binds to the cytosolic portion of the mannose-6-phosphate receptor, thereby directing proteins destined for lysosomes into clathrin-coated vesicles.



Incorporation of lysosomal proteins into clathrin-coated vesicles

The coats of COPI- and COPII-coated vesicles are composed of distinct protein complexes, which function analogously to clathrin and adaptor proteins in vesicle budding. Interestingly, components of the COPI coat interact with the KKXX motif responsible for the retrieval of ER proteins from the Golgi apparatus, consistent with a role for COPI-coated vesicles in recycling from the Golgi to the ER.

The assembly of vesicle coats also requires GTP-binding proteins, which appear to regulate the binding of coat proteins to the membrane. The budding of both clathrin-coated and COPI-coated vesicles from the Golgi complex requires a GTP-binding protein called ARF (ADP-ribosylation factor), while the budding of COPII-coated vesicles from the ER requires a distinct GTP-binding protein called Sar1. The role of these proteins is illustrated by the function of ARF in assembly of COPI-coated vesicles (Figure 3.2). The first step in vesicle formation is the association of ARF bound to GDP with the Golgi membrane. Proteins in the Golgi membrane then stimulate the exchange of the GDP bound to ARF for GTP, and the COPI coat proteins bind to the ARF/GTP complex. Assembly of the coat is then followed by deformation of the membrane and vesicle budding. ARF then hydrolyzes its bound GTP, leading to the conversion of ARF to the GDP-bound state and the dissociation of coat proteins from the vesicle membrane.

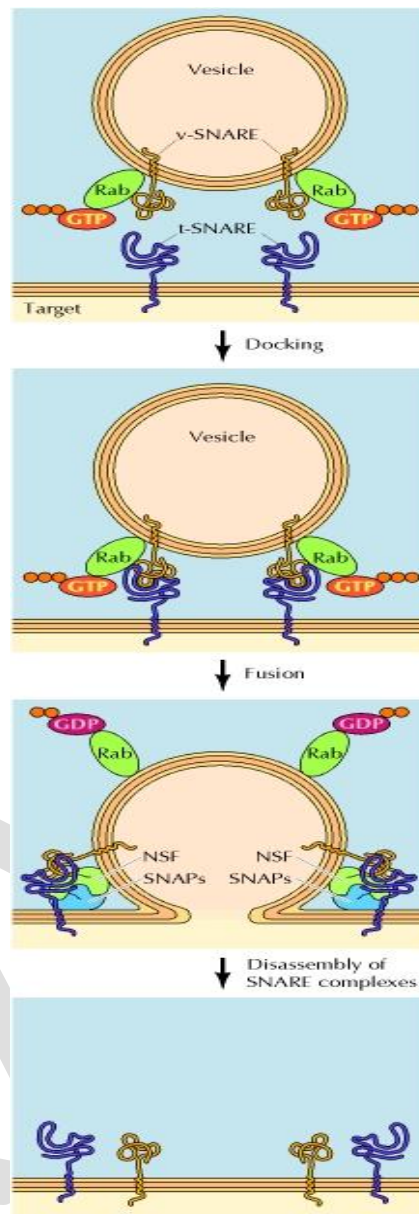


Role of ARF in the formation of COP-coated vesicles

Vesicle Fusion

The fusion of a transport vesicle with its target involves two types of events. First, the transport vesicle must specifically recognize the correct target membrane; for example, a vesicle carrying lysosomal enzymes has to deliver its cargo only to lysosomes. Second, the vesicle and target membranes must fuse, thereby delivering the contents of the vesicle to the target organelle. Research over the last several years has led to development of a model of vesicle fusion in which specific recognition between a vesicle and its target is mediated by interactions between unique pairs of transmembrane proteins, followed by fusion between the phospholipid bilayers of the vesicle and target membranes.

Proteins involved in vesicle fusion were initially identified in James Rothman's laboratory by biochemical analysis of reconstituted vesicular transport systems from mammalian cells. Analysis of the proteins involved in vesicle fusion in these systems led Rothman and his colleagues to propose a general model, called the **SNARE hypothesis**, in which vesicle fusion is mediated by interactions between specific pairs of proteins, called SNAREs, on the vesicle and target membranes (v-SNAREs and t-SNAREs, respectively) (Figure 3.3). This hypothesis was supported by the identification of SNAREs that were present on synaptic vesicles and by the finding of yeast secretion mutants that appeared to encode SNAREs required for a variety of vesicle transport events. For example, transport from the ER to the Golgi in yeast requires specific SNAREs that are located on both the vesicle and target membranes. The formation of complexes between v-SNAREs on the vesicle and t-SNAREs on the target membranes then leads to membrane fusion, by mechanisms which remain to be fully understood.



Vesicle fusion

In addition to SNAREs, vesicle fusion requires at least two other types of proteins. The Rab proteins are a family of small GTP-binding proteins that are related to the Ras proteins, which were discussed in Chapter 7. More than 30 different Rab proteins have been identified and shown to function in specific vesicle transport processes. They may function in several steps of vesicle trafficking, including interacting with SNAREs to regulate and facilitate the formation of v-SNARE/t-SNARE complexes.

Following the formation of complexes between complementary SNAREs and membrane fusion, a complex of two additional proteins (the NSF/ SNAP complex) is needed to complete the process of vesicle transport. The NSF/SNAP proteins are recruited to membranes following the

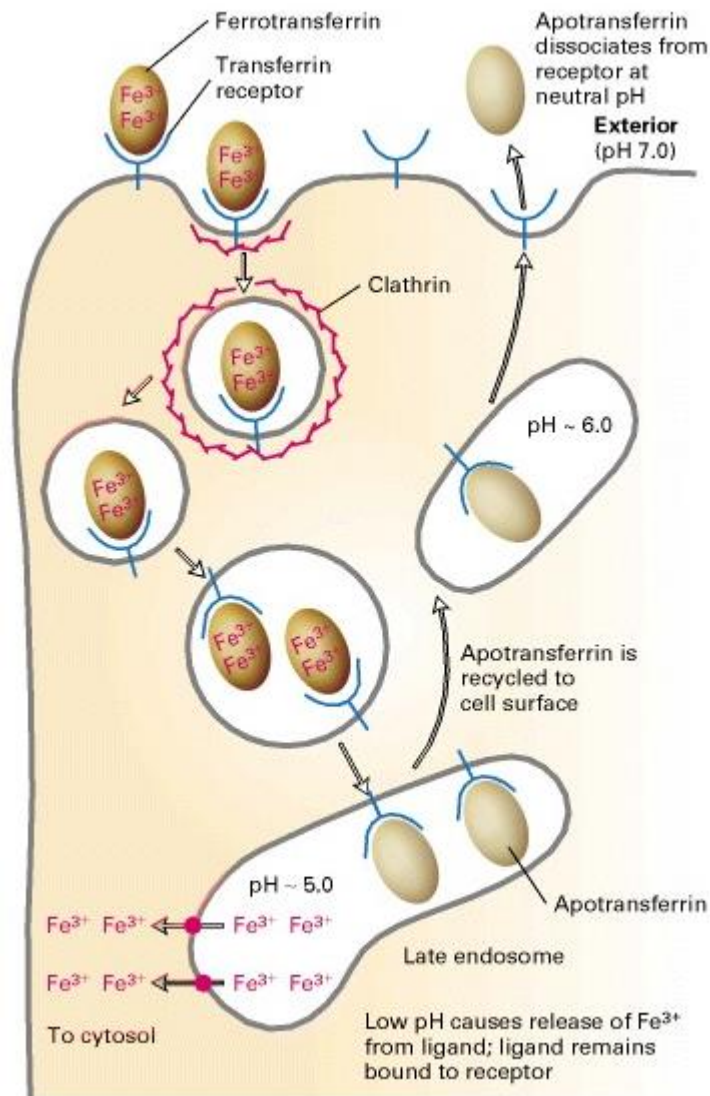
formation of v-SNARE/t-SNARE complexes, and they are not required directly for either vesicle/target pairing or for the fusion of paired membranes. Instead, the NSF/SNAP proteins act after membrane fusion to disassemble the SNARE complex, thereby allowing the SNAREs to be reutilized for subsequent rounds of vesicle transport.

Receptor-Mediated Endocytosis of transferrin

Some of the integral membrane proteins that a cell displays at its surface are receptors for particular components of the ECF. For example, iron is transported in the blood complexed to a protein called transferrin. Cells have receptors for transferrin on their surface. When these receptors encounter a molecule of transferrin, they bind tightly to it. The complex of transferrin and its receptor is then engulfed by endocytosis. Ultimately, the iron is released into the cytosol. The strong affinity of the transferrin receptor for transferrin (its ligand) ensures that the cell will get all the iron it needs even if transferrin represents only a small fraction of the protein molecules present in the ECF. Receptor-mediated endocytosis is many thousand times more efficient than simple pinocytosis in enabling the cell to acquire the macromolecules it needs.

The endocytic pathway involving the transferrin receptor and its ligand differs from the LDL pathway in that the receptor-ligand complex does not dissociate in late endosomes. Nonetheless, changes in pH also mediate the sorting of receptors and ligands in the transferrin pathway, which functions to deliver iron to cells.

Transferrin, a major glycoprotein in the blood, transports iron to all tissue cells from the liver (the main site of iron storage in the body) and from the intestine (the site of iron absorption). The iron-free form, *apotransferrin*, binds two Fe^{3+} ions very tightly to form *ferrotransferrin*. All growing cells contain surface transferrin receptors that avidly bind ferrotransferrin at neutral pH, after which the receptor-bound ferrotransferrin is subjected to endocytosis. Like the components of LDL, the two bound Fe^{3+} atoms remain in the cell, but there the similarity with the fate of other endocytosed ligands, including LDL, ends: the apotransferrin part of the ligand is secreted from the cell within minutes, carried in the bloodstream to the liver or intestine, and reloaded with iron.



The explanation for the behavior of the transferrin receptor – ligand complex lies in the unique ability of apotransferrin to remain bound to the transferrin receptor at the low pH (5.0 – 5.5) of late endosomes. At a pH of less than 6.0, the two bound Fe^{3+} atoms dissociate from ferrotransferrin and are transported from the late endosome vesicle into the cytosol (in an unknown manner). The apotransferrin formed by the dissociation of the iron atoms remains bound to the transferrin receptor and is recycled back to the surface along with the receptor. Remarkably, although apotransferrin binds tightly to its receptor at a pH of 5.0 or 6.0, it does not bind at neutral pH. Hence the bound apotransferrin dissociates from its receptor when the recycling vesicles fuse with the plasma membrane and the receptor-ligand complex encounters the neutral pH of the extracellular interstitial fluid or growth medium. The surface receptor is then free to bind another molecule of ferrotransferrin.

The Laws of Thermodynamics

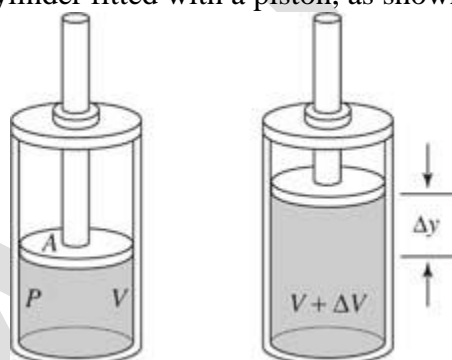
The laws of thermodynamics involve the relations between heat and mechanical, electrical, and other forms of energy or work. The laws are valid only when applied to systems in thermal equilibrium and *not* for systems in the process of rapid change or with complicated states of transition. A system very nearly in equilibrium all the time is called a reversible system.

The first law of thermodynamics

The **first law of thermodynamics** is the restatement of conservation of energy. Mathematically, it reads $\Delta Q = \Delta U + \Delta W$, where ΔQ is the heat energy supplied to the system, ΔU is the change in the internal energy, and ΔW is the work done by the system against external forces. It must be emphasized that these quantities are defined in general terms. The internal energy includes not only mechanical energy, but also the rotational and vibrational energy of the molecules, as well as the chemical energy stored in interatomic forces. Work is not only mechanical work but includes other forms, such as work done by electrical currents.

Work

Imagine a system of gas in a cylinder fitted with a piston, as shown in Figure



A cylinder filled with gas, with a piston.

As the gas in the cylinder expands, the force exerted by the gas on the piston is $F = PA$. The piston moves up a distance Δy ; therefore, the work done by the gas is $W = F\Delta y = PA\Delta y$, or $W = P\Delta V$ because $A\Delta y$ is the increase in volume (V) of the gas. In general, work done by an expanding gas equals the area under a pressure-volume curve.

The second law of thermodynamics

The second law of thermodynamics can be stated thus: It is impossible to construct a heat engine that only absorbs heat from a heat source and performs an equal amount of work. In other words, no machine is ever 100 percent efficient; some heat must be lost to the environment.

The second law also determines the order of physical phenomenon. Imagine viewing a film where a pool of water forms into an ice cube. Obviously, the film is running backward from the way in which it was filmed. An ice cube melts as it heats but never spontaneously cools to form an ice cube again; thus, this law indicates that certain events have a preferred direction of time, called the **arrow of time**. If two objects of different temperatures are placed in thermal contact, their final temperature will be between the original temperatures of the two objects. A second way to state the second law of thermodynamics is to say that heat cannot spontaneously pass from a colder to a hotter object.

Entropy

Entropy is the measure of how much energy or heat is unavailable for work. Imagine an isolated system with some hot objects and some cold objects. Work can be done as heat is transferred from the hot to the cooler objects; however, once this transfer has occurred, it is impossible to extract additional work from them alone. Energy is always conserved, but when all objects have the same temperature, the energy is no longer available for conversion into work.

The change in entropy of a system (ΔS) is defined mathematically as

$$\Delta S = \frac{\Delta Q}{T}$$

The equation states the following: The change in entropy of a system is equal to the heat flowing into the system divided by the temperature (in degrees Kelvin).

The entropy of the universe increases or remains constant in all natural processes. It is possible to find a system for which entropy decreases, but only due to a net increase in a related system. For example, the originally hotter objects and cooler objects reaching thermal equilibrium in an isolated system may be separated, and some of them put in a refrigerator. The objects would again have different temperatures after a period of time, but now the system of the refrigerator would have to be included in the analysis of the complete system. No net decrease in entropy of all the related systems occurs. This is yet another way of stating the second law of thermodynamics.

The concept of entropy has far-reaching implications that tie the order of our universe to probability and statistics. Imagine a new deck of cards in order by suits, with each suit in numerical order. As the deck is shuffled, no one would expect the original order to return. There is a probability that the randomized order of the shuffled deck would return to the original format, but it is exceedingly small. An ice cube melts, and the molecules in the liquid form have less order than in the frozen form. An infinitesimally small probability exists that all of the slower moving molecules will aggregate in one space so that the ice cube will reform from the pool of water. The entropy and disorder of the universe increase as hot bodies cool and cold bodies warm. Eventually, the entire universe will be at the same temperature, so the energy will be no longer usable.

State Functions

State functions are "variables" that define the state of a system. When you have a system you need to be able to define the conditions in which it exists before and after a change. We typically referred to these as the initial and final states. By states we mean the system can be described by a set of properties. For example, the state of a system might be that I have 1 mole of argon in a 10 L container with a temperature of 300 K. Here the state of the system is defined by the "state functions" of volume and temperature as well as the amount of the gas. Likewise, the pressure is also a state function. We'll also see later in thermodynamics that there are a number of variables related to energy that are also state functions.

If I take my 1 mole of gas and do something to it, I might end up in a new state where for example the volume is now 20 L and the temperature is 600 K. For such a process I can look at the change in the state functions. In this case the initial volume, V_i , was 10 L and the final volume, V_f , was 20 L. So my change in volume is given by

$$\Delta V = V_f - V_i = 20\text{L} - 10\text{L} = 10\text{L}$$

The key to this idea is that the change in volume has nothing to do with the particulars of the mystery process that brought me from my initial state to my final state. The difference in volume

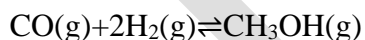
will always be the same. That is, if I started with a volume of 10 L and ended with a volume of 20 L the difference is always + 10 L. This might seem frightfully obvious. However, when we start to think about abstract state functions related to energy it can be more difficult to wrap your head around the ideas. But it is important to know that the concept is exactly the same. If you know the initial state and you know the final state, then you can calculate the change (regardless of the process by which you achieved the change).

Equilibrium constant

There are two types of equilibrium reactions. It is important to understand the difference between the two, because their equilibrium constants are expressed differently.

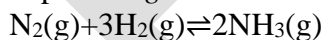
Homogeneous

The simpler one, a homogeneous reaction, is one where the states of matter of the products and reactions are all the same (the word "homo" means "same"). In most cases, the solvent determines the state of matter for the overall reaction. For example, the synthesis of methanol from a carbon monoxide-hydrogen mixture is a *gaseous* homogeneous mixture, which contains two or more substances:



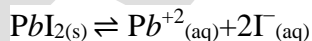
At equilibrium, the rate of the forward and reverse reaction are equal, which is demonstrated by the arrows. The equilibrium constant, however, gives the ratio of the units (pressure or concentration) of the products to the reactants when the reaction is at equilibrium.

The synthesis of ammonia is another example of a *gaseous* homogeneous mixture:

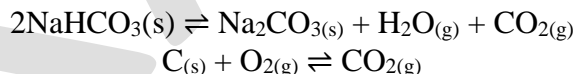


Heterogeneous

A heterogeneous reaction is one where one or more states within the reaction differ (the Greek word "heteros" means "different"). For example, the formation of an aqueous solution of lead(II) iodide creates a heterogeneous mixture dealing with molecules in both the *solid* and *aqueous* states:



The decomposition of sodium hydrogen carbonate (baking soda) at high elevations is another example of a heterogeneous mixture, this reaction deals with molecules in both the *solid* and *gaseous* states:

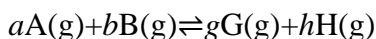


Once again, this difference is emphasized so that students remember that equilibrium constant calculations are different from heterogeneous mixtures.

Equilibrium Constants

An equilibrium constant is obtained by letting a single reaction proceed to equilibrium and then measuring the concentrations of each molecule involved in that reaction and creating a ratio of the product concentrations to reactant concentrations. Because the concentrations are measured at equilibrium, the equilibrium constant is remain the same for a given reaction independent of initial concentrations, which determine the rate (for an ideal reaction). This knowledge allowed scientists to derive a model expression that can serve as a "template" for any reaction. This basic "template" form of a homogeneous equilibrium constant is examined here.

This discussion makes use of the following hypothetical reaction:



*The lower case letters represent the number of moles of each molecule, the upper case letters represent the molecule itself, and the letters in the parenthesis always represents the state of matter of the molecule.

Equilibrium Constant of Concentration

The equilibrium constant of concentration gives the ratio of concentrations of products over reactants for a reaction that is at equilibrium. This is usually used when the state of matter for the reaction is (aq). The equilibrium constant expression is written as K_c , as in the expression below:

$$K_c = \frac{[G]^g [H]^h}{[A]^a [B]^b}$$

- If $K > 1$ then equilibrium favors products
- If $K < 1$ then equilibrium favors the reactants

Here, the letters inside the brackets represent the concentration of each molecule. Notice the mathematical product of the chemical products raised to the powers of their respective coefficients is the numerator of the ratio and the mathematical product of the reactants raised to the powers of their respective coefficients is the denominator. This is the case for every equilibrium constant. Keep in mind that this expression was obtained by a homogeneous equilibrium reaction. K represents an equilibrium constant and c represents concentration (e.g., K_c). This means that every species shows up in the expression, as long as it is a solution or a gas.

Equilibrium Constant of Pressure

Gaseous reaction equilibria are not expressed in terms of concentration, but instead in terms of partial pressures. The equilibrium constant of pressure gives the ratio of pressure of products over reactants for a reaction that is at equilibrium (again, the concentrations of all species are raised to the powers of their respective concentrations). The equilibrium constant is written as K_p , as shown below:

$$K_p = \frac{p_G^g p_H^h}{p_A^a p_B^b}$$

- Where p can have units of pressure (e.g., atm or bar).

The procedure for this is the same as the procedure for the concentration constant above.

Conversion of K_c to K_p

To convert K_c to K_p , the following equation is used:

$$K_p = K_c (RT)^{\Delta n_{gas}}$$

where:

- $R = 0.0820575 \text{ L atm mol}^{-1} \text{ K}^{-1}$ or $8.31447 \text{ J mol}^{-1} \text{ K}^{-1}$
- T = Temperature in Kelvin
- Δn_{gas} = Moles of gas (product) - Moles of Gas (Reactant)

Reaction Quotient

Another quantity of interest is the reaction quotient, Q_c , which is the same ratio constant at any point in the reaction at which the reaction is not at equilibrium. The reaction quotient is calculated the same way as is K_c , but is not necessarily equal to K_c . It is used to determine which way the reaction will proceed at any given point in time.

$$Q_c = \frac{[G]^g [H]^h}{[A]^a [B]^b}$$

- $Q_c > K_c$, then the reactions shifts to the left
- $Q_c < K_c$, then the reactions shifts to the right
- $Q_c = K_c$ then the reaction is still at equilibrium

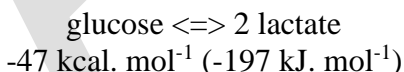
The same process is employed when calculating Q_p

Coupled reactions

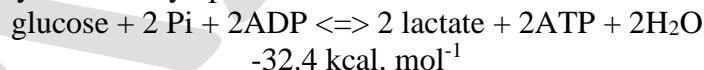
Coupling between reactions

A change of state of a system which occurs independently of coupling to the surroundings cannot conserve the work potential associated with the change. For a chemical reaction in a test-tube, all the work potential (change in free-energy, or the $-DG$ of the process) is squandered as heat. If the system is able to exchange heat with the surroundings, this heat appears in the surroundings as an increase in entropy. In order to conserve energy, a change in state of the system must be coupled to a change in the surroundings which increases the work potential of a part of the surroundings (work is performed on the surroundings, or a separate system undergoes a change in state with $+DG$). In the context of biological energy conservation, this means that the work potential (as represented by the $-DG$) for a biochemical reaction will be lost unless the reaction occurs in concert with (is coupled to) another reaction which has a $+DG$.

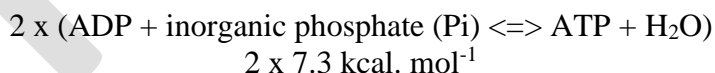
For example, the reaction of fermentation in an organism producing lactate as the sole product of glucose metabolism can be written as:



The reaction of glycolysis in the cytoplasm can be written as:



The difference between these two reactions is:



If we sum the free energy changes for the fermentation reaction and ATP synthesis ($-47 \text{ kcal. mol}^{-1} + 14.6 \text{ kcal. mol}^{-1}$) we get $-32.4 \text{ kcal. mol}^{-1}$, the free energy change from the glycolysis reaction.

We can see that the change in the system represented by the fermentation reaction, with a $-DG$, is coupled to a change in the surroundings (the change in a separate system represented by the phosphorylation of ADP to ATP), with $+DG$.

When two systems are coupled in this way, it is often convenient to treat them as a single

system. In this example, the new system is the reaction represented by the glycolysis equation, with a $-DG$ equal to the sum of values for the two processes contributing.

From this example, it will be apparent that we can, from a thermodynamic perspective, treat metabolic processes in several ways. We can treat individual reactions as separate systems, or treat a set of coupled reactions (including the complete set representing the metabolism of the organism as a whole) as a single system. The choice is one of convenience, and the important points are that the system should be carefully defined, the reaction equation balanced in conformity with the Law of conservation of mass, and the energy equation balanced in accordance with the First Law of thermodynamics, and the properties of variables of state.

Energy charge

Energy charge is an index used to measure the energy status of biological cells. It is related to ATP, ADP and AMP concentrations. Energy charge was first defined by Atkinson and Walton who found that it was necessary to take into account the concentration of all three nucleotides, rather than just ATP and ADP, to account for the energy status in metabolism.

Many reactions in metabolism are controlled by the energy status of the cell. One index of the energy status is the *energy charge*, which is proportional to the mole fraction of ATP plus half the mole fraction of ADP, given that ATP contains two anhydride bonds whereas ADP contains one. Hence the energy charge is defined as:

$$\text{Energy charge} = \frac{[\text{ATP}] + \frac{1}{2}[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$$

The energy charge can have a value ranging from 0 (all AMP) to 1 (all ATP). Daniel Atkinson showed that ATP-generating (catabolic) pathways are inhibited by a high energy charge. In plots of the reaction rates of such pathways versus the energy charge, the curves are steep near an energy charge of 0.9, where they usually intersect. It is evident that control of these pathways has evolved to maintain the energy charge within rather narrow limits. In other words the energy charge like the pH of a cell is buffered. The energy charge of most cells range from 0.8 to 0.95.

ATP Cycle

Adenosine Triphosphate (ATP).

This molecule acts as the short-term energy currency of the cell and provides the source of energy used in individual synthetic (nonspontaneous) reactions.

ATP collects small packets of energy from the food-burning power plants of the cell and transports this energy to where it is needed.

Some energy in ATP is released to do work, such as move muscles or force a seedling out of the ground. At other times, ATP gives up its energy to a nonspontaneous synthetic reaction, such as the formation of sucrose.

ATP is used to close the energy gap between energy-releasing reactions (food breakdown) and energy-requiring reactions (synthesis).

When a molecule of fatty acid is burned, energy is given off. Some of this energy is trapped in molecules of ATP, and some is lost in the form of heat. Each ATP molecule can then be transported elsewhere within the cell and used where needed.

The energy-carrying part of an ATP molecule is the triphosphate "tail". Three phosphate groups are joined by covalent bonds. The electrons in these bonds carry energy.

Within the power plants of the cell (mitochondria), energy is used to add one molecule of inorganic phosphate (P) to a molecule of adenosine diphosphate (ADP).

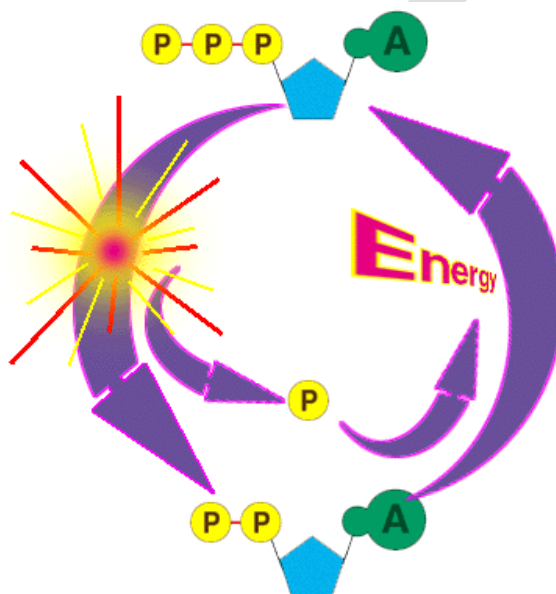


The amount of energy stored is about 7,300 calories for every mole of ATP formed.

At the energy-requiring site, the last phosphate group in the tail is broken off and the energy in the bond is liberated.



Again, about 7,300 calories of energy per mole is released. The ADP and the phosphate are then free to return to the power plant and be rejoined. In this way, ATP and ADP are constantly being recycled.



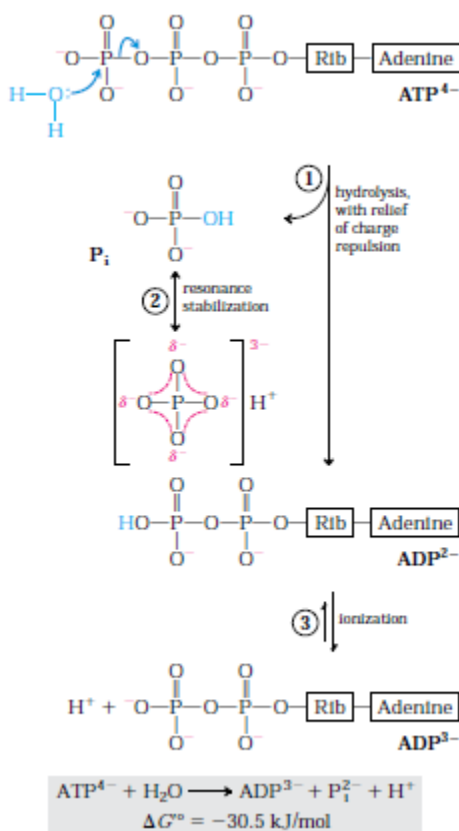
Phosphoryl Group Transfers and ATP

Having developed some fundamental principles of energy changes in chemical systems, we can now examine the energy cycle in cells and the special role of ATP as the energy currency that links catabolism and anabolism. Heterotrophic cells obtain free energy in a chemical form by the catabolism of nutrient molecules, and they use that energy to make ATP from ADP and P_i . ATP then donates some of its chemical energy to endergonic processes such as the synthesis of metabolic intermediates and macromolecules from smaller precursors, the transport of substances across membranes against concentration gradients, and mechanical motion. This donation of energy from ATP generally involves the covalent participation of ATP in the reaction that is to be driven, with the eventual result that ATP is converted to ADP and P_i or, in some reactions, to AMP and 2 P_i . We discuss here the chemical basis for the large free-energy changes that accompany hydrolysis of ATP and other high-energy phosphate compounds, and we show that most cases of energy donation by ATP involve group transfer, not simple hydrolysis of ATP. To illustrate the range of energy transductions in which ATP provides the energy, we consider the synthesis of information-rich macromolecules, the transport of solutes across membranes, and motion

produced by muscle contraction.

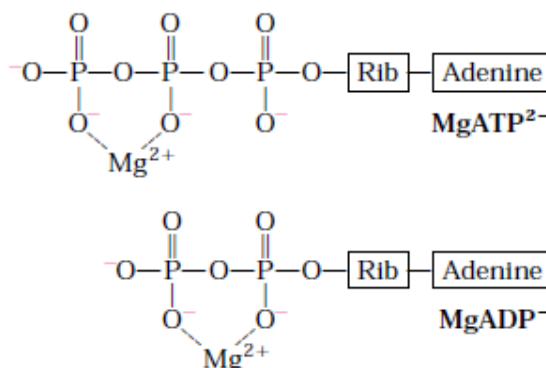
The Free-Energy Change for ATP Hydrolysis

The hydrolytic cleavage of the terminal phosphoric acid anhydride (phosphoanhydride) bond in ATP separates one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP; the P_i (HPO_4^{2-}) released is stabilized by the formation of several resonance forms not possible in ATP; and ADP^{2-} , the other direct product of hydrolysis, immediately ionizes, releasing H^+ into a medium of very low $[H^+]$ ($\sim 10^{-7}$ M). Because the concentrations of the direct products of ATP hydrolysis are, in the cell, far below the concentrations at equilibrium (Table 13–5), mass action favors the hydrolysis reaction in the cell.



Chemical basis for the large free-energy change associated with ATP hydrolysis.

Although the hydrolysis of ATP is highly exergonic ($\Delta G^\circ = -30.5 \text{ kJ/mol}$), the molecule is kinetically stable at pH 7 because the activation energy for ATP hydrolysis is relatively high. Rapid cleavage of the phosphoanhydride bonds occurs only when catalyzed by an enzyme.

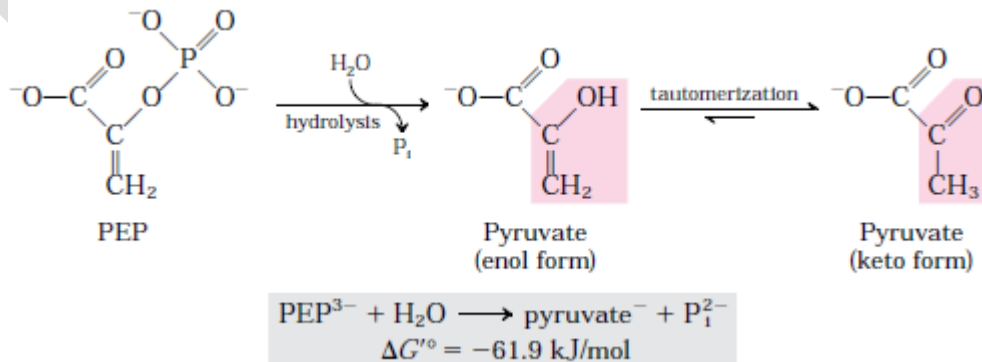


Mg²⁺ and ATP.

The free-energy change for ATP hydrolysis is -30.5 kJ/mol under standard conditions, but the *actual* free energy of hydrolysis (ΔG) of ATP in living cells is very different: the cellular concentrations of ATP, ADP, and Pi are not identical and are much lower than the 1.0 M of standard conditions. Furthermore, Mg²⁺ in the cytosol binds to ATP and ADP, and for most enzymatic reactions that involve ATP as phosphoryl group donor, the true substrate is MgATP²⁻. The relevant ΔG is therefore that for MgATP²⁻ hydrolysis. In intact cells, ΔG for ATP hydrolysis, usually designated ΔG_p , is much more negative than ΔG° , ranging from -50 to -65 kJ/mol. ΔG_p is often called the phosphorylation potential. In the following discussions we use the standard free-energy change for ATP hydrolysis, because this allows comparison, on the same basis, with the energetics of other cellular reactions. Remember, however, that in living cells ΔG is the relevant quantity for ATP hydrolysis and all other reactions and may be quite different from ΔG° .

Other Phosphorylated Compounds and Thioesters Also Have Large Free Energies of Hydrolysis

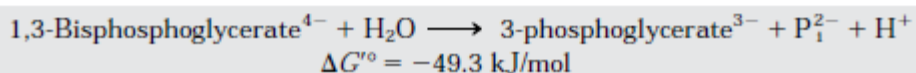
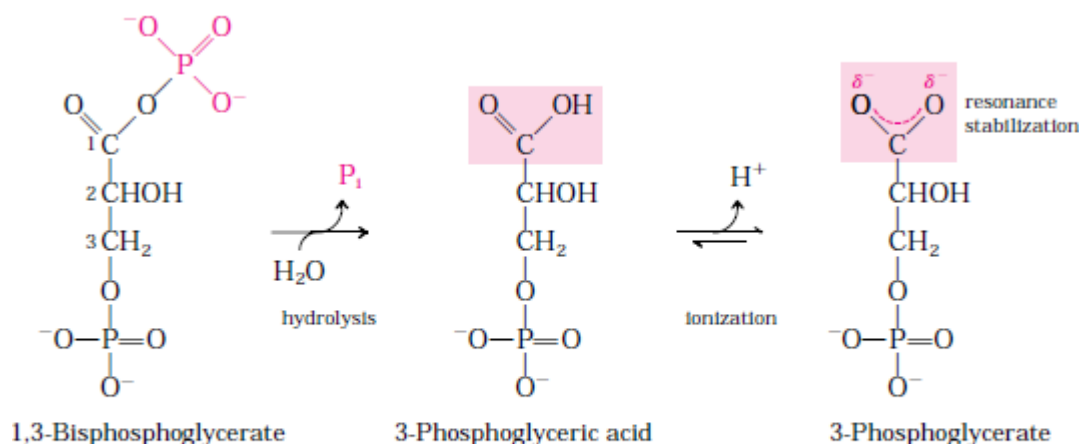
Phosphoenolpyruvate contains a phosphate ester bond that undergoes hydrolysis to yield the enol form of pyruvate, and this direct product can immediately tautomerize to the more stable keto form of pyruvate. Because the reactant (phosphoenolpyruvate) has only one form (enol) and the product (pyruvate) has two possible forms, the product is stabilized relative to the reactant. This is the greatest contributing factor to the high standard free energy of hydrolysis of phosphoenolpyruvate: $\Delta G^\circ = -61.9$ kJ/mol.



Hydrolysis of phosphoenolpyruvate (PEP)

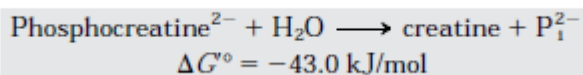
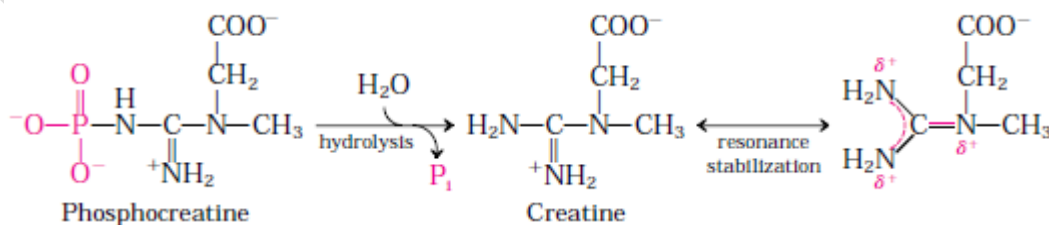
Another three-carbon compound, 1,3-bisphosphoglycerate, contains an anhydride bond

between the carboxyl group at C-1 and phosphoric acid. Hydrolysis of this acyl phosphate is accompanied by a large, negative, standard free-energy change ($\Delta G'^{\circ} = -49.3 \text{ kJ/mol}$), which can, again, be explained in terms of the structure of reactant and products. When H_2O is added across the anhydride bond of 1,3-bisphosphoglycerate, one of the direct products, 3-phosphoglyceric acid, can immediately lose a proton to give the carboxylate ion, 3-phosphoglycerate, which has two equally probable resonance forms. Removal of the direct product (3-phosphoglyceric acid) and formation of the resonance-stabilized ion favor the forward reaction.



Hydrolysis of 1,3- bisphosphoglycerate.

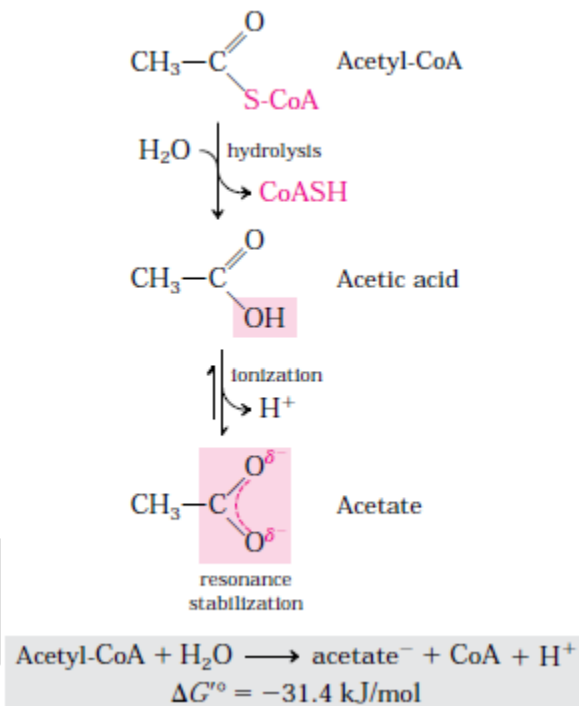
In phosphocreatine (Fig. 13–5), the PON bond can be hydrolyzed to generate free creatine and P_i . The release of P_i and the resonance stabilization of creatine favor the forward reaction. The standard free-energy change of phosphocreatine hydrolysis is again large, -43.0 kJ/mol . In all these phosphate-releasing reactions, the several resonance forms available to P_i stabilize this product relative to the reactant, contributing to an already negative free-energy change.



Hydrolysis of phosphocreatine.

Thioesters, in which a sulfur atom replaces the usual oxygen in the ester bond, also have large, negative, standard free energies of hydrolysis. Acetyl-coenzyme A, or acetyl-CoA, is one of many thioesters important in metabolism. The acyl group in these compounds is activated for transacylation, condensation, or oxidation-reduction reactions. Thioesters undergo much less

resonance stabilization than do oxygen esters; consequently, the difference in free energy between the reactant and its hydrolysis products, which *are* resonance-stabilized, is greater for thioesters than for comparable oxygen esters. In both cases, hydrolysis of the ester generates a carboxylic acid, which can ionize and assume several resonance forms. Together, these factors result in the large, negative $\Delta G'^{\circ}$ (-31 kJ/mol) for acetyl-CoA hydrolysis.



Hydrolysis of acetyl-coenzyme A.

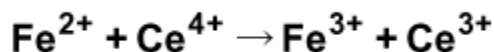
3.11. Redox reaction

Redox reactions are reactions in which one species is reduced and another is oxidized. Therefore the oxidation state of the species involved must change. These reactions are important for a number of applications, including energy storage devices (batteries), photographic processing, and energy production and utilization in living systems including humans.

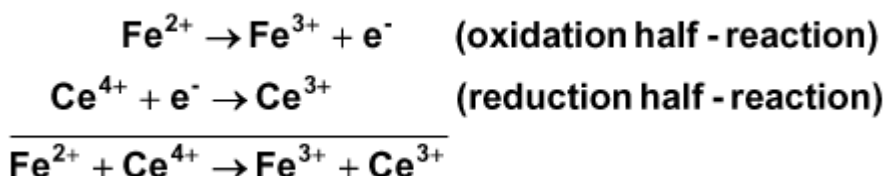
Reduction: A process in which an atom gains an electron and therefore decreases (or reduces its oxidation number). Basically the positive character of the species is reduced.

Oxidation: A process in which an atom loses an electron and therefore increases its oxidation number. In other words, the positive character of the species is increased.

Historically, the term "oxidation" was used because the redox reactions that were first systematically investigated took place in oxygen, with oxygen being reduced and the other species being oxidized, hence the term oxidation reaction. However, it was later realized that this case (oxidation reactions involving oxygen) was just one possible scenario. For example consider the redox reaction shown below.

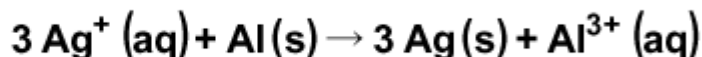


In this process the Fe^{2+} ion is oxidized, but there is no oxygen involved in this reaction. The Ce^{4+} ion, which is reduced acts as the oxidizing agent. So oxidation reactions need not involve oxygen. This redox reaction is actually the sum of two separate half-reactions (a reduction half-reaction and an oxidation half-reaction).



Example

In the following redox reaction, which species is being oxidized? Which one is being reduced?



$\text{Al} (\text{s})$ is being oxidized.

$\text{Ag}^+ (\text{aq})$ is being reduced.

A mnemonic you might find helpful to remember the definitions of oxidation and reduction is: *Leo the lion goes ger*. Leo: lose electron(s) = oxidation. Ger: gain electron(s) = reduction.

Oxidation State: The condition of a species with a specified oxidation number. An element with a given oxidation number exists in the corresponding oxidation state.

Assigning Oxidation Numbers

The following rules for assignment of oxidation numbers are listed in hierarchical order.

1. Pure elements (in their natural, standard state): ox. # = 0.
2. Monatomic ions: ox. # = ionic charge.
3. F is always F (-I) in compounds.
4. Alkali metals (those in the 1st column of the periodic table): ox. # = I.
5. Alkaline-earth metals (those in the 2nd column of the periodic table): ox # = II.
6. Hydrogen is almost always H (I). The exception is in metal hydrides (MH_x).
7. Oxygen is almost always O (-II) in compounds. Exceptions are O-O and O-F.

The sum of all oxidation numbers in the species will equal the total charge of that species.

Standard redox potentials

The standard reduction potential is the tendency for a chemical species to be reduced, and is measured in volts at standard conditions. The more positive the potential is the more likely it

will be reduced.

Introduction

The standard reduction potential is in a category known as the standard cell potentials or standard electrode potentials. The standard cell potential is the potential difference between the cathode and anode. For more information view Cell Potentials. The standard potentials are all measured at 298 K, 1 atm, and with 1 M solutions.

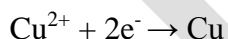
Standard Reduction Potentials

As stated above, the standard reduction potential is the likelihood that a species will be reduced. It is written in the form of a reduction half reaction. An example can be seen below where "A" is a generic element and C is the charge.

- Standard Reduction Potential



For example, copper's Standard Reduction Potential of $E^{\circ}=+0.340V$ is for this reaction:



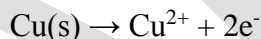
Standard Oxidation Potentials

The standard oxidation potential is much like the standard reduction potential. It is the tendency for a species to be *oxidized* at standard conditions. It is also written in the form of a half reaction, and an example is shown below.

- Standard Oxidation Potential (SOP) under standard conditions:



Copper's Standard Oxidation Potential



$$E_0^{\circ}(SOP) = -0.34 V$$

The standard oxidation potential and the standard reduction potential are opposite in sign to each other for the same chemical species.

- Relation Between Standard Reduction Potential (SRP) and the Standard Oxidation Potential (SOP)

$$E_0^{\circ}(SRP) = -E_0^{\circ}(SOP)$$

Nernst equation

Walther H. Nernst, notable for the development of the Nernst equation and winner of 1920 Nobel Prize in chemistry, was a major contributor to the study of membrane potential. He developed the Nernst equation to solve for the equilibrium potential for a specific ion.

The *Nernst Equation* is derived from the Gibbs free energy under standard conditions.

$$E^{\circ} = E^{\circ}_{\text{reduction}} - E^{\circ}_{\text{oxidation}}$$

ΔG is also related to E under general conditions (standard or not) via

$$\Delta G = -nFE$$

with

- n is the number of electrons transferred in the reaction (from balanced reaction),

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- F is the Faraday constant (96,500 C/mol), and
- E is potential difference.

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POSSIBLE QUESTIONS

UNIT-I

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. What is/are the main function(s) of clathrin?
2. Define Redox reaction
3. What are the different types of vesicular transport?
4. What is Nernst equation?
5. What is membrane fusion?
6. What is clathrin?
7. What is vesicular transport?
8. What is phosphorylation potential?

PART-C (8 MARKS)

1. Explain receptor mediated endocytosis of transferrin.
2. Write a note on redox reactions and Nernst equation.
3. Explain different laws of thermodynamics.
4. Explain the molecular mechanism of vesicular transport.
5. Write a note on universal electron carriers.
6. Elaborate on COPI and COPII coated vesicles.

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18BCU103 - Membrane Biology and Bioenergetics **PART A (20 X 1 = 20 MARKS) - Online MCQ questions**

Unit 3

S.No	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	ATP transforms after breakdown of two-high	CPM	AMP	ADP	PDA	ADP
2	Gain of hydrogen atoms from a molecule	loss of neutrons	loss of protons	loss of electron	gain of electrons	gain of electrons
3	Ribose, adenine and phosphate group in linear	sodium chloride	ATP molecule	amino acids	calcium phosphate	ATP molecule
4	Reactions by which C-H bonds of fuel	dilute-concentrated	light-dark reactions	solute-solvent reactions	oxidation-reduction	oxidation-reduction
5	ATP was discovered by Karl Lohmann in	1829	1929	1949	1910	1929
6	During common energy reactions, number of	two	three	four	six	two
7	Double-ringed nitrogenous base present in ATP	adenine	ribose	phosphate group	adenosine	adenine
8	Nucleotide which is energy currency of all cells	adenosine	adenosine diphosphate	adenosine triphosphate	adenosine bimagnese	adenosine
9	Form of investment of energy in respiration is	dark energy	kinematic energy	glycol-bond energy	bond energy	bond energy
10	Direct source of energy in life processes of life	catabolize reaction	anatomize reaction	redox reaction	saturated reaction	redox reaction
11	Bonds present in fuel molecules that are broke	C-H bonds	D-H bonds	M-H bonds	O-H bonds	C-H bonds
12	Energy released by breakdown of one	5.8 kcal	7.3 kcal	8.3 kcal	9.2 kcal	7.3 kcal
13	Form of investment of energy in	light energy	kinetic energy	potential energy	dark energy	light energy
14	ADP can be built from	AMP	ATP	CMP	TPM	AMP
15	-----is the simultaneous transmembrane	Antiport	Symport	Osmosis	Diffusion	Antiport
16	The movement of a lipid within one leaflet of a	Lateral diffusion	Osmosis	Antiport	Reverse osmosis	Lateral diffusion
17	Oxidoreductases involved in biological	Dehydrogenases	Transaminases	Oxygenases	Hydroperoxidases	Transaminases
18	All of the electron carriers are components of	NADP ⁺	NAD ⁺	FAD	co-enzyme Q	NADP ⁺
19	The energy content of ATP molecule is	1000 calories	5000 calories	8000 calories	12000 calories	8000 calories
20	All the following are high energy compounds	Phosphor enol	Glucose-6 phosphate	ATP	Creatine phosphate	Creatine phosphate
21	The energy rich process of ATP are related to	Sugar groups	N-glycoside linkage	Heterocyclic ring	Phosphate anhydride	Phosphate
22	Which of the following form part of the	Outer chloroplast	Outer hydrogenosome	Lysosomal membrane	Golgi apparatus	Outer chloroplast
23	Which of the following statements is correct?	Coatomer-coated II	With reference to	The G protein, guanine	Active ADP ribosylation	Active ADP
24	Secretory cells that release relatively large	Rough endoplasmic	Smooth endoplasmic	Golgi apparatus	Mitochondria	Mitochondria
25	What is the name of the following statement:	First Law of	Second Law of	Mechanical equivalent of	Zeroth Law of	Zeroth Law of

26	The average molecular kinetic energy of a gas	Pressure	Volume	Temperature	Number of moles	Temperature
27	The second law of thermodynamics essentially	heat is energy	motion energy	at the atomic level,	entropy increases	entropy increases
28	Oxidation involves	loss of hydrogen	loss of oxygen	gain in hydrogen	gain in electrons	loss of hydrogen
29	Redox reactions involves exchange of	electrons between	protons between	neutrons between atoms	neurons between	electrons between
30	Redox reaction in living organisms involves	carbon atoms	nitrogen atoms	hydrogen atoms	oxygen atoms	hydrogen atoms
31	Hydrolysis of phosphate groups in ATP is an	exergonic process	endergonic process	endothermic process	both A and C	exergonic process
32	ATP is hydrolyzed in to	ADP	inorganic phosphate	both A and B	organic phosphate	both A and B
33	Reaction by which chemical energy that has	ATP phosphorylation	ATP dehydrogenation	ATP hydrogenation	ATP hydrolysis	ATP hydrolysis
34	A compound which is found in all living cells	ADP	ATP	chlorophyll	granum	ATP
35	Chlorophyll converts light energy into	heat energy	chemical energy	potential energy	electrical energy	chemical energy
36	There is a chemical link between anabolism	ADP	ATP	both A and B	ASP	ATP
37	Thylakoid membrane produces ATP by process	photosynthesis	chemiosmosis	chemosynthesis	respiration	chemiosmosis
38	Oxidizing agent which removes hydrogen from	FAD	NAD	NADP	NADPH	FAD
39	There is reduction as well as oxidation during	redox process	oxidative reaction	reduction	neutral process	redox process
40	Synthesis of ATP by initiation of reaction with	photophosphorylation	photolysis	photo system	photosynthesis	photophosphorylati
41	Energy transformation in living systems is	photosynthesis	respiration	kreb's cycle	translocation	photosynthesis
42	Splitting of water molecule releasing oxygen is	photosynthesis	photolysis	photolytic	photophosphorylation	photolysis
43	Specialized molecule which traps high energy	primary acceptor	electron acceptor	primary electron acceptor	electron acceptor	primary electron
44	Study of energy relationships and energy	microbiology	biotechnology	bioenergetics	biophysics	bioenergetics
45	If left hand side of chemical equation is ADP +	AMP + P _i + energy	APM + M _i + energy	BMP + B _i + energy	TDA + T _i + energy	AMP + P _i + energy
46	Major source of energy to perform cellular	ATP	BTP	PTA	APT	ATP
47	Biologist who discovered ATP is	Daniel Olive	Daniel Koshland	Karl Lohmann	Emil August	Karl Lohmann
48	Nobel Prize winner biologist who proposed	Fritz Lipmann	Emil August	Daniel Koshland	Karl Lohmann	Fritz Lipmann
49	Nobel Prize winner Fritz Lipmann who	1949	1935	1941	1929	1941
50	Sunlight absorbed by chlorophylls is converted	synthetic energy	potential energy	kinetic energy	chemical energy	chemical energy
51	Energy released by breakdown of bonds is	hydra energy	thermal energy	potential energy	kinetic energy	kinetic energy
52	Released kinetic energy by breakdown of	potential energy	artificial energy	hydra energy	thermal energy	potential energy
53	Gain of electrons is known as	oxidation	reduction	anabolism	metabolism	reduction
54	ADP is recombined with P _i to form	adenine	adenosine triphosphate	ribose triphosphate	adenine diphosphate	adenosine
55	Energy received from sunlight is absorbed by	chromatin	chromatids	chlorophyll	chloroplasts	chlorophyll
56	Important factor which plays role in ability of	pH structure	cellular structure	nuclear structure	molecular structure	molecular structure
57	Factors that affect electrons on being an	arrangement of atoms	location	arrangement of molecules	both a and b	both a and b
58	High-energy molecules that are made by	AMP and ADP	AMP and ATP	NADPH and ATP	ATP and DNA	NADPH and ATP
59	Deficiency or absence of any environmental	limiting factor	defeating factor	winning factor	metabolic factor	limiting factor

60	Loss of hydrogen atoms from a molecule	loss of electron	gain of electrons	gain of protons	gain of neutrons	loss of electron
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UNIT-IV

SYLLABUS

Oxidative phosphorylation: Mitochondria. Electron transport chain- its organization and function. Inhibitors of ETC and uncouplers. Peter Mitchell's chemiosmotic hypothesis. Proton motive force. Fo F1ATP synthase, structure and mechanism of ATP synthesis. Metabolite transporters in mitochondria. Regulation of oxidative phosphorylation. ROS production and antioxidant mechanisms. Thermogenesis. Alternative respiratory pathways in plants.

Oxidative Phosphorylation

Mitochondria

- Mitochondria are rod-shaped organelles that can be considered the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP).
- ATP is the chemical energy "currency" of the cell that powers the cell's metabolic activities. This process is called aerobic respiration and is the reason animals breathe oxygen.
- Without mitochondria (singular, mitochondrion), higher animals would likely not exist because their cells would only be able to obtain energy from anaerobic respiration (in the absence of oxygen), a process much less efficient than aerobic respiration.
- In fact, mitochondria enable cells to produce 15 times more ATP than they could otherwise, and complex animals, like humans, need large amounts of energy in order to survive.

Mitochondria Structural Features

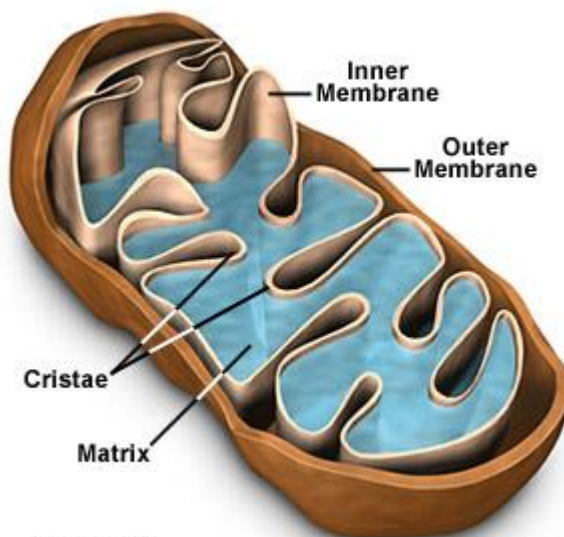


Figure 1

The number of mitochondria present in a cell depends upon the metabolic requirements of that cell, and may range from a single large mitochondrion to thousands of the organelles.

Mitochondria, which are found in nearly all eukaryotes, including plants, animals, fungi, and protists, are large enough to be observed with a light microscope and were first discovered in the 1800s. The name of the organelles was coined to reflect the way they looked to the first scientists to observe them, stemming from the Greek words for "thread" and "granule." For many years after their discovery, mitochondria were commonly believed to transmit hereditary information. It was not until the mid-1950s when a method for isolating the organelles intact was developed that the modern understanding of mitochondrial function was worked out.

Electron transport chain

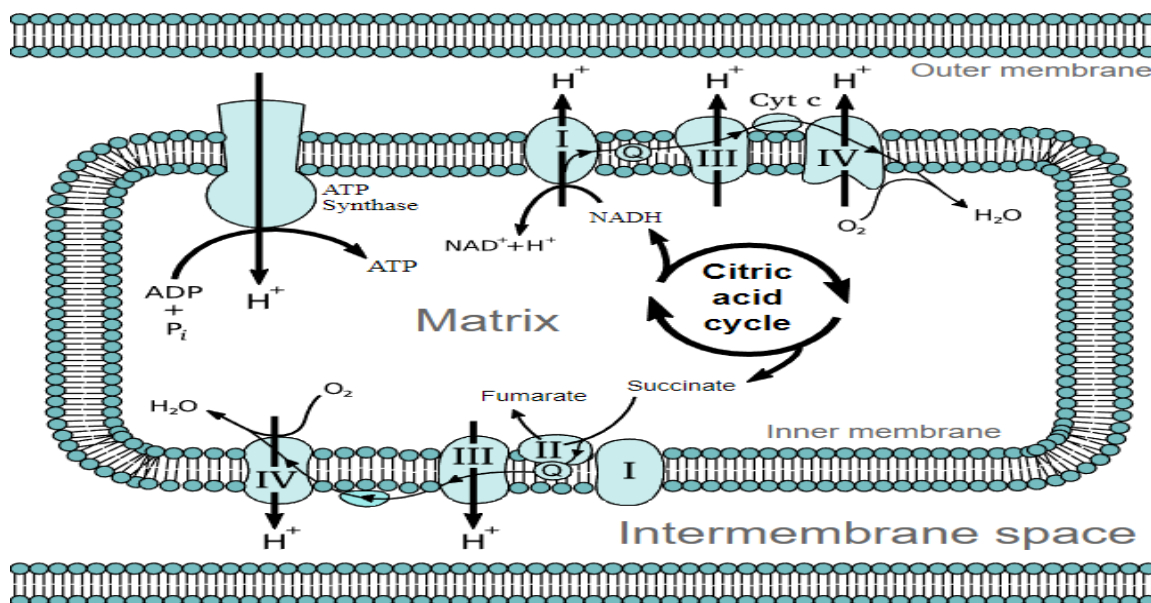
The electron transport chain (aka ETC) is a process in which the NADH and [FADH₂] produced during glycolysis, β -oxidation, and other catabolic processes are oxidized thus releasing energy in the form of ATP. The mechanism by which ATP is formed in the ETC is called chemiosmotic phosphorylation.

Introduction

The byproducts of most catabolic processes are NADH and [FADH₂] which are the reduced forms. Metabolic processes use NADH and [FADH₂] to transport electrons in the form of hydride ions (H⁻). These electrons are passed from NADH or [FADH₂] to membrane bound electron carriers which are then passed on to other electron carriers until they are finally given to oxygen resulting in the production of water. As electrons are passed from one electron carrier to another hydrogen ions are transported into the intermembrane space at three specific points in the chain. The transportation of hydrogen ions creates a greater concentration of hydrogen ions in the intermembrane space than in the matrix which can then be used to drive ATP Synthase and produce ATP (a high energy molecule).

Overview

In the diagram located below there are the major electron transporters responsible for making energy in the ETC.



The Electron Carriers

- **I** (NADH-ubiquinone oxidoreductase): An integral protein that receives electrons in the form of hydride ions from NADH and passes them on to ubiquinone
- **II** (Succinate-ubiquinone oxidoreductase *aka succinate dehydrogenase* from the TCA cycle): A peripheral protein that receives electrons from succinate (an intermediate metabolite of the TCA cycle) to yield fumarate and [FADH₂]. From succinate the electrons are received by [FAD] (a prosthetic group of the protein) which then become [FADH₂]. The electrons are then passed off to ubiquinone.
- **Q** (Ubiquinone/ ubiquinol): Ubiquinone (the oxidized form of the molecule) receives electrons from several different carriers; from I, II, Glycerol-3-phosphate dehydrogenase, and ETF. It is now the reduced form (ubiquinol) which passes its electron off to III.
- **III** (Ubiquinol-cytochrome c oxidoreductase): An integral protein that receives electrons from ubiquinol which are then passed on to Cytochrome c
- **IV** (Cytochrome c oxidase): An integral protein that that receives electrons from Cytochrome c and transfers them to oxygen to produce water within the mitochondria matrix.
- **ATP Synthas**: An integral protein consisting of several different subunits. This protein is directly responsible for the production of ATP via chemiosmotic phosphorylation. It uses the proton gradient created by several of the other carriers in the ETC to drive a mechanical rotor. The energy from that rotor is then used to phosphorylate ADP to ATP.

Inhibitors of Electron Transport:

These are the inhibitors that arrest respiration by combining with members of the respiratory chain, rather than with the enzymes that may be involved in coupling respiration with ATP synthesis.

They appear to act at 3 loci that may be identical to the energy transfer sites I, II and III. The given below are the inhibitors of Electron transport chain.

1. Rotenone:

- It is the non-toxic inhibitors of Electron transport chain.
- These compound extracted from roots of tropical plant *Derris elliptica* and *Lonchocarpus nicou*.
- It binds at Complex I between Fe-S protein and Ubiquinone.
- This is non-toxic to mammals because poorly absorbed. Shows toxic effect in fishes.

2. Piericidin A:

- It is an Antibiotic.
- It is produced by species of *streptomyces*.
- The action is similar to Rotenone.

3. Barbiturates (Amytal, Seconal):

- It blocks NADH dehydrogenase and Coenzyme.Q

4. Antimycins:

- These are antibiotic, produced by *Streptomyces*. One of the inhibitor in Electron transport chain.
- IT inhibits around site II and block electron flow between cytochromes b and c1, which prevents ATP synthesis coupled to the generation of a proton gradient. at site II.
- About 0.07 micromole of antimycin A per gram of mitochondrial protein is effective.

5. Dimercaprol:

- It is identical in action to the antimycins.

6. Cyanides:

- The cyanide ion (CN^-) combines tightly with cytochrome oxidase, leading to

7. Azide:

- Azide blocks the electron flow between the cytochrome oxidase complex and oxygen.
- Azide reacts with the ferric form (Fe_3^+) of this carrier.

8. Hydrogen Sulfide:

- H_2S is toxic, with disagreeing odour gives warning.
- It inhibits Cytochrome Oxidase.

9. Carbon Monoxide:

- It blocks between cytochrome oxidase and Oxygen.
- It inhibits Fe_2^+

Inhibitors of Oxidative Phosphorylation:

The given below are the list of inhibitors in Oxidative Phosphorylation.

1. Oligomycins:

- Is a polypeptide antibiotic are obtained from various species of "Streptomyces.
- They inhibit the transfer of high-energy phosphate to ADP and also inhibit electron transfers coupled to phosphorylation.
- The antibiotic is potent inhibitor to ATP synthase complex.

2. Rutamycin:

- This antibiotic also inhibits both ETC and oxidative phosphorylation.

3. Atractylate:

- It backs oxidative phosphorylation by compelling with ATP & ADP for a site on the ADP-ATP antiport of the mitochondrial membranes. One of the inhibitors list which blocks the oxidative phosphorylation.

4. Bongkrekate:

- It is a toxin formed by bacteria (*Pseudomonas*) in a coconut preparation from Java.1
- It also blocks the ADP-ATP antiport.

Uncouplers of Oxidative Phosphorylation:

Uncouplers can be defined as *A substance that uncouples phosphorylation of ADP from electron transfer.*

Uncoupling agents are compounds which dissociate the synthesis of ATP from the transport of electrons through the cytochrome system. This means that the electron transport continues to function, leading to oxygen consumption but phosphorylation of ADP is inhibited.

Below are few uncoupling agents,

1. 2,4-Dinitrophenol:

- A classic uncoupler of oxidative phosphorylation.
- The substance carries protons across the inner mitochondria membrane.
- In the presence of these uncouplers, electron transport from NADH to O₂ proceeds normally, but ATP is not formed by the mitochondria. ATP are because the proton motive force across the inner mitochondrial membrane is dissipated.
- DNA and other uncouplers are very useful in metabolic studies because of their specific effect on oxidative phosphorylation.

2. Dicoumarol (Vitamin.K analogue):

- Used as anticoagulant.

3. Calcium:

Transport of Ca⁺² ion into mitochondria can cause uncoupling.

- Mitochondrial transport of Ca⁺² is energetically coupled to oxidative phosphorylation.
- It is coupled with uptake of pⁱ
- When calcium is transported into mitochondria, electron transport can proceed but energy is required to pump the 4 Ca⁺² into the mitochondria. Hence, no energy is stored as ATP.

4. CCCP (Chloro carbonyl cyanide phenyl hydrazone):

- Most active uncoupler
- These lipid soluble substances can carry protons across the inner mitochondrial membrane.

5. Physiological un-couplers:

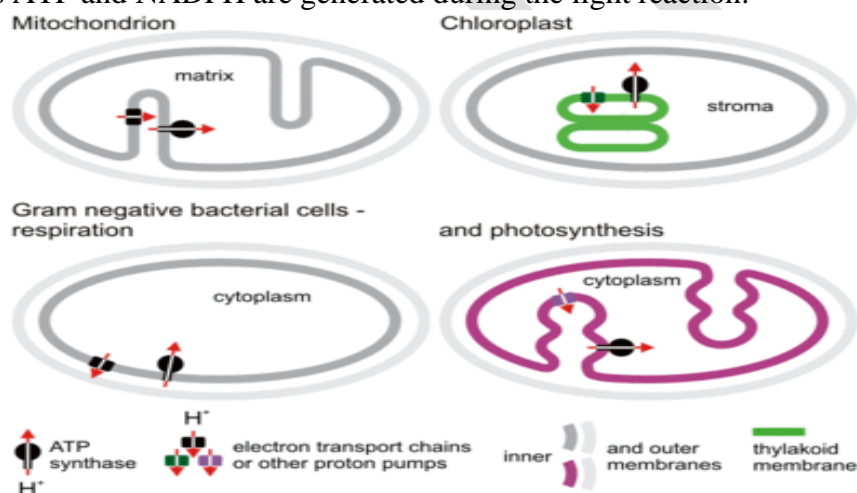
- Excessive thyroxine hormone
- EFA deficiency
- Long chain FA in brown adipose tissue
- Unconjugated hyperbilirubinaemia

6. Valinomycin:

- This is the example to Ionophore of oxidative phosphorylation.
- Produced by a type of streptomyces
- It is a repeating macrocyclic molecule made up of four kinds of residues (L-lactate, L-Valine, D-hydroxyisovalerate and D-Valine) taken 3 times.
- Transports K^+ from the cytosol into matrix and H^+ from matrix to cytosol, thereby decreasing the proton gradient.

Peter Mitchell's chemiosmotic hypothesis

In 1961, Peter Mitchell postulated the Chemiosmotic hypothesis. It explains the mechanism of ATP synthesis within chloroplast during photosynthesis. During photochemical phase or light reaction, ATP and NADP are generated. These are the key components and used in the dark reaction for the production of a final product of photosynthesis i.e. sugar molecules. Let us see how this ATP and NADPH are generated during the light reaction.



Proton Motive Force

Proton motive force refers to the energy obtained from the proton gradient created by several of the electron carriers. Only three of the four mentioned electron carriers are capable of transporting protons from the matrix to the intermembrane space: I, III, and IV. It is this proton gradient that drives phosphorylation of ADP to ATP as well as several other important transport systems. As proton concentration builds up in the intermembrane space a gradient is created and protons are transported from high to low concentration. The energy from the transfer of protons is used to change ADP into ATP through phosphorylation. ATP synthase is the protein responsible for ADP phosphorylation.

It is also important for proper concentrations of substrates to be maintained within and without the mitochondria to allow for chemiosmotic phosphorylation. The two main types of proteins responsible for maintaining proper substrate concentrations are pyruvate and phosphate symporters and ADP/ATP antiporters.

Fo F1 ATP synthase

ATP Synthase: Structure and Mechanism

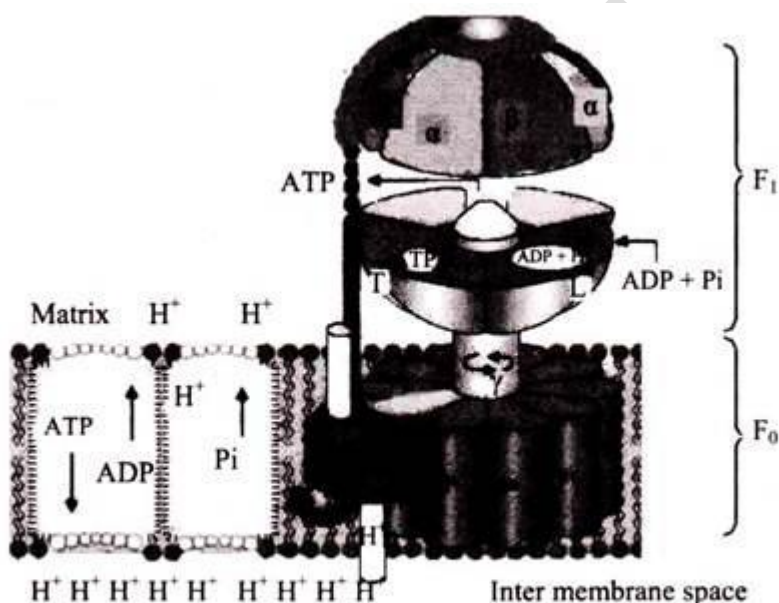
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Boyer and Walker received the Nobel Prize in 1997 for elucidating the mechanism of ATP synthase. This is all-important reactions in which the proton-motive force, produced by proton translocation, is coupled to the synthesis of ATP from ADP and phosphate. ATP synthase is a complex structure consisting of two domains F_0 and F_1 . F_1 is a spherical structure, sticks out into the matrix and is anchored to the membrane, consists of three α - and three β - subunits, all of which can bind nucleotides, but only the β -subunits can take part in the reactions. F_0 is a cylindrical structure capable of rotation when driven by translocated protons and is linked to a central stalk that can revolve inside F_1 .



structure of ATP synthase (F_1 particle is the catalytic subunit; The F_0 particle attaches to F_1 and is embedded in the inner membrane)

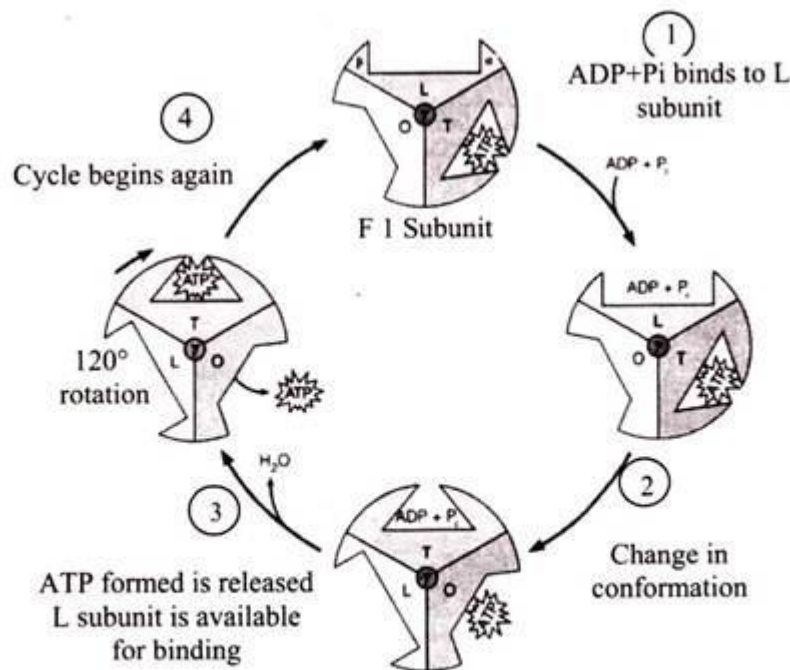
In F_1F_0 ATP synthase, the F_0 portion is within the membrane and the F_1 portion is above the membrane. The F_1 fraction derives its name from the term "Fraction 1" and F_0 (written as a subscript "O", not "zero") derives its name from being the oligomycin binding fraction. The antibiotic oligomycin inhibits the F_0 unit of ATP synthase. A soluble portion, the F_1 ATP-ase, contains 5 subunits, in a stoichiometry of $3\alpha:3\beta:1\gamma:1\delta:1\epsilon$. Three substrate binding sites are in the β -subunits. Additional adenine nucleotide binding sites in α -subunits are regulatory.

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The binding-change mechanism of Paul Boyer, rotation of the γ -subunit relative to α , β -ring induces a change in the binding affinities of reactants. ATP forms spontaneously from tightly bound ADP and Pi

According to the current model of ATP synthesis (known as the alternating catalytic model), the proton-motive force across the inner mitochondrial membrane, generated by the electron transport chain, drives the passage of protons through the membrane via the F_O region of ATP synthase. A portion of the F_O (the ring of C-subunits) rotates as the protons pass through the membrane. The ring is tightly attached to the asymmetric central stalk (consisting primarily of the gamma subunit) which rotates within the $\alpha_3\beta_3$ of F₁ causing the 3 catalytic nucleotide binding sites to go through a series of conformational changes that leads to ATP synthesis.

The mechanism that drives ATP synthesis seems to depend upon a binding change conception in which catalytic sites on the β -subunits have different affinities for nucleotides and are designated loose (L), tight (T), and open (O). The loose (L) sites bind the substrates (ADP and phosphate) reversibly. The T sites then bind the reactants so tightly that ATP is formed. The O sites, which have a low affinity for substrates, then release the ATP already formed in the T state. The central stalk is driven by the retro-location of protons through F_O (counter-clockwise, as seen from above), and rotates in 120° stages.

At each stage, each of the β -subunits in turn changes conformation: L changes to T (after binding ADP and phosphate), T to O, and O to L (after releasing ATP). The new L site then binds new ADP and phosphate and begins a new reaction sequence. One complete revolution of F_O, therefore, results in the formation of 3 ATP, one from each of the β -subunits (~3.3 H⁺ needed for the formation of one ATP from ADP and Pi).

Metabolite transporters in mitochondria

Mitochondria have diverse metabolic functions, which vary from tissue to tissue. In liver mitochondria, a wide variety of externally added substrates can be processed by the mitochondrial metabolic machinery. The enzymes are predominantly in the matrix, requiring that substrates be transported across the mitochondrial inner membrane. The transport is catalysed by a superfamily of proteins, which show strong sequence homology across the whole spectrum of the eukaryote world, but no obvious prokaryote precursor. The metabolite transporters generally catalyse neutral exchanges, but some are electrogenic, or involve H^+ cotransport or OH^- exchange, so that the transport equilibrium is determined by the proton gradient.

The swelling of mitochondria in the ammonium salts of weak acids or metabolites

The transport activities for the metabolite systems were first demonstrated by use of a simple and elegant technique introduced by Brian Chappell. The technique depends on the following:

1. Mitochondria behave as perfect osmometers; they respond to changes in osmotic pressure of the suspending medium by shrinking (or swelling) as water flows across the membrane to compensate for the difference in activity when the osmolytes are added to (or removed from) the external medium.
2. The mitochondrial membrane is impermeable to small ions such as H^+ , K^+ , Na^+ , NH_4^+ , Cl^- , NO_3^- , etc.
3. The mitochondrial membrane is permeable to small uncharged molecules such as O_2 , CO_2 , N_2 , 3-C or 4-C sugars, etc.
4. Although the membrane is impermeable to small ions (item 2), and therefore to acetate $^-$, and NH_4^+ , it is permeable to acetic acid, and to NH_3 , which are neutral small molecules (item 3).

As a consequence of these permeability properties, mitochondria will not swell in NH_4Cl , or in K-acetate, because the membrane is impermeable to the ions. However, they will swell if suspended in NH_4 -acetate, because the neutral forms can cross the membrane.

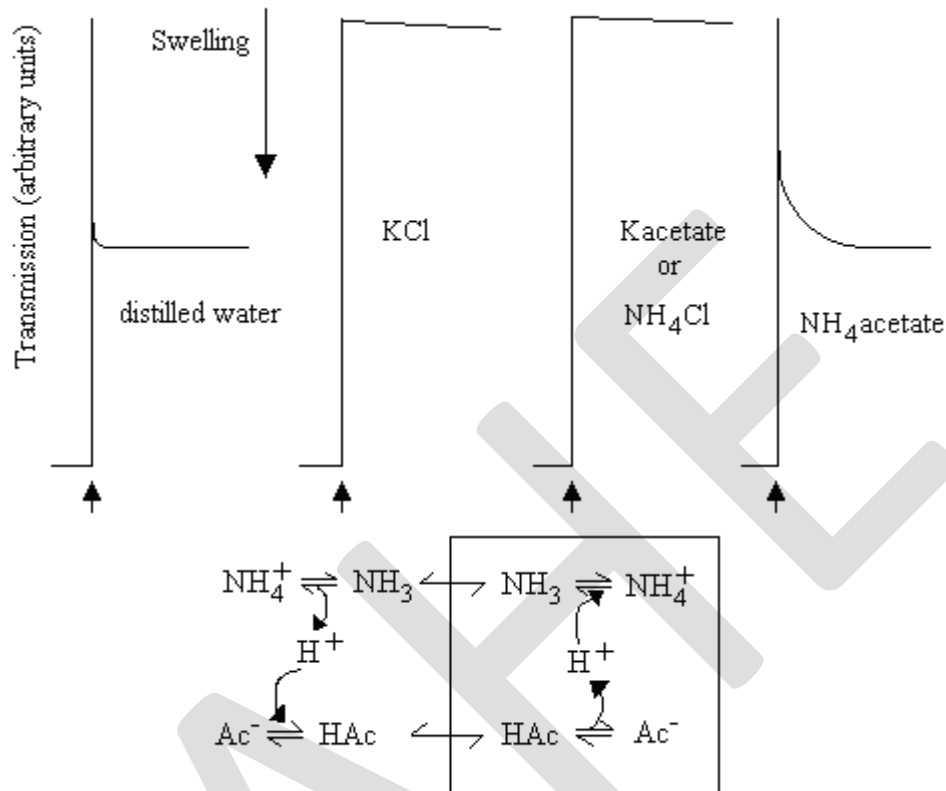
In the picture below, mitochondrial swelling was measured in a simple light-scattering photometer. On addition of aliquots of mitochondrial suspension to distilled water, or 100 mM of the salts shown (pH adjusted to 7.0), after decreasing to an initial level due to absorption of light by the added mitochondria, the transmission increased as the mitochondria swelled; very rapidly in water, very slowly in KCl, K-acetate or NH_4Cl , rapidly in NH_4 -acetate:

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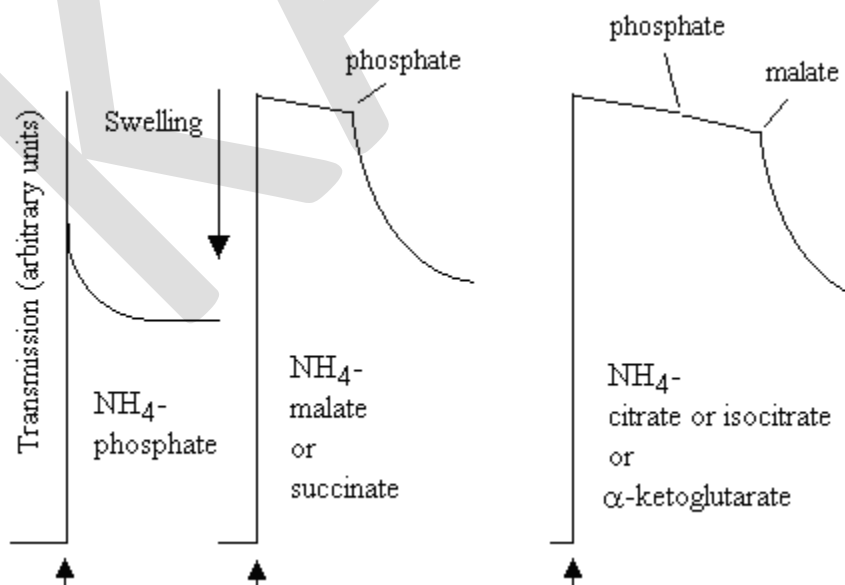
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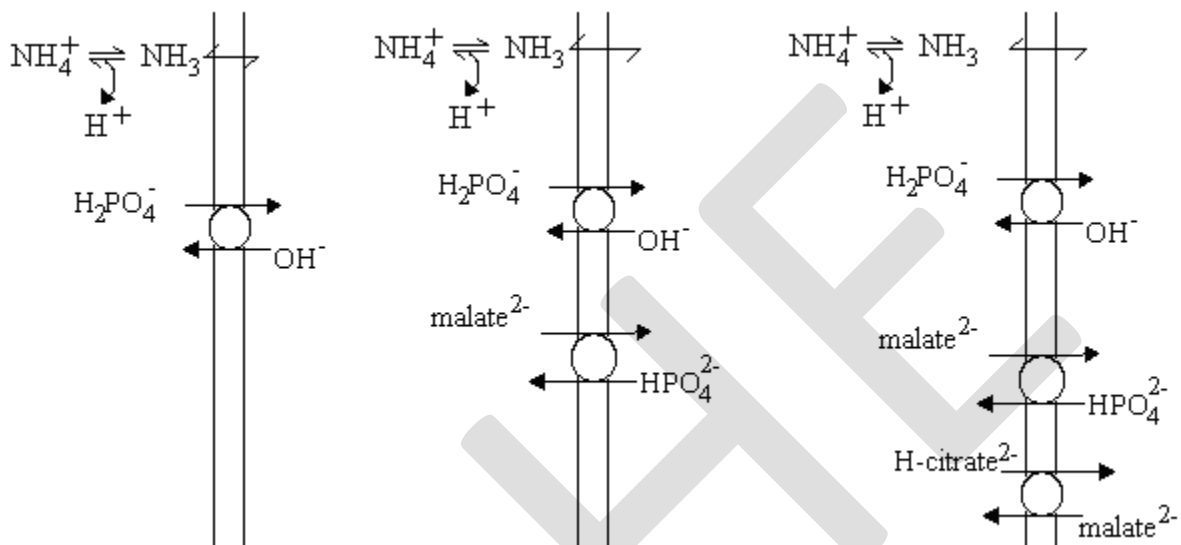
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In similar experiments, mitochondria were added to the NH₄-salts of various mitochondrial substrates:



The mitochondria swelled rapidly in NH_4 -phosphate, but not in the other NH_4 -salts. However, on addition of catalytic amounts of K-phosphate to the malate or succinate experiment, or of K-phosphate and K-malate to the citrate, isocitrate or α -ketoglutarate experiment, rapid swelling was observed. The results were interpreted as shown in the scheme below:



- Left: The presence of a phosphate transporter allowed H_2PO_4^- to exchange for OH^- across the membrane. This effectively allowed phosphate to cross the membrane as if it were a weak acid, so the situation was as for acetate.
- Center: The malate $^{2-}$ - HPO_4^{2-} exchange transporter allowed malate to enter, but only if a catalytic amount of external phosphate was present to allow rapid turn-over of the phosphate transporter. Effectively, the activity of the two carriers allows malate $^{2-}$ to enter in exchange to 2OH^- .
- Right: The citrate transporter allowed H-citrate $^{2-}$ (one of the three carboxylate groups is protonated) to enter in exchange for malate $^{2-}$, but this could only occur rapidly if sufficient external malate and phosphate were present to allow rapid turn-over of their carriers. Effectively, citric acid enters as a neutral species (or in exchange for 3OH^-).

In each case, the carriers allow the metabolites to enter as the neutral species; the anion exchanges for an equivalent number of OH^- charges, and this allows an equivalent number of NH_3 molecules to enter and be protonated to NH_4^+ , allowing net entry of the NH_4 -salt, and swelling of the mitochondria.

Family, sequences, structure

The family of proteins which includes the five members discussed above (phosphate transporter, adenine nucleotide translocator, tricarboxylate transporter, malate transporter, 2-oxoglutarate transporter) has been extensively studied in recent years. There are many sequences available, of which a sample can be found at this link.

The family also includes an interesting protein which is active in the mitochondria from brown adipose tissue. "Brown-fat" mitochondria are uncoupled, - their expression is thought to allow for the warming up of hibernating animals, by generation of heat. The mechanism of heat generation involves activation of the uncoupling protein, which acts as a proton carrier.

The ancestry of the mitochondrial transporter family is obscure. Recently, the genome sequencing project on *Rickettsia* has shown a protein with sequence similarity to the ADP/ATP translocator of plant mitochondria. This makes it likely that the mitochondria were derived from an ancestor common to *Rickettsia*, which is an endosymbiotic bacterium, and that the ADP/ATP translocator came from this source. The ADP/ATP translocator is sufficiently different in sequence from the other metabolite transporters (phosphate, citrate/isocitrate, malate/a-KG, brown fat uncoupling protein, carnitine/acyl carnitine, etc.) that this ancestry cannot be generalized to cover all the mitochondrial transporters. It is possible that this latter set represent a specifically eukaryotic invention to facilitate integration of the mitochondrial endosymbiont into the metabolism of the host.

No tertiary structure for a mitochondrial transport protein is yet available. The mitochondrial family of metabolite transporters seem to have a conserved secondary structure, with three well defined hydrophobic spans which are likely transmembrane helices, and a fourth span with hydrophobic and amphipathic character, which might also be transmembrane. There has been much speculation about the role of this and other amphipathic spans in the transport mechanism.

Regulation of Oxidative Phosphorylation

Since electron transport is directly coupled to proton translocation, the flow of electrons through the electron transport system is regulated by the magnitude of the PMF. The higher the PMF, the lower the rate of electron transport, and vice versa. Under resting conditions, with a high cell energy charge, the demand for new synthesis of ATP is limited and, although the PMF is high, flow of protons back into the mitochondria through ATP synthase is minimal. When energy demands are increased, such as during vigorous muscle activity, cytosolic ADP rises and is exchanged with intramitochondrial ATP via the transmembrane adenine nucleotide carrier ADP/ATP translocase. Increased intramitochondrial concentrations of ADP cause the PMF to become discharged as protons pour through ATP synthase, regenerating the ATP pool. Thus, while the rate of electron transport is dependent on the PMF, the magnitude of the PMF at any moment simply reflects the energy charge of the cell. In turn the energy charge, or more precisely ADP concentration, normally determines the rate of electron transport by mass action principles. The rate of electron transport is usually measured by assaying the rate of oxygen consumption and is referred to as the cellular respiratory rate. The respiratory rate is known as the state 4 rate when the energy charge is high, the concentration of ADP is low, and electron transport is limited by ADP. When ADP levels rise and inorganic phosphate is available, the flow of protons through ATP synthase is elevated and higher rates of electron transport are observed; the resultant respiratory rate is known as the state 3 rate. Thus, under physiological conditions mitochondrial respiratory activity cycles between state 3 and state 4 rates.

ROS production and antioxidant mechanisms.

ROS Formation

Reactive oxygen species are formed by several different mechanisms:

- the interaction of ionizing radiation with biological molecules

- as an unavoidable byproduct of cellular respiration. Some electrons passing "down" the electron transport chain leak away from the main path (especially as they pass through complexes I and III) and go directly to reduce oxygen molecules to the superoxide anion (#2 above).
- synthesized by dedicated enzymes in phagocytic cells like neutrophils and macrophages
 - **NADPH oxidase** (in both type of phagocytes)
 - **myeloperoxidase** (in neutrophils only)

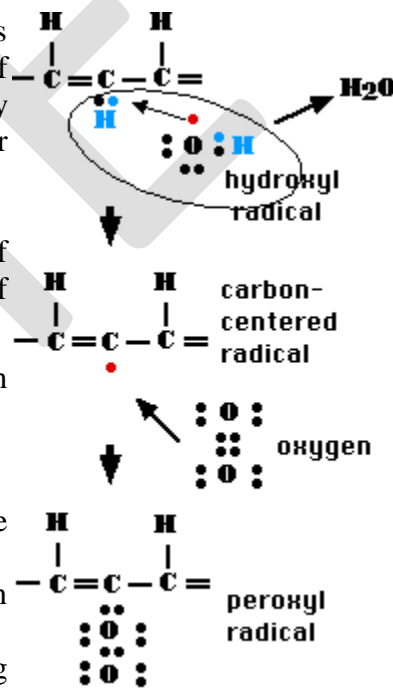
ROS Activity

Strong oxidants like the various ROS can damage other molecules and the cell structures of which they are a part.

Among the most important of these are the actions of free radicals on the fatty acid side chains of lipids in the various membranes of the cell, especially mitochondrial membranes (which are directly exposed to the superoxide anions produced during cellular respiration).

The figure shows one common series of reactions.

- A hydroxyl radical removes a hydrogen atom from one of the carbon atoms in the fatty acid chain (only a portion of which is shown) forming
- a molecule of water and leaving the carbon atom with an unpaired electron (in red); thus now a radical.
- Several possible fates await it.



One of the most likely (and shown here) is to react with a molecule of oxygen (O_2) forming a peroxyl radical.

This might then steal a hydrogen atom from a nearby side chain making it now a radical.

One of the insidious things about free radicals is that in interacting with other molecules to gain a stable configuration of electrons, they convert that target molecule into a radical. So a chain reaction begins that will propagate until two radicals meet each other and each contributes its unpaired electron to form a covalent bond linking the two.

Two common examples:

The peroxyl radical may interact with:

- another peroxyl radical on a nearby side chain crosslinking them with a covalent bond.
- another nearby carbon-centered radical crosslinking them covalently.

In both these latter cases, radical formation comes to an end but with the result that the fatty acid side chains of membrane lipids may have become so deformed as to damage the membrane.

The **lipofuscin** so characteristic of aging cells may be formed by these mechanisms [Link].

- Superoxide dismutase (SOD) converts this into hydrogen peroxide, which kills off the engulfed bacteria (except those that manufacture enough catalase to protect themselves).
- Neutrophils (but not macrophages) also kill off engulfed pathogens by using the enzyme myeloperoxidase which catalyzes the reaction of hydrogen peroxide (made from superoxide anions) with chloride ions to produce the strongly antiseptic hypochlorite ion (OCl^- , #6 above).
 $\text{H}_2\text{O}_2 + \text{Cl}^- \rightarrow \text{HOCl} \text{ (hypochlorous acid)} + \text{OH}^-$
 $\text{HOCl} \rightarrow \text{H}^+ + \text{OCl}^-$

Thermogenesis

- Thermogenesis is the adjustment of metabolic heat production to maintain body temperature; generating bodyheat through muscle activity.
- Example: moths “shivering” to warm themselves up before flying; shivering is a way of warming up.
- Nonshivering thermogenesis – hormones causing an increase in metabolic activity as opposed to muscles.
- Endotherms can vary their insulation to acclimatize to seasonal temperature changes. They produce “antifreeze” compounds to prevent ice formation in cells during subzero conditions.
- Hypothalamus – the region of the brain that controls thermoregulation; triggers heat loss or heat generating mechanisms.
- Fever is the result of a change in the set point for a biological thermostat.

Energy Requirements

Bioenergetics – overall flow and transformation of energy in animals; determines how much food an animal needs and it relates to an animal’s size, activity, and environment.

Animals harvest energy from food – ATP production (also produces heat).

4.10. Alternative respiratory pathways in plants.

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- C3 plants fix carbon exclusively through the Calvin Cycle, because the three carbon PGA is formed.
- Plants that do not produce the three carbon PGA live in hot or dry climates and utilize alternative pathways.
 - C4 pathway enables plants to fix into four carbon compounds. Examples include: corn, sugar cane, and crabgrass.
 - Cactuses, pineapples, and certain other plants that live in hot, dry climates fix carbon through the CAM pathway.

POSSIBLE QUESTIONS

UNIT-I

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Name the different complexes of electron transport chain.
2. Name the different inhibitors of electron transport chain.
3. What is oxidative phosphorylation?
4. Define electron transport chain.
5. Define ETC.
6. What is thermogenesis?
7. Define proton motive force.

PART-C (8 MARKS)

1. Explain the structure and functions of mitochondria.
2. Describe electron transport chain.
3. Explain alternative respiratory pathways in plants.
4. Detail the regulatory mechanisms of oxidative phosphorylation.
5. Write a note on FoF1ATP synthase.
6. How oxidative phosphorylation is regulated?
7. Explain the significance of metabolite transporters in mitochondria.
8. Write a note on the structure and mechanism of ATP synthesis.

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18BCU103 - Membrane Biology and Bioenergetics PART A (20 X 1 = 20 MARKS) - Online MCQ questions

Unit 4

S.No	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	If the mitochondria were blocked at the site of	Zero	One less than normally	Same as that normally	One more than	Same as that
2	-If the oxidative phosphorylation was	decreased	Increased inorganic	decreased oxidative	decreased production	Increased inorganic
3	If the rotenone is added to the mitochondrial	P: O ratio of NADH is	Rate of NADH oxidation is	Succinate oxidation	Oxidative phosphorylation	Succinate oxidation
4	If 2, 4 dinitro phenol is added to tightly coupled	Electron flow will continue	Electron flow will continue	Electron flow will cease,	Both Electron flow and ATP	Electron flow will
5	The prosthetic group of NADH dehydrogenase:	FMN	NADH	FAD	NADPH	FMN
6	In substrate level phosphorylation	The substrate reacts to	ATP synthesis is linked to	High energy intermediate	Oxidation of one molecule	The substrate
7	The chemiosmotic hypothesis involves all of	Electron transport by the	Proton flow in to the	ATPase activity is	Only proton transport is	Only proton
8	The effect of Valinomycin on oxidative	The net yield of ATP	Rate of oxygen	Excessive heat is released	pH gradient across the	pH gradient across
9	Which of the following ETC components accept	Coenzyme Q	Cytochrome b	FAD	FMN	Cytochrome b
10	Which of the following has highest redox	O ₂	Ubiquinone	NAD	FAD	O ₂
11	All of the following except one are NAD +	Acyl co A dehydrogenase	Glyceraldehyde-3-P	Pyruvate dehydrogenase	Malate dehydrogenase	Acyl co A
12	Which one of the following enzymes catalyzes	Malate dehydrogenase	Succinate Thiokinase	Succinate dehydrogenase	Alpha keto dehydrogenase	Succinate
13	The major metabolic consequence of	Increased production of	Increased oxidation of	Increased reduction of O ₂	Decreased regeneration of	Decreased
14	Inhibition of oxidative phosphorylation by	Gluconeogenesis to	Transport of ADP into the	Utilization of fatty acids	Lactic acid in the blood	Lactic acid in the
15	Choose a statement out of the following that	Uncouple ATP synthesis	Uncouple ATP/ADP	Uncouple electron	Uncouple electron	Uncouple electron
16	Which of the following statements best	It inhibits NADH	It is an uncoupler of	It inhibits ATP/ADP	It blocks the flow of	It blocks the flow
17	ADP transport in to the mitochondrial matrix–	is an active transport	is directly inhibited by	is carried out through a	is accompanied by ATP	is accompanied by
18	Which of the following enzymes catalyzes the	Super oxide dismutase	Cytochrome P 450	Catalase	Hydratase	Catalase
19	The rate of respiration of mitochondria can be	ADP	ATP	FMN	NAD	ADP
20	Atractyloside inhibits oxidative	NADH-Q oxidoreductase	Q-cytochrome c	Succinate Q reductase	ADP/ATP transporter	ADP/ATP
21	The enzyme that catalyzes the direct transfer	Oxidase	Oxygenase	Peroxidase	Reductase	Oxygenase
22	Choose a site along the electron transport	NADH- coenzyme Q (CoQ)	Succinate-Co Q reductase	Cytochrome bc1 reductase	Cytochrome oxidase.	Succinate-Co Q
23	Which of the following ETC components	Oxygen	FMN	FAD	Cytochrome b	Cytochrome b
24	Which of the following is a component of	Niacin	FMN	FAD	Coenzyme	FAD
25	MELAS is a mitochondrial disorder	1	2	4	8	2

26	Which of the following enzymes catalyzes a	Glycerol-3-P-dehydrogenase	Succinate dehydrogenase	Malate dehydrogenase	Lactate dehydrogenase	Succinate
27	Which of the following compounds inhibits	Malonate	Oxamate	Atractyloside	Barbiturates	Atractyloside
28	Which of the components of electron transport	NADH dehydrogenase	Cytochrome a-a3 oxidase	Succinate dehydrogenase	Cytochrome bc1 -c	Cytochrome a-a3
29	Aspirin in a high dosage produces	Aspirin affects	It uncouples oxidative	It stimulates metabolism	It is itself metabolized to	It uncouples
30	Choose the correct statement out of the	Iron-sulfur proteins	These may contain five	Iron may be linked through	The Fe-S take part in two	Iron may be linked
31	Out of the following respiratory chain	Cytochrome oxidase	NADH-Q reductase	Ubiquinone	Succinate dehydrogenase	Ubiquinone
32	Choose the incorrect statement about redox	The redox potential of a	The components of	NADH/NAD + redox pair	Oxygen/H2O redox pair has	NADH/NAD + redox
33	The chemical energy required for the synthetic	Phosphorylation of ATP	Dephosphorylation of ATP	Phosphorylation of ADP	Dephosphorylation of ADP	Dephosphorylation
34	All except one are incorrect about oxidases	Oxidases catalyze	Oxidases catalyze reaction	Oxidases catalyze	Oxidases catalyze reactions	Oxidases catalyze
35	Which of the components of electron transport	NADH dehydrogenase	Cytochrome a-a3 oxidase	Succinate dehydrogenase	Cytochrome bc1 -c	Cytochrome a-a3
36	A child has accidentally ingested a chemical	Cyanide	Malonate	2,4 dinitrophenol	Rotenone	2,4 dinitrophenol
37	-A 32- year female working in a laboratory	Complex I	Complex II	Complex III	Complex IV	Complex IV
38	The enzymes of mitochondrial matrix include	Enzymes of fatty acid	Creatine kinase	Enzymes of TCA cycle	Pyruvate dehydrogenase	Creatine kinase
39	Patients with inherited defects of	Myopathy	Lactic acidosis	Encephalopathy	Hepatomegaly	Hepatomegaly
40	The inner mitochondrial membrane is rich in	Cardiolipin	Lecithin	Cephalin d	None of the above	Cardiolipin
41	All are true about ATP synthase complex,	F1projects into the inter	F0 spans the membrane	F0 is inhibited by	F1contains the	F1projects into the
42	The energy yield during the conversion of	2ATP	1ATP	3ATP	No ATP	2ATP
43	Which of the following best describes the	Increased fatty acid	Increased muscular activity	Elevated consumption of	Uncoupling of oxidative	Uncoupling of
44	The electron flow from complex I to complex III	Cytochrome c	Ubiquinone	Complex II	Complex IV	Ubiquinone
45	Which of the following best describes the	Acts as an inhibitor of ETC	Acts as an uncoupler	Acts as an inhibitor of	Inactivates ATP synthase	Acts as an inhibitor
46	-For each H2O molecule formed in ETC around	4	2	10	None of the above	10
47	Which one of the following enzymes catalyzes	Malate dehydrogenase	Succinate Thiokinase	Succinate dehydrogenase	Alpha keto glutarate	Succinate
48	An 18- year -old cricket player sustains a	Complex I	Complex II	Complex III	ATP synthase complex	ATP synthase
49	The cytochromes are iron-containing	NADH-Q oxidoreductase	Q-cytochrome c	cytochrome c oxidase	Succinate Q reductase	cytochrome c
50	Which of the following occurs in non shivering	Glucose is oxidized to	Fatty acids uncouple	Fatty acids uncouple	ATP is spent for heat	Glycogen is
51	One out of the following is an inhibitor of	Rotenone	H2S	BAL	CN	Rotenone
52	Which out of the following statements	Oxygen directly oxidizes	Succinate dehydrogenase	All of the components are	Cyanide does not inhibit	Cyanide does not
53	The free energy released during the transport	7.3 Kcal/mol	52.6 Kcal/mol	21.9 Kcal/mol	None of the above	7.3 Kcal/mol
54	Magnitude of proton motive force depends	number of ribosomes in	permeability of membrane	both A and B	energy charge of cell	energy charge of
55	Rate of electron transfer when proton motive	slower	faster	moderate	zero	slower
56	Regulation of oxidative phosphorylation	slower	Magnitude of electron	Magnitude of ion motive	none of above	slower
57	Coenzyme Q is involved in electron transport	directly to O2	a water-soluble electron	covalently attached	a lipid-soluble electron	a lipid-soluble
58	Which of the following is not a feature of	Direct transfer of	An electrochemical	A membrane bound ATP	A protonmotive force	Direct transfer of
59	Which of the following is correct sequence of	Krebs cycle - glycolysis -	Glycolysis - Krebs cycle -	Electron transport - Krebs	Krebs cycle - electron	Glycolysis - Krebs

60	In electron transport, electrons ultimately pass	ADP	cytochrome b	oxygen	none of these	oxygen
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[illegible]

[illegible]



UNIT-V

SYLLABUS

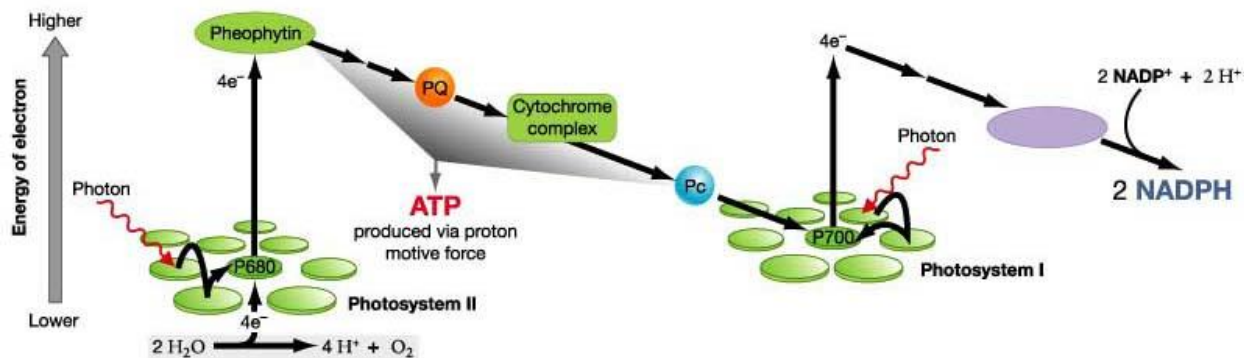
Photophosphorylation: General features of photophosphorylation, historical background, Hills reaction, photosynthetic pigments, light harvesting systems of plants and microbes and resonance energy transfer. Bacterial photophosphorylation in purple bacteria, Green sulfur bacteria and *Halobacterium salinarum*. Photophosphorylation in plants - structure of chloroplast, molecular architecture of Photosystem I and Photosystem II, Z-scheme of photosynthetic electron flow, oxygen evolving complex and action of herbicides. Cyclic photophosphorylation and its significance. Photo inhibition. Evolution of oxygenic photosynthesis.

Photophosphorylation

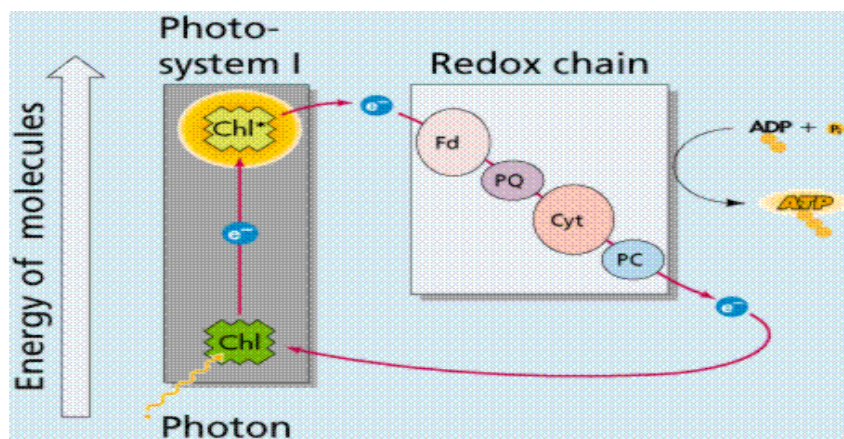
Cyclic and non cyclic photophosphorylation:

Photophosphorylation is the process of creating ATP using a Proton gradient created by the Energy gathered from sunlight. The process of creating the Proton gradient resembles that of the electron transport chain of Respiration. But since formation of this proton gradient is light-dependent, the process is called Photophosphorylation.

Noncyclic Photophosphorylation really refers to the ATP generated by Protons moved across the Thylakoid membranes during the Z-scheme. The Cytb6-f complex acts as an electron transport chain. As the electrons lose Energy (during a series of re/dox reactions) Protons are moved into the Thylakoid space. This Proton gradient can be used to generate ATP chemiosmotically.

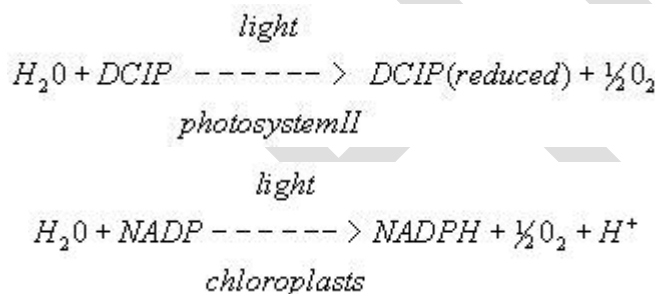


During Cyclic Photophosphorylation the electrons are recycled, hence the name cyclic photophosphorylation. The excited electrons resulting from the absorption of light in photosystem I are received by the primary electron acceptor and then transferred to the cytb6-f complex which acts as an electron transport chain. The electrons return back to the reaction center of Photosystem I, where the cycle is ready to start all over. The electrons are used to translocate Protons which the ATPase uses to synthesize ATP. No reduction of NADP+ occurs in Cyclic Photophosphorylation.



Hill's reaction

Photolysis of water and production of reducing power can be demonstrated by the Hill reaction. DCPIP can substitute for NADP, receiving electrons and becoming reduced.



In the presence of illuminated chloroplasts DCPiP becomes reduced. It does this by accepting excited electrons released from chlorophyll molecules. It may also accept hydrogen ions from the photolysis of water. We know this occurs due to a colour change. DCPiP is blue whereas reduced DCPiP is colourless.

In the Hill reaction the isolated chloroplasts in the experiment have to be suspended in an ice-cold buffered 2% sucrose solution

- The low temperature is to slow down enzyme activity
- 2% sucrose solution is to prevent water leaving entering or leaving the chloroplasts by osmosis

Photosynthetic pigments

Chlorophyll

Chlorophylls are greenish pigments which contain a porphyrin ring. This is a stable ring-shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll "captures" the energy of sunlight.

There are several kinds of chlorophyll, the most important being chlorophyll "a". This is the molecule which makes photosynthesis possible, by passing its energized electrons on to molecules which will manufacture sugars. All plants, algae, and cyanobacteria which photosynthesize contain chlorophyll "a". A second kind of chlorophyll is chlorophyll "b", which occurs only in "green algae" and in the plants. A third form of chlorophyll which is common is (not surprisingly) called chlorophyll "c", and is found only in the photosynthetic members of the Chromista as well as the dinoflagellates. The differences between the chlorophylls of these major groups was one of the first clues that they were not as closely related as previously thought.

Carotenoids

Carotenoids are usually red, orange, or yellow pigments, and include the familiar compound carotene, which gives carrots their color. These compounds are composed of two small six-carbon rings connected by a "chain" of carbon atoms. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll. For this reason, they are called accessory pigments. One very visible accessory pigment is fucoxanthin the brown pigment which colors kelps and other brown algae as well as the diatoms.

Phycobillin

Phycobilins are water-soluble pigments found in the stroma of chloroplast organelles that are present only in Cyanobacteria and Rhodophyta. The two classes of phycobilins include phycocyanin and phycoerythrin. Phycocyanin is a bluish pigment found in primarily cyanobacteria (blue-green algae) to aid in absorption of light in photosynthesis, while phycoerythrin is a pigment found in Rhodophyta (red algae) that is responsible for its characteristic red color. It is an accessory pigment that allows red algae to carry out photosynthesis in deep water where wavelengths of blue light are most abundant by absorbing blue light and reflecting red light.

In contrast to algae and higher plants which are oxygenic (i.e., they evolve O_2 during photosynthesis and have two photo-systems that act in tandem or series, the photosynthetic bacteria are anoxygenic (i.e., they do not evolve O_2 during photosynthesis and have comparatively simple photo transduction machinery with only one type of photosystem and reaction centre.

Purple bacteria have Type II Reaction Centre which passes electrons through bacteriopheophytin (bacteriochlorophyll lacking central Mg^{2+} ion) to a quinone. Green sulphur bacteria have Type I Reaction Centre that passes electrons to an Fe-s protein.

Bacterial photophosphorylation

(1) Type II Reaction Centre (The Bacteriopheophytin – Quinone Reaction Centre):

In purple bacteria, P-870 constitutes the reaction centre of the only one pigment system present. When P-870 (B. Chl.a) receives a photon of light, it gets excited (*). An electron with extra energy is ejected from it which is immediately (within pico seconds) captured by bacteriopheophytin a (B. Pheo).

Thus, charge separation occurs with a positive charge on bacteriochlorophyll and negative charge on bacteriopheophytin:

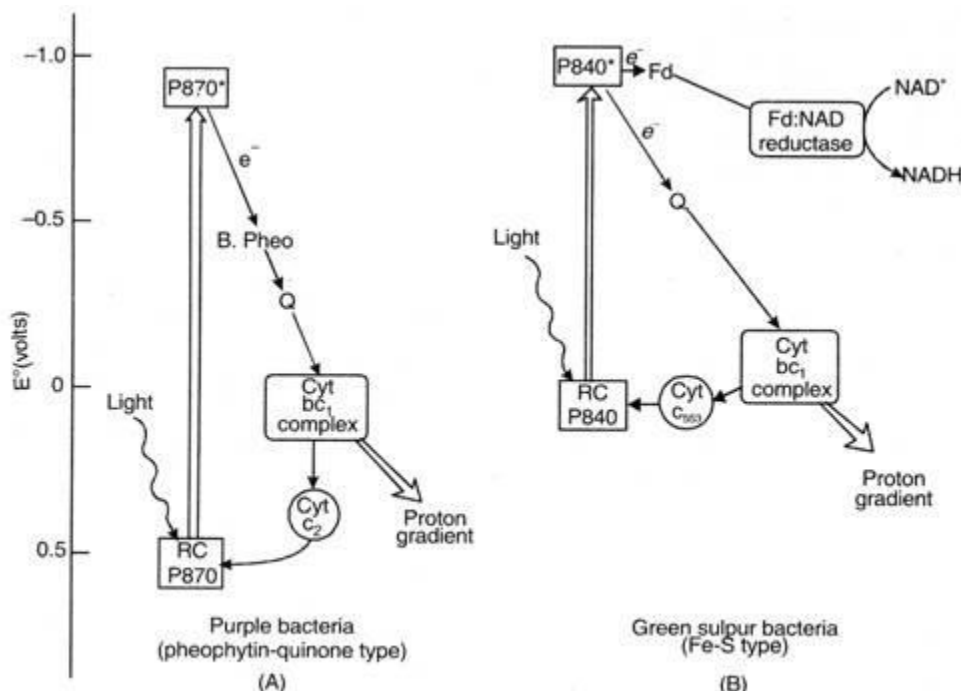
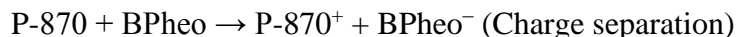
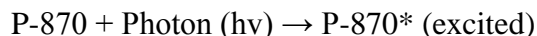


Fig. 11.31 Photosynthetic electron transport in photosynthetic bacteria. (A), In purple bacteria. (B), In green sulphur bacteria. See Text for details.

From BPheo⁻, the electron is transferred within micro-seconds to a tightly bound molecule of quinone (Q_A), converting it into a semi-quinone radical (Q_A[•]). The electron from Q_A is taken by another quinone (Q_B) which is loosely bound to the membrane. Two such electron transfers convert Q_B to its fully reduced anionic form Q_B²⁻. The latter also takes two protons (2H⁺) from the cytosol and is converted into fully reduced uncharged form, the hydroquinone (Q_BH₂). The latter now freely diffuse in the membrane bi-layer.

From Q_BH₂, the electrons (one by one) are transferred to Cyt. c₂ through Cyt. b₆c₁ complex and finally back to the reaction centre, thus completing the cycle (Fig. 11.31 A) (Cyt b₆c₁ complex is homologous to complex III in mitochondria).

The energy of electrons flow through the Cyt b₆c₁ complex causes proton pumping across the membrane, producing a proton motive force that powers synthesis of ATP from ADP + Pi by ATP synthase (photophosphorylation). The proton electrochemical gradient across the membrane in purple bacteria is from outside (periplasm) to inside (cytosol).

(2) Type I Reaction Centre (The Fe-S Reaction Centre):

In green sulphur bacteria, P-840 constitutes the reaction centre of the only one pigment system

present. Contrary to the cyclic photosynthetic electron transport of purple bacteria, the photosynthetic electron transport in green sulphur bacteria appears to involve both cyclic and non-cyclic routes (Fig. 11.31B).

(i) Cyclic photosynthetic electron transport:

Excitation of P-840 in pigment system by a photon of light results in transfer of an electron from the reaction centre to Cyt bc_1 complex through a quinone (Q) and back to the reaction centre via Cyt C_{553} . The electron transport through the Cyt bc_1 complex causes proton pumping across the membrane, producing a proton motive force that powers synthesis of ATP from ADP + Pi by ATP synthase (photophosphorylation).

(ii) Non-cyclic photosynthetic electron transport:

During this process, some electrons flow from excited P-840 to an Fe-S protein Ferredoxin (Fd), which in turn passes electrons to NAD^+ through Fd: NAD-reductase and ultimately forming NADH (Fig. 11.31B). The electrons from the reaction centre which reduce $NAD^+ \rightarrow NADH$, are compensated by electrons coming from oxidation of H_2S to elemental S and then to SO_4^{2-} (not shown in figure). This process is chemically analogous to oxidation of H_2O by oxygenic plants.

(B) Carbon Assimilation:

Like algae and higher green plants, photosynthetic bacteria also utilise the assimilatory power ($NADH_2 + ATP$) generated during light reaction to reduce CO_2 to synthesize organic matter. However, some photosynthetic bacteria may also reduce simple organic compounds and photosynthesize complex organic matter in the cells.

Three categories of carbon assimilation in photosynthetic bacteria have been recognised:

(i) The Calvin Cycle:

Certain photosynthetic bacteria e.g., *Rhodospirillum rubrum* make use of this cycle to synthesize carbohydrates by reducing CO_2 . However, since these bacteria do not store or utilise carbohydrates, lesser amount of sugar phosphates have been detected in them during photosynthesis.

(ii) Reductive Carboxylic Acid Cycle:

In some photosynthetic bacteria such as *Chlorobium*, *Chromatium* etc. another carbon reduction cycle is known to operate which is called as Reductive Carboxylic Acid Cycle (Fig. 11.32).

Similarly, photo assimilation of succinic acid, propionic acid etc. leads to the accumulation of glycogen like polysaccharides. Photosynthesis of these carbon reserves inside the bacterial cells appears to be analogous to the formation of starch in algae and higher green plants.

PHOTOSYNTHESIS IN HALOBACTERIUM:

Some archaea are able to use light as a source of energy. Instead of using chlorophyll, these microbes use a membrane protein called **bacteriorhodopsin** (more correctly called archaeorhodopsin). One such archaeon is the halophile *Halobacterium salinarum*. *H. salinarum* normally depends on aerobic respiration for the release of energy from an organic energy source. It cannot grow anaerobically by anaerobic respiration or fermentation. However, under conditions of low oxygen and high light intensity, it synthesizes bacteriorhodopsin, a deep-purple pigment that closely resembles the rhodopsin found in the rods and cones of vertebrate eyes. Bacteriorhodopsin's chromophore is retinal, a type of carotenoid. The chromophore is covalently attached to the pigment protein, which is embedded in the plasma membrane in such a way that the retinal is in the center of the membrane. Bacteriorhodopsin functions as a light-driven proton pump. When retinal absorbs light, a proton is released and the bacteriorhodopsin undergoes a sequence of conformation changes that translocate the proton into the periplasmic space. The light-driven proton pumping generates a pH gradient that can be used to power the synthesis of ATP by chemiosmosis. This phototrophic capacity is particularly useful to *Halobacterium* because oxygen is not very soluble in concentrated salt solutions and may decrease to an extremely low level in *Halobacterium*'s habitat. When the surroundings become temporarily anoxic, the archaeon uses light energy to synthesize sufficient ATP to survive until oxygen levels rise again. Note that this type of phototrophy does not involve electron transport. It had been thought that rhodopsin-based phototrophy is unique to *Archaea*. However, proton-pumping rhodopsins have recently been discovered in some proteobacteria (proteorhodopsin) and a fungus.

DARK REACTION: Many autotrophs obtain energy by trapping light during the light reactions of photosynthesis, but some derive energy from the oxidation of inorganic electron donors. Autotrophic CO₂ fixation is crucial to life on Earth because it provides the organic matter on which heterotrophs depend.

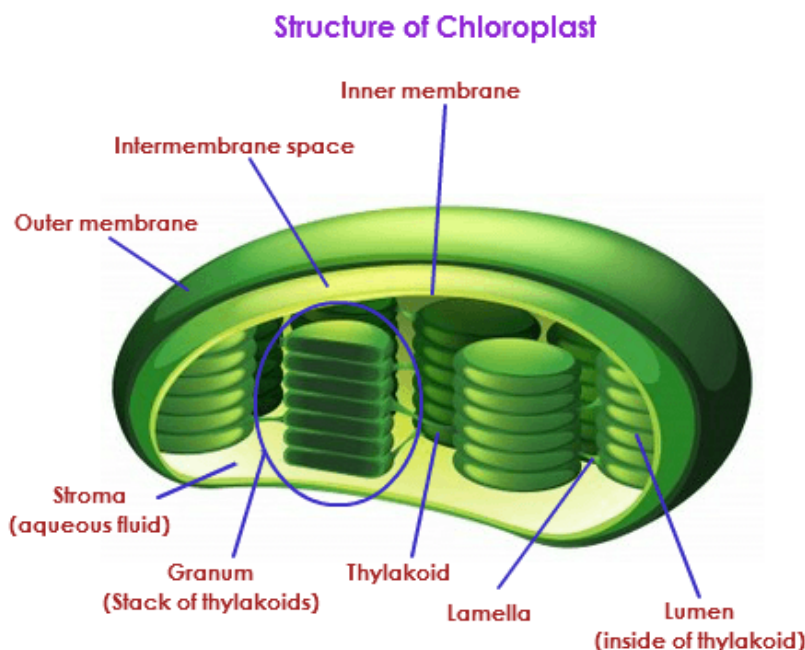
Four different CO₂-fixation pathways have been identified in microorganisms. Most autotrophs use the **Calvin cycle**, which is also called the Calvin-Benson cycle or the **reductive pentose phosphate cycle**. The Calvin cycle is found in photosynthetic eucaryotes and most photosynthetic bacteria. It is absent in some obligatory anaerobic and microaerophilic bacteria. Autotrophic archaea also use an alternative pathway for CO₂ fixation.

Photophosphorylation in plants

Chloroplast Structure

- Chloroplasts found in higher plants are generally biconvex or planoconvex shaped. In different plants chloroplasts have different shapes, they vary from spheroid, filamentous saucer-shaped, discoid or ovoid shaped.

- They are vesicular and have a colorless center. Some chloroplasts are in shape of club, they have a thin middle zone and the ends are filled with chlorophyll. In algae a single huge chloroplast is seen that appears as a network, a spiral band or a stellate plate.
- The size of the chloroplast also varies from species to species and it is constant for a given cell type. In higher plants, the average size of chloroplast is 4-6 μ m in diameter and 1-3 μ m in thickness.



The chloroplast are double membrane bound organelles and are the site of photosynthesis. The chloroplasts have a system of three membranes: the outer membrane, the inner membrane and the thylakoid system. The outer and the inner membrane of the chloroplast enclose a semi-gel-like fluid known as the stroma. This stroma makes up much of the volume of the chloroplast, the thylakoids system floats in the stroma.

Outer membrane - It is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.

Intermembrane Space - It is usually a thin intermembrane space about 10-20 nanometers and it is present between the outer and the inner membrane of the chloroplast.

Inner membrane - The inner membrane of the chloroplast forms a border to the stroma. It regulates passage of materials in and out of the chloroplast. In addition of regulation activity, the fatty acids, lipids and carotenoids are synthesized in the inner chloroplast membrane.

Stroma

Stroma is an alkaline, aqueous fluid which is protein rich and is present within the inner membrane

of the chloroplast. The space outside the thylakoid space is called the stroma. The chloroplast DNA, chloroplast ribosomes and the thylakoid system, starch granules and many proteins are found floating around the stroma.

Thylakoid System

- The thylakoid system is suspended in the stroma. The thylakoid system is a collection of membranous sacks called thylakoids. The chlorophyll is found in the thylakoids and is the site for the process of light reactions of photosynthesis to happen. The thylakoids are arranged in stacks known as grana.
- Each granum contains around 10-20 thylakoids.
- Thylakoids are interconnected small sacks, the membranes of these thylakoids is the site for the light reactions of the photosynthesis to take place. The word 'thylakoid' is derived from the Greek word "thylakos" which means 'sack'.
- Important protein complexes which carry out light reaction of photosynthesis are embedded in the membranes of the thylakoids. The Photosystem I and the Photosystem II are complexes that harvest light with chlorophyll and carotenoids, they absorb the light energy and use it to energize the electrons.
- The molecules present in the thylakoid membrane use the electrons that are energized to pump hydrogen ions into the thylakoid space, this decreases the pH and becomes acidic in nature. A large protein complex known as the ATP synthase controls the concentration gradient of the hydrogen ions in the thylakoid space to generate ATP energy and the hydrogen ions flow back into the stroma.
- Thylakoids are of two types - granal thylakoids and stromal thylakoids. Granal thylakoids are arranged in the grana as pancake shaped circular discs, which are about 300-600 nanometers in diameter. The stromal thylakoids are in contact with the stroma and are in the form of helicoid sheets.
- The granal thylakoids contain only photosystem II protein complex, this allows them to stack tightly and form many granal layers with granal membrane. This structure increases stability and surface area for the capture of light.
- The photosystem I and ATP synthase protein complexes are present in the stroma. These protein complexes act as spacers between the sheets of stromal thylakoids.

Photosystems I and II

The structural and photochemical properties of the minimum particles capable of performing light reactions I and II have received much study. Treatment of lamellar fragments with neutral detergents releases these particles, designated photosystem I and photosystem II,

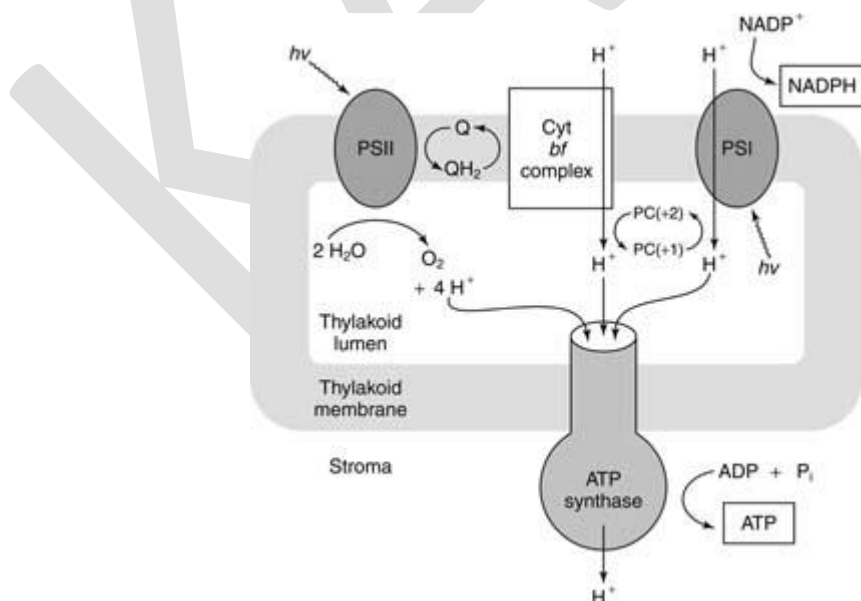
respectively. Subsequent harsher treatment (with charged detergents) and separation of the individual polypeptides with electrophoretic techniques have helped identify the components of the photosystems. Each photosystem consists of a light-harvesting complex and a core complex. Each core complex contains a reaction centre with the pigment (either P_{700} or P_{680}) that can be photochemically oxidized, together with electron acceptors and electron donors. In addition, the core complex has some 40 to 60 chlorophyll molecules bound to proteins. In addition to the light absorbed by the chlorophyll molecules in the core complex, the reaction centres receive a major part of their excitation from the pigments of the light-harvesting complex.

Quantum requirements

The quantum requirements of the individual light reactions of photosynthesis are defined as the number of light photons absorbed for the transfer of one electron. The quantum requirement for each light reaction has been found to be approximately one photon. The total number of quanta required, therefore, to transfer the four electrons that result in the formation of one molecule of oxygen via the two light reactions should be four times two, or eight. It appears, however, that additional light is absorbed and used to form ATP by a cyclic photophosphorylation pathway. (The cyclic photophosphorylation pathway is an ATP-forming process in which the excited electron returns to the reaction centre.) The actual quantum requirement, therefore, probably is 9 to 10.

Z-Scheme of Photosynthesis

The “Z-scheme” describes the oxidation/reduction changes during the light reactions of photosynthesis. The vertical axis in the figure represents the reduction potential of a particular species—the higher the position of a molecular species, the more negative its reduction potential, and the *more easily it donates electrons*.



In the Z-scheme, electrons are removed from water (to the left) and then donated to the lower (non-excited) oxidized form of P680. Absorption of a photon excites P680 to P680*, which “jumps” to a more actively reducing species. P680* donates its electron to the quinone-cytochrome bf chain, with proton pumping. The electron from cytochrome bf is donated to PSI, converting P700 to P700*. This electron, along with others, is transferred to NADP, forming NADPH. Alternatively, this electron can go back to cytochrome bf in cyclic electron flow.

Photosystem II PsbU, oxygen evolving complex

Oxygenic photosynthesis uses two multi-subunit photosystems (I and II) located in the cell membranes of cyanobacteria and in the thylakoid membranes of chloroplasts in plants and algae. Photosystem II (PSII) has a P680 reaction centre containing chlorophyll 'a' that uses light energy to carry out the oxidation (splitting) of water molecules, and to produce ATP via a proton pump. Photosystem I (PSI) has a P700 reaction centre containing chlorophyll that takes the electron and associated hydrogen donated from PSII to reduce NADP⁺ to NADPH. Both ATP and NADPH are subsequently used in the light-independent reactions to convert carbon dioxide to glucose using the hydrogen atom extracted from water by PSII, releasing oxygen as a by-product.

PSII is a multisubunit protein-pigment complex containing polypeptides both intrinsic and extrinsic to the photosynthetic membrane. Within the core of the complex, the chlorophyll and beta-carotene pigments are mainly bound to the antenna proteins CP43 (PsbC) and CP47 (PsbB), which pass the excitation energy on to the reaction centre proteins D1 (Qb, PsbA) and D2 (Qa, PsbD) that bind all the redox-active cofactors involved in the energy conversion process. The PSII oxygen-evolving complex (OEC) oxidises water to provide protons for use by PSI, and consists of OEE1 (PsbO), OEE2 (PsbP) and OEE3 (PsbQ). The remaining subunits in PSII are of low molecular weight (less than 10 kDa), and are involved in PSII assembly, stabilisation, dimerisation, and photo-protection.

In PSII, the oxygen-evolving complex (OEC) is responsible for catalysing the splitting of water to O₂ and 4H⁺. The OEC is composed of a cluster of manganese, calcium and chloride ions bound to extrinsic proteins. In cyanobacteria there are five extrinsic proteins in OEC (PsbO, PsbP-like, PsbQ-like, PsbU and PsbV), while in plants there are only three (PsbO, PsbP and PsbQ), PsbU and PsbV having been lost during the evolution of green plants.

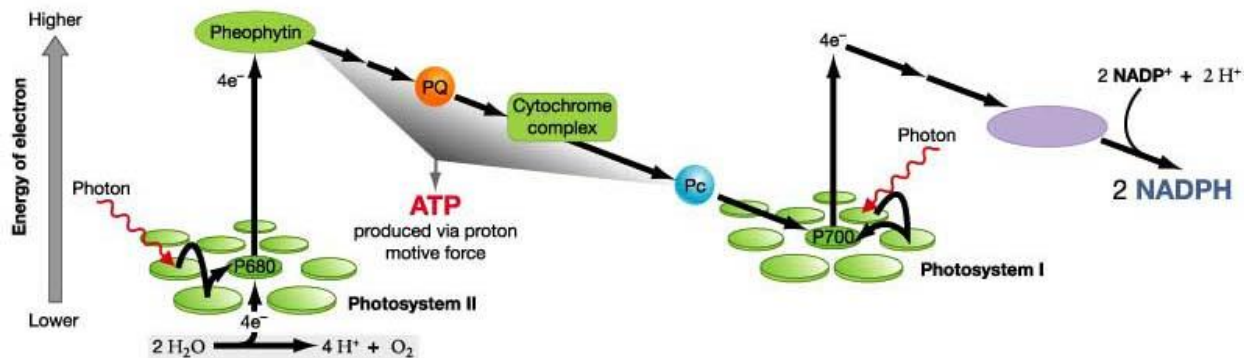
This family represents the PSII extrinsic protein PsbU, which forms part of the OEC in cyanobacteria and red algae. PsbU acts to stabilise the oxygen-evolving machinery of PSII against heat-induced inactivation, which is crucial for cellular thermo-tolerance.

Cyclic and non cyclic photophosphorylation:

Photophosphorylation is the process of creating ATP using a Proton gradient created by the Energy gathered from sunlight. The process of creating the Proton gradient resembles that of the electron transport chain of Respiration. But since formation of this proton gradient is light- dependent, the process is called Photophosphorylation.

Noncyclic Photophosphorylation really refers to the ATP generated by Protons moved across the Thylakoid membranes during the Z-scheme. The Cytb6-f complex acts as an electron transport chain. As the electrons lose Energy (during a series of re/dox reactions) Protons are moved into

the Thylakoid space. This Proton gradient can be used to generate ATP chemiosmotically.



During Cyclic Photophosphorylation the electrons are recycled, hence the name cyclic photophosphorylation. The excited electrons resulting from the absorption of light in photosystem I are received by the primary electron acceptor and then transferred to the cytb₆-f complex which acts as an electron transport chain. The electrons return back to the reaction center of Photosystem I, where the cycle is ready to start all over. The electrons are used to translocate Protons which the ATPase uses to synthesize ATP. No reduction of NADP⁺ occurs in Cyclic Photophosphorylation.

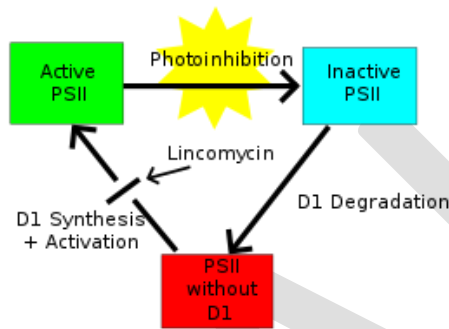
Significance of Photochemical Phase/Non cyclic and cyclic Photophosphorylation

Major Points to remember with respect to Significance of Photochemical Phase/Non cyclic and cyclic Photophosphorylation are as under:-

- Photochemical Phase i.e. light reaction (consisting of non-cyclic and cyclic photophosphorylation reactions) is essential for the following dark reaction (Phase-II) of photosynthesis.
- It utilizes the unending and unlimited source of energy i.e. solar energy (light) and converts it into the biologically usable form i.e. ATP energy (Chemical Energy) with the help of PS-I and PS-II.
- Light reaction of photosynthesis is the primary source of ATP energy (chemical energy) for all organisms in the world.
- It produces the assimilatory power "ATP and NADPH₂" needed for the synthesis of carbohydrates from CO₂ during the Dark reaction (Phase-II).
- It releases oxygen in the atmosphere during photolysis of water.
- Light reaction of photosynthesis is the only source of oxygen in nature. It is responsible for maintaining the percentage of oxygen in the atmosphere.

Photoinhibition

- ✓ Photoinhibition is light-induced reduction in the photosynthetic capacity of a plant, alga, or cyanobacterium. Photosystem II (PSII) is more sensitive to light than the rest of the photosynthetic machinery, and most researchers define the term as light-induced damage to PSII.
- ✓ In living organisms, photoinhibited PSII centres are continuously repaired via degradation and synthesis of the D1 protein of the photosynthetic reaction center of PSII.
- ✓ Photoinhibition is also used in a wider sense, as dynamic photoinhibition, to describe all reactions that decrease the efficiency of photosynthesis when plants are exposed to light.



The mechanism(s) of photoinhibition are under debate, several mechanisms have been suggested.

1. Acceptor-side photoinhibition

Strong light causes the reduction of the plastoquinone pool, which leads to protonation and double reduction (and double protonation) of the Q_A electron acceptor of Photosystem II. The protonated and double-reduced forms of Q_A do not function in electron transport. Furthermore, charge recombination reactions in inhibited Photosystem II are expected to lead to the triplet state of the primary donor (P_{680}) more probably than same reactions in active PSII. Triplet P_{680} may react with oxygen to produce harmful singlet oxygen.

2. Donor-side photoinhibition

If the oxygen-evolving complex is chemically inactivated, then the remaining electron transfer activity of PSII becomes very sensitive to light. It has been suggested that even in a healthy leaf, the oxygen-evolving complex does not always function in all PSII centers, and those ones are prone to rapid irreversible photoinhibition.

3. Manganese mechanism

A photon absorbed by the manganese ions of the oxygen-evolving complex triggers inactivation

of the oxygen-evolving complex. Further inhibition of the remaining electron transport reactions occurs like in the donor-side mechanism. The mechanism is supported by the action spectrum of photoinhibition.

4. Singlet oxygen mechanisms

Inhibition of PSII is caused by singlet oxygen produced either by weakly coupled chlorophyll molecules or by cytochromes or iron-sulfur centers.

5. Low-light mechanism

Charge recombination reactions of PSII cause the production of triplet P_{680} and, as a consequence, singlet oxygen. Charge recombination is more probable under dim light than under higher light intensities.

Evolution of oxygenic photosynthesis

When did oxygenic photosynthesis evolve? The question is significant because photosynthetic oxygen production by cyanobacteria led to oxygenation of the atmosphere and oceans, in turn allowing aerobic respiration and the evolution of large, complex and ultimately intelligent organisms (Catling *et al.* 2005). However, the answer is not self-evident, because it is not clear that the first appearance of oxygenic photosynthesis necessarily coincided with the first signs of oxygen in the environment. Indeed, there are three schools of thought on the matter, which are as follows.

- i. Oxygenic photosynthesis evolved hundreds of millions of years before the atmosphere became significantly oxygenated, because it took aeons to oxidize the continued production of reduced volcanic gases, hydrothermal fluids and crustal minerals (Catling & Claire 2005).
- ii. It arose at the *ca* 2.4Ga (billion years ago) 'Great Oxidation Event', causing immediate environmental change (Kopp *et al.* 2005).
- iii. Biogenic oxygen production began very early in Earth's history, before the start of the geological record, leading to an Archaean (greater than 2.5 Ga) atmosphere that was highly oxygenated (Ohmoto 1997).

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I B.SC., BIOCHEMISTRY

COURSE NAME: MEMBRANE BIOLOGY AND BIOENERGETICS

COURSE CODE: 18BCU103 UNIT: V BATCH-2018-2021

POSSIBLE QUESTIONS

UNIT-I

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Write any two general features of photophosphorylation.
2. Give any two significance of photophosphorylation.
3. What is photophosphorylation?
4. Write a note on Hills reaction.
5. Define photosystem.
6. What is photoinhibition?
7. Define proton motive force.
8. Why do we observe plant as green?

PART-C (8 MARKS)

1. Explain cyclic photophosphorylation and its significance.
2. Write a note on the Z-scheme of photosynthetic electron flow.
3. Differentiate between PS I and PS II.
4. Write a note on bacterial photophosphorylation.
5. Write a note on the different photosynthetic pigments.
6. Describe the molecular architecture of photosystem I.
7. Describe the molecular architecture of photosystem II.

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I B.Sc BIOCHEMISTRY
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18BCU103 - Membrane Biology and Bioenergetics

PART A (20 X 1 = 20 MARKS) - Online MCQ questions

Unit 5

S.No	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	Which among the following element is	Nitrogen	Chlorine	Carbon	Oxygen	Nitrogen
2	Plants are known as purifiers of air due to the	Respiration	Photosynthesis	Transpiration	Dessication	Transpiration
3	When the CO ₂ uptake cannot be increased by	Extinction point	CO ₂ saturation point	Light saturation point	Light compensation point	Extinction point
4	Which among the following conditions are	Aerobic condition only	Aerobic and low light	Aerobic and optimumk light	Anaerobic and low light	Aerobic and
5	When pigment system II is illuminated in	Hill reaction	Cyclic	Dark reaction	All of these	Dark reaction
6	Which among the following plant is used in the	Chlorella	Asparagus	Garden Pea	Acetabularia	Chlorella
7	The number of ATP and NADPH ₂ required to	3 and 2	2 and 3	5 and 3	3 and 5	5 and 3
8	Formation of energy currency of cell during	Substrate level	Phosphorylation	Photophosphorylation	Oxidative phosphorylation	Photophosphor
9	The red drop phenomenon is due to the	Pigment system I	Pigment system II	Carotenoids	PSI and PS II	Carotenoids
10	The first step in photosynthesis is the	Formation of ATP	Ionisation of water	Excitement of an electron of	Joining of two 3-carbon	Excitement of
11	The electron gap formed in PS-I during non	Pigment system I	Photolysis of water	Pigment system II	Oxidative phosphorylation	Pigment system
12	Who among the following stated for the first	Van Neil	Blakslee	Melvin Calvin	Van Helmont	Blakslee
13	The inhibitory effect of oxygen on the rate of	Warburg effect	Pasteur effect	Richmond-Lang effect	Blackman effect	Richmond-Lang
14	Photosynthetically active radiation is	640 - 650 nm	600-960 nm	400-700 nm	340-450	600-960 nm
15	During photorespiration reaction between	Mitochondria	Chloroplast	Peroxisome	Nuclues	Chloroplast
16	Where does the light reaction of	Quantasomes in	Outer membrane of	Inner membrane of	Stroma	Inner
17	Solar energy brings which of the following	Photolysis of Water	Reduction of CO ₂	Reduction of O ₂	Activation of chlorophyll	Photolysis of
18	Which among the following is the	Only pigment system II is	Photolysis of water is	Both pigment system I and II	Phosphorylation only takes	Phosphorylation
19	The concept of quantosome and	Emerson	Warburg	Arnold	Priestly	Emerson
20	Which among the following pigment is called	Carotene	Xanthophyll	Phycobilins	All the above	All the above
21	The pigment molecule which act as the	Chlorophyll	Carotenoids	Carotenes	Phycobilins	Chlorophyll
22	Photosynthesis is	Oxidative, anabolic,	Reductive, anabolic,	Reductive, ababolic,	Oxidative catabolic	Reductive,
23	P 700 is a special form of the following pigment	Chlorophyll -a	Carotene	Xanthophyll	Chlorophyll-b	Chlorophyll -a
24	The first stable product of dark reaction in C ₄	Phosphoglycolic acid	Glycolic acid	Oxaloacetic acid	Phosphoglyceric acid	Phosphoglyceric
25	The scientist who discovered that the site of	Arnon	Joseph Priestly	Julius Sachs	Robert Mayer	Robert Mayer

26	When green algae are illuminated, motile	Light	Oxygen	Algae	CO2	Oxygen
27	A photosynthetic organism which does not	Blue green algae	Green algae	Green sulphur Bacterium	Lichen	Green sulphur
28	90% of the total photosynthesis is carried out	algae	Mesophytes	Pteridophytes	Xerophytes	algae
29	The site of Dark reaction of photosynthesis is	grana	stroma	thylakoids	Both (a) and (b)	Stroma
30	Which of the following is least effective in	Blue light	Green light	Red light	Sunlight	Green light
31	The hydrogen donor in bacterial	Water	Ammonia	Sulphur	Hydrogen sulphide	Hydrogen
32	Light is necessary in the process of	Split carbon dioxide	Produce ATP and a	Release energy	combine carbon dioxide and	Produce ATP
33	The light reaction of photosynthesis does not	chemiosmosis	oxygen liberation	charge separation	electron transport	oxygen
34	The final product of the Calvin cycle is	RuPB	PGA	ATP	G3P	G3P
35	Photosynthesis takes place in the membranes	thylakoids	grana	photosystems	photon	thylakoids
36	The dark reaction in photosynthesis is limited	CO2, temperature, and	CO2, light, and water	water, temperature, and	oxygen, water, and	CO2,
37	Colors of light most useful in photosynthesis	green, yellow, and	red, violet, and blue	infrared, red, and yellow	red, white, and blue	red, violet, and
38	The pigment molecules responsible for	Mitochondria	cytoplasm of the cell	stroma of the chloroplast	thylakoid membrane of the	thylakoid
39	Both carotenoids and chlorophylls	are pigments	absorb photons of all	contain porphyrin rings	all of the above	are pigments
40	Which of the following is the correct sequence	P680 → P700 → water →	water → P700 →	P700 → P680 → NADP+ →	water → P680 → P700 →	water → P680 →
41	During what stage of photosynthesis are ATP	the light-dependent	the light-independent	both of the above	none of the above	the
42	Water vapor exits and CO2 enters a leaf	stomata	grana	porphyrin rings	photons	stomata
43	Which of the following organisms have the	C4 plants	Heterotrophs	C3 plants	CAM plants	C3 plants
44	High-energy photons	have long wavelengths	have short	are more likely to produce	cannot be absorbed	have short
45	During photosynthesis, photons raise electrons	H2O	ATP	RuBP	Chlorophyll	Chlorophyll
46	Which of the following occurs during the	electron transport	chemiosmosis	splitting of water	all of the above	all of the above
47	The oxygen that is released as O2 during	carbon dioxide	water	glucose	chlorophyll	water
48	Compared to retinal, chlorophyll can be	narrow absorption range	narrow absorption	wide absorption range but	wide absorption range but	narrow
49	Which of the following is mismatched?	Photosystem I - uses the	PGA - a 3-carbon	antenna complex - contains	CAM plants - open their	CAM plants -
50	Which of the following statements about	the light-dependent	photorespiration is	the light-dependent	all of the above are true	the
51	Which of the following statements accurately	photosynthesis occurs	photosynthesis uses	photosynthesis involves the	the primary function of	photosynthesis
52	In sulfur bacteria, how many pigment electrons	one	two	three	four	three
53	The primary form of sugar transported from	glucose	fructose	ribulose 1,5-bisphosphate	sucrose	sucrose
54	Light is required for the light dependent	it is the source for	it splits the water	it energizes electrons in the	it splits ATP molecules	it energizes
55	Which of the following statements are	The reaction of PS I is P	The reaction centre of	Both photosystems are	PSI is located on the	Both
56	Which of the following statements are	light reaction is called as	light reaction takes	CO2 fixation to	ATP and NADPH are	CO2 fixation to
57	All plastids have similar structure because they	store starch, lipids and	gets transformed from	be present together	perform some function	gets
58	Chlorophyll consists of	75% chlorophyll a and	75% chlorophyll b and	60% chlorophyll a and 40%	100% chlorophyll a	75% chlorophyll
59	Chlorophyll in Chloroplast is located in	grana	stroma	thylakoids	both grana and stroma	thylakoids

60	Which color of the spectrum is reflected by	Green	Blue	Yellow	Red	Green
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