M.Sc. Chemistry **2018-2019**

ELECTIVE-III **Semester - III**

18CHP305C **INDUSTRIAL CHEMISTRY** **4H 4C**

(APPLIED BIOINORGANIC CHEMISTRY, INORGANIC DRUG TARGETS AND METALS IN MEDICINE)

Instruction Hours/week:L: 4 T:0 P:0 **Marks: Internal:40 External: 60 Total:100**

External Semester Exam: 3 Hours

Course Objectives

1. To give the knowledge of the role of metals in human body
2. To learn about the physical methods in bioinorganic chemistry, metal biomolecules interactions, complexes, and drug discovery.
3. To give knowledge in Binding of Metal Ions and Complexes to Biomolecules
4. Learnt about complexes and chelating agents
5. Provide fundamental knowledge in Drug Discovery and Design

Course Outcomes

The student understood

1. The role of metals in human body
2. The various physical methods used in bioinorganic chemistry
3. The knowledge of Binding of Metal Ions and Complexes to Biomolecules
4. Nature of complexes and chelating agents
5. The process of Drug Discovery and Design

UNIT- I

Metals in the Human Body: General principles - the elements in the human body - biological significance, storage and transport of Fe, Zn, Cu, Mo, Co, Cr, V and Ni - metal functions in metalloproteins -metallo enzyme functions -supplying elements to the body - metals and human health.

UNIT- II

Physical Methods in Bioinorganic Chemistry: X-ray methods - magnetic resonance methods - mossbauer spectroscopy - magnetic measurements -other instrumental methods -atomic force microscopy - fast and time-resolved methods - stopped-flow kinetic methods - flash photolysis - time-resolved crystallography.

UNIT- III

Binding of Metal Ions and Complexes to Biomolecules: Nucleic acid structures - fundamental interactions with nucleic acids - binding interactions of tris(phenanthroline) metal complexes with DNA - techniques to monitor binding - applications of metal complexes that bind to nucleic acids -biopolymer promoted metal ligand interactions.

UNIT- IV

Complexes and Chelating Agents: Labile and inert complexes - metal-ligand selectivity-HSAB approach-chelate effect and Irving-William series -survey of metals used for diagnosis and chemotherapy-radiodiagnostic agents-Magnetic Resonance Imaging (MRI) - gold and other metal phosphines-main-group and transition metal compounds - miscellaneous metals in medicine-chelating agents and therapy - EDTA-evolution, chemical properties, *in vivo* chelation of radionuclides, dosage and toxicity .

UNIT-V

Drug Discovery and Design: Outline- therapeutic index, chemotherapeutic index, structure- activity relationship (SAR) and quantitative structure-activity relationship (QSAR)-Factors governing drug design- computer aided drug design-cancer chemotherapy-bioinorganic chemistry (DNA binding) of platinum anticancer drugs (cisplatin and carboplatin)-mechanism of action studies-clinical trials and their significance- production and quality control- patent protection.

SUGGESTED READINGS:

1. Taylor, D. M., & Williams, D. R. (1995). *Trace Element Medicine and Chelation Therapy* (I Edition). United Kingdom: The Royal Society of Chemistry.
2. AshutoshKar, (2000). *Medicinal Chemistry*. New Delhi: New Age International Publishers.
3. Gareth Thomas, (2000). *Medicinal Chemistry*. United Kingdom: John-Wiley & Sons Ltd.
4. Bertini, I., Gray, H. B., Lippard, S. J., & Valentine, J. S. (1994). *Bioinorganic Chemistry*. California: University Science books.
5. Roat-Malone, R. M. (2002). *Bioinorganic Chemistry*. NJ: John Wiley & Sons. Inc.

KARPAGAM ACADEMY OF HIGHER EDUCATION

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Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF CHEMISTRY

STAFF NAME: Dr. E. YAMUNA
SUBJECT NAME: INDUSTRIAL CHEMISTRY

SUB.CODE: 18CHP305C

SEMESTER: III CLASS: II-M.Sc (CHEMISTRY)

S.No.	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I (Metals in the Human Body)	
1	1	General principles	T1:16
2	1	the elements in the human body	T1:16-20
3	1	biological significance	T2:1-12
4	1	storage and transport of Fe, Zn, Cu, Mo, Co, Cr, V and Ni	T2:1-12 T2:12-30
5	1	metal functions in metalloproteins	T2:35-40
6	1	metallo enzyme functions	T2:40-54
7	1	supplying elements to the body	T1:20-21
8	1	metals and human health	T1:20-21
9	1	Recapitulations and discussion of important questions	
	Total No of Hours Planned For Unit 1=9		
		UNIT-II (Physical Methods in Bioinorganic Chemistry)	
1	1	X-ray methods - magnetic resonance methods	T1: 73-110
2	1	mossbauer spectroscopy	T1:114-116
3	1	magnetic measurements	T1: 116-117 T1:119
4	1	other instrumental methods	T1: 121

5	1	atomic force microscopy stopped	T1: 121-123
6	1	fast and time-resolved methods	T1: 123
7	1	flow kinetic methods	T1: 123
8	1	flash photolysis - time-resolved crystallography	T1: 124
9	1	Recapitulations and discussion of important questions	
	Total No of Hours Planned For Unit II=9		
		UNIT-III (Binding of Metal Ions and Complexes to Biomolecules)	
1	1	Nucleic acid structures	T1: 455-458
2	1	fundamental interactions with nucleic acids	T1: 459-467
3	1	binding interactions of tris(phenanthroline) metal complexes with DNA	T1:468-472
4	1	techniques to monitor binding	T1:472-478
5	1	applications of metal complexes that bind to nucleic acids	T1:478-485
6	1	biopolymer promoted metal ligand interactions.	T1:485-487
7	1	Recapitulations and discussion of important questions	
8	1	Previous year question paper discussion	
	Total No of Hours Planned For Unit III=8		
		UNIT-IV (Complexes and Chelating Agents)	
1	1	Labile and inert complexes-metal-ligand selectivity	T2:26-31
2	1	HSAB approach-chelate effect and Irving-William series	T2: 32-55
3	1	survey of metals used for diagnosis and chemotherapy	T1: 514
4	1	Radio diagnostic agents	T1: 514-517
5	1	Magnetic Resonance Imaging (MRI)	T1:517-520
6	1	gold and other metal phosphines	T1:520-521
7	1	main-group and transition metal compounds	T1:520-521
8	1	miscellaneous metals in medicine-chelating agents and therapy	T1:521

9	1	EDTA-evolution, chemical properties, *in vivo* chelation of radionuclides, dosage and toxicity .	T2:81-88
10	1	Recapitulations and discussion of important questions	
11	1	Revision & Discussion of important questions	
	Total No of Hours Planned For Unit IV=11		
		UNIT-V (Drug Discovery and Design)	
1	1	Outline- therapeutic index, chemotherapeutic index	T1: 2-3
2	1	structure- activity relationship (SAR) and quantitative structure-activity relationship (QSAR)	T1: 22-23
3		Factors governing drug design	T1: 4-6
4	1	computer aided drug design	T1: 95-98
5	1	cancer chemotherapy	T1: 794-800
6	1	bioinorganic chemistry (DNA binding) of platinum anticancer drugs (cisplatin and carboplatin)	T2: 93
7	1	mechanism of action studies	T1: 522-537
8	1	clinical trials and their significance	T1: 1-3
9	1	production and quality control- patent protection	W1
10	1	Recapitulations and discussion of important questions	
11	1	Previous year question paper discussion	
	Total No of Hours Planned for Unit V=11		
Total Planned Hours	**48**		

Text Book:

1. Taylor, D. M., & Williams, D. R. (1995). *Trace Element Medicine and Chelation Therapy* (I Edition). United Kingdom: The Royal Society of Chemistry.
2. Ashutosh Kar, (2000). *Medicinal Chemistry*. New Delhi: New Age International Publishers.
3. Gareth Thomas, (2000). *Medicinal Chemistry*. United Kingdom: John-Wiley & Sons Ltd.
4. Bertini, I., Gray, H. B., Lippard, S. J., & Valentine, J. S. (1994). *Bioinorganic Chemistry*. California: University Science books.
5. Roat-Malone, R. M. (2002). *Bioinorganic Chemistry*. NJ: John Wiley & Sons. Inc.

W1: https://en.wikipedia.org/wiki/Quality_control, https://en.wikipedia.org/wiki/Patent

1. In what form is iron stored in the body	**as transferring**	**as ferritin**	**as haemoglobin**	**d) as a siderophore**	**as haemoglobin2**
How does carbon monoxide affect the human body	It does not allow binding of oxygen with haemoglobin	It reduces the surface area of the alveoli and disrupts gaseous transfers	It causes the liver to malfunction, increasing bile secretion	It reduces the body's tendency to absorb water thereby making us feel dehydrated	It does not allow binding of oxygen with haemoglobin
How does nitrogen affect the human body	Increases v ulnerability to pathogens	Destroys the macrophages	Injures the defence mechanism of the lungs	All of the mentioned	All of the mentioned
How does lead affect the human body?	Increases blood pressure	Damages the cerebellum, liver and kidney	Leads to reproductive disorders and osteoporosis	All of the mentioned	All of the mentioned
Iron is component of myoglobin in	white blood cells	muscle cells	ligament cells	red blood cells	muscle cells
Iron is component of hemoglobin in	red blood cells	white blood cells	muscle cells	ligament cells	red blood cells
Disease anemia is caused by	deficiency of phosphorus	deficiency of magnesium	deficiency of iron	deficiency of calcium	**deficiency of iron**
Essential element for maintenance and development of teeth and bones is	ethyl oxide	methyl alcohol	calcium	acetyl	calcium
Sources of calcium includes	cabbage and nuts	milk and beans	cheese and egg yolk	all of above	all of above
What is the name of the iron containing protein that gives red blood vessels their colour?	Hemocyanin	Pyrite	Hemoglobin	Myoglobin	Hemoglobin
The…..produes red blood cells, which transport….and some….	Liver,oxygen; mineral ions	Liver,oxygen; carbondioxide	Bone marrow;oxygen;hormones	Bone marrow;oxygen;carbondioxide	Bone marrow;oxygen;carbondioxide
During the formation of the peptide bond which of the following takes place?	Hydroxyl group is lost from its carboxyl group of one amino acid and a hydrogen atom is lost from its amino group of another amino acid	Hydrogen atom is lost from its carboxyl group of one amino acid and a hydroxyl group is lost from its amino group of another amino acid	Hydroxyl group is lost from its carboxyl group of one amino acid and a hydroxyl group is lost from its amino group of another amino acid	Hydrogen atom is lost from its carboxyl group of one amino acid and a hydrogen atom is lost from its amino group of another amino acid	Hydroxyl group is lost from its carboxyl group of one amino acid and a hydrogen atom is lost from its amino group of another amino acid
Which of the following is not the classified form of conjugated proteins?	Lipoproteins	Glycoproteins	Metalloproteins	Complete proteins	Glycoproteins
Other than dairy products, which of the following food groups	Meat	Fish	Cereals	Vegetables	Cereals

provides a rich source of calcium in the UK?					
Which of the following factors inhibits non-haem iron bioavailability the most?	Phytic acid	Citric acid	Vegetable protein	Calcium	Phytic acid
How is iron transported in the circulation from the intestine to the sites of metabolism in the body?	As simple Fe2+ in the serum	Bound to albumin	Bound to ferritin	Bound to transferrin	Bound to transferrin
Which of the following in not a zinc-dependent enzyme?	Superoxide dismutase	Alkaline phosphatase	Glutathione peroxidase	DNA polymerase	Glutathione peroxidase
Which of the following inborn errors of metabolism gives rise to zinc deficiency?	Acrodermatiti senteropathica	Wilson's disease	Menkes disease	Haemochroma tosis	Acrodermatitis enteropathica
Which of the following foods might be considered a "goitrogen"?	Fish	Meat	Rice	Brassicas	Brassicas
Which of the following statements about iodine is correct?	50% of adults consume iodine at levels below the RNI	Dairy products are a poor source of iodine	The iodine content of organic milk is generally lower that the level in non-organic milk	UK dietary reference values recommend an increase in iodine intake in pregnancy	The iodine content of organic milk is generally lower that the level in non-organic milk
In which form is selenium found in the 25 human selenoproteins?	Selenophosph ate	Selenocysteine	Selenohistidin e	Selenate	Selenocysteine
Zinc finger motifs are a particular characteristic of proteins with which one of the following functions?	Biochemical catalysis. (The proteins are enzymes)	Formation of the cell cytoskeleton. (The proteins are structural proteins.)	Gene regulation. (The proteins are DNA-binding proteins.)	Signal transduction across the cell membrane. (The proteins are transmembran e proteins.)	Gene regulation. (The proteins are DNA-binding proteins.)
Metals are unable to exhibit metallic bonding in biological systems. True or false?	TRUE	FALSE		Lithium	TRUE
Which of the following elements are classed as metals? Please select all that apply.	Oxygen	Iron	Calcium	Chlorine	Iron, Lithium, Calcium
Which of the following metal is involved in the transmission of nerve impulses?	Lithium	Iron	Potassium	Zinc	Potassium

Which metal forms part of the haem group, to which oxygen binds in haemoglobin?	Zinc	Copper	Manganese	Iron	Iron
Haemoglobin can only bind oxygen. True or false?	TRUE	FALSE			FALSE
In the active sites of many enzymes, metals are coordinated by the amino acid histidine. Which element in histidine donates the electrons that form the coordinate bond?	Carbon	Oxygen	Nitrogen	Sulfur	Nitrogen
Which of the following chemical is responsible for London smog episode?	Sulphur dioxide	Sulphur	Sulphur trioxide	Sulphur oxide	Sulphur dioxide
Environmental disease outbreak in Toyama, Japan was due to ____	Lead	Cadmium	Mercury	Zinc	Cadmium
Which of the following chemical is responsible for acute lung disease from Bhopal gas tragedy?	Methylisocyanate	Methylisocyanade	Methyl	Methylcyanate	Methylisocyanate
Heavy metals like Arsenic, Cadmium and Cyanide effects _____	Immune system	Nervous system	Skin	Respiratory system	Nervous system
In oxyhaemoglobin, the iron centre is best described by which of the following?	high-spin Fe(III)	high-spin Fe(II)	low-spin Fe(III)	low-spin Fe(II)	low-spin Fe(III)
In oxyhaemoglobin, the coordinated dioxygen is best described by which of the following?	molecular O2 with linear Fe–O–O	molecular O2 with bent Fe–O–O	[O2]–	[O2]2–	[O2]–
Which statement best describes haemerythrin?	A haem protein	A metalloprotein containing two active sites: one, a haem Fe and one, a non-haem Fe	A non-haem protein with one Fe centre at the active site	A non-haem protein with two Fe centres at the active site	A non-haem protein with two Fe centres at the active site
Which statement correctly describes the function of cytochromes P-	Cytochromes P-450 act as monooxygenases and catalyse the	Cytochromes P-450 couple to cytochrome c in the mitochondrial	Cytochromes P-450 act as dioxygenases	Cytochromes P-450 contain high-spin Fe(III); this directly binds	Cytochromes P-450 act as monooxygenases and catalyse the insertion of

450?	insertion of O into a C–H bond	electron-transfer chain		O2 and acts as an O2 carrier	O into a C–H bond
Which statement is incorrect about Types 1, 2 and 3 centres in blue copper proteins?	A Type 1 centre exhibits an intense LMCT band in the electronic spectrum	A Type 2 centre does not give rise to an EPR signal	A Type 3 centre contains two Cu centres which are antiferromagnetically coupled	A plastocyanin contains a Type 1 Cu centre	A Type 2 centre does not give rise to an EPR signal
Which statement about Fe–S proteins is incorrect?	A rubredoxin contains an FeS4 unit, each S coming from a Cys residue	A [2Fe–2S] ferredoxin contains six S donors, two of which are S2– ligands	A [4Fe–4S] ferredoxin contains a cubane core	In a [4Fe–4S] ferredoxin, four redox couples that make use of the four Fe centres are accessible in Nature	In a [4Fe–4S] ferredoxin, four redox couples that make use of the four Fe centres are accessible in Nature
The terminal member of the mitochondrial electron-transfer chain is:	cytochrome P-450	cytochrome c oxidas	cytochrome c	cytochrome c1	cytochrome c oxidase
Which statement about the [Fe-Fe]-hydrogenases from bacteria isolated from C. pasteurianum and D. desulfuricans is correct?	Fe-only hydrogenase contains [4Fe–4S] units and a unit consisting of an Fe4S4-cluster connected by a Cys residue to an Fe2S2-group	Fe-only hydrogenase contains four [4Fe–4S] units as the active sites	The active site in an Fe-only hydrogenase is an Fe2(Cys)2(His)2 unit	The active site in an Fe-only hydrogenase consists of an Fe4S4-cluster connected to an Fe2S2(Cys)4-group	Fe-only hydrogenase contains [4Fe–4S] units and a unit consisting of an Fe4S4-cluster connected by a Cys residue to an Fe2S2-group
Nitrogenase contains:	an Fe-protein and an FeMo-protein that operate in conjunction with each other	an FeMo-protein in which the active site consists of a single [3FeMo-4S] cluster	an FeMo-protein in which the P-cluster is in an irreversibly reduced state	an Fe-only containing protein in which the P-cluster is the active site	an Fe-protein and an FeMo-protein that operate in conjunction with each other
Cytochrome c oxidase contains all but one of the following. Which one is the odd one out?	A Cu2 centre, bridged by two Cys residues	A haem unit with two axial His units	A Cu centre coordinated by three Cys residues	A haem unit with one axial His residue, sited near a Cu centre coordinated by three His residues	A Cu centre coordinated by three Cys residues
Cytochrome c:	is a haem protein	accepts an electron from cytochrome c oxidase in the mitochondrial electron-transfer chain	is a non-haem protein	is irreversibly oxidized in the mitochondrial electron-transfer chain	is a haem protein
Which of the following is not involved in electron transfer?	Carbonic anhydrase	cytochrome b	rubredoxin	Rieske protein	Carbonic anhydrase
Each part of this answer lists properties of Zn2+, which may	Zn2+ is a hard metal centre; it acts as a Lewis acid; it	Zn2+ is a hard metal centre; it acts as a Lewis acid; it favours	Zn2+ is a d10 ion; it is a soft metal centre; it acts as a Lewis	Zn2+ is a d10 ion; it is a soft metal centre; it tolerates	Zn2+ is a hard metal centre; it acts as a Lewis acid; it

or may not be correct. Which list gives properties that are correct and relevant to Zn-containing enzymes?	tolerates different coordination geometries	4-coordination	acid	different coordination geometries	tolerates different coordination geometries
Studies of Zn(II)-containing proteins often make use of Co(II)-for-Zn(II) substitution. Which statement is correct?	Tetrahedral coordination is one of several environments observed for both Co2+ and Zn2+	TTetrahedral Co2+ and Zn2+ are both diamagnetic	The ionic radius of Co2+ is significantly smaller than that of Zn2+	The visible spectra of complexes of Co2+ are similar to those of related complexes of Zn2+	Tetrahedral coordination is one of several environments observed for both Co2+ and Zn2
Thioneins are rich in which of the following amino acid residues?	cysteine	histidine	glycine	threonine	cysteine
Haemocyanins are O2-carrying copper-containing proteins in:	mammals	molluscs	bacteria	fungi	molluscs
In studies of blue copper proteins, EPR spectroscopy is useful because:	Cu(I) has one unpaired electron	Cu(II) is paramagnetic	Cu(II) and Cu(I) are both paramagnetic	Cu(II) and Cu(I) have one and two unpaired electrons, respectively	Cu(II) is paramagnetic
Which of the following equilibria has the largest value of K? (Hae = haemoglobin)	Which of the fHae + O2 = Hae(O2)ollow ing equilibria has the largest value of K? (Hae = haemoglobin)	Hae(O2) + O2 = Hae(O2)2	Hae(O2)2 + O2 = Hae(O2)3	Hae(O2)3 + O2 = Hae(O2)4	Hae(O2)3 + O2 = Hae(O2)4
Anemia affects what percentage of the population?	12%	18%	27%	32%	27%
ron deficiency anemia (IDA) is the most common type of anemia. True or false?	TRUE	FALSE			TRUE
Anemia is of particular concern in	Young children	Pregnant women	Older individuals	All of the above	All of the above
Although anemia is a significant global health issue, it is not associated with high mortality or morbidity. True or false?	TRUE	FALSE			FALSE
Iron deficiency anemia is common in adolescents with:	Asthma	Bulimia	Heavy menstrual bleeding	Obesity	Heavy menstrual bleeding
Anemia is prevalent in high-risk patients undergoing transcatheter aortic valve	TRUE	FALSE			TRUE

implantation (TAVI), and impacts mortality following the procedure. True or false?					
Unexplained IDA may be linked to:	Celiac disease	Crohn's disease	Diverticulitis		Celiac disease
Which of the following statements about red blood cells (RBCs) is correct?	RBCs contain hemoglobin	Mature RBCs lack nuclei	Mature RBCs lack ribosomes	all the above	all the above
Which dietary component is needed for the synthesis of DNA and influences the production of RBCs?	Calcium	iron	folic acid	Vitamin A	folic acid
What percentage of the body weight (in kg) is composed of blood? What percentage of this blood is composed of plasma?	20%; 55 %	20%; 45%	7%; 45%	7%; 55%	7%; 55%

UNIT-I

Metals in the Human Body:

General principles-the elements in the human body- metal functions in metalloproteins - metallo enzyme functions -supplying elements to the body - metals and human health-biological significance, storage and transport of Fe, Zn, Cu, Mo, Co, Cr, V and Ni.

The elements in the human body

The average amounts of some essential and non-essential elements in the human body are shown in Table 2.1. This shows that the weights of some of the essential elements in the body vary by six or more orders of magnitude. Oxygen, at- 45 kg, is by far the most abundant element with the majority being present as one simple inorganic compound: water. Some of the elements listed in Table 2.1, and marked with an asterisk, have no known beneficial function and are present in the body simply because they are present in rocks and soils and find their way into water and foodstuffs and from thence into the human body; many of these elements are present in only minute quantities.

Each of the elements shown in the three groups in Table 2.1, irrespective of whether it is essential, beneficial or even potentially toxic, has its own individual pattern of intake into the body, transfer to the blood, utilization in the tissues and finally excretion from the body. For example, hydrogen taken into the body by the ingestion of water, or the inhalation of water vapour, is rapidly and completely transferred to the blood, from where it passes into the tissues to participate in many different types of reaction, before being excreted from the body with an equivalent half-time of about 10 days. The equivalent biological half- time assumes that the material is being lost at a constant rate from a single compartment, whereas in fact more that one compartment and rate of loss may be involved. In contrast iron is taken into the body in foods or drugs, but its absorption from the gastrointestinal tract is closely controlled to meet the physiological needs of the body. However, the iron which is absorbed into the blood stream is effectively all retained in the body for a very long time. Some illustrative, nominal values for the gastrointestinal absorption and the notional equivalent half-times of excretion from the body tissues for some important elements are listed in Table 2.2.

Table 2.1 *The elemental composition of a 70 kg 'reference' person*

Elements	*Mass*	
	g	*Moles*
A. Main group non-metals		
Hydrogen	7000	3500
Carbon	12 600	1050
Nitrogen	2100	75
Oxygen	45 500	1425
Phosphorus	700	22.5
Sulfur	175	5.5
Fluorine	0.8	0.02
Chlorine	105	3.0
Bromine	0.2	0.025
Iodine	0.013	0.0001
B. Main group metals		
Lithium	0.0007	0.0001
Boron	0.01	0.0009
Sodium	105	4.6
Potassium	140	3.6
Rubidium*	1.1	0.013
Caesium*	0.0015	0.00001
Aluminium	0.1	0.0037
Zinc	2.3	0.035
Silicon	1.4	0.05
Arsenic	0.014	0.0002
Antimony	0.07	0.0006
Selenium	0.02	0.003
Tin	0.03	0.0002
Lead	0.08	0.0004
Cadmium	0.03	0.0003
Magnesium	35	1.4
Calcium	1050	26
Strontium	0.14	0.0016
Barium*	0.016	0.00012
Radium*	3×10^{-11}	1.4×10^{-13}
Uranium*	9×10^{-5}	3.8×10^{-14}
Plutonium*	6×10^{-18}	$2.5.10^{-20}$
C. Transition series metals		
Titanium	0.01	0.0002
Vanadium	0.02	0.0004
Chromium	0.005	0.0001
Manganese	0.02	0.00036
Iron	4.2	0.075
Cobalt	0.0007	0.0001
Nickel	0.01	0.0002
Copper	0.11	0.0016
Zirconium	0.3	0.003
Niobium	0.1	0.001
Molybdenum	0.005	0.00005

* Elements with no recognized physiological role.

Some elements are essential for life and others, although not truly essential, contribute to our general well-being. Some elements, though apparently harmless at 'normal' concentrations, may be toxic if they are present in rather larger amounts. For example, the natural total body content

Table 2.2 *The fractional absorption (f_1) and the notional equivalent half-time (T_{eq}) of retention in the human body of some main group and transition series metals. The f_1 is the fraction of an orally administered element which passes from the gastrointestinal tract to the blood stream; the notional T_{eq} assumes a constant rate of loss from a single compartment*

Element	f_1	T_{eq}/days
	Main group metals	
Lithium	1	1
Sodium	1	10
Potassium	1	30
Rubidium*	1	60
Caesium*	1	110
Magnesium	0.5	0.8
Calcium	0.5	10000
Strontium	0.3	10000
Barium*	0.2	10000
Radium*	0.2	10000
Uranium*	0.01	10000
Plutonium*	0.0005	10000
	Transition series metals	
Manganese	0.1	45
Iron	0.15	2000
Cobalt	0.1	40
Copper	0.5	40
Zinc	0.5	400
Molybdenum	0.8	45
Nickel	0.05	1

* Elements with no recognized physiological role.

of barium is about 2 mg, of which about 90% is locked up in the hydroxy apatite of the bone mineral but about 400 times this amount (- 800 mg) could cause death. However, because it is present as a species which is not bio-available, the so-called *Barium Meal* containing up to 200 g of the highly insoluble barium sulfate (solubility product 1.07 **X** lo-" mol dm-3), is routinely, and safely, administered orally to humans as a contrast medium for the radiological investigation of gastrointestinal disorders. The proper functioning of the human body requires an adequate supply of the essential and beneficial elements and this is met from the diet, and in the case of some inorganic elements to a lesser extent from drinking water. For metallic elements the adequacy of the diet with respect to the requirements for a specific metal will depend on two factors: the concentration of the element of interest in the food and water and its bio-availability. The bio-availability is the extent to which the metal concerned is transferred from the gastrointestinal tract to the blood and this depends on the chemical behaviour of the

element in the gastrointestinal tract, where the pH may vary from - 2 in the stomach to about pH 8 in the small intestine, where high concentrations of complexing ligands are also present. The bio-availability of metals is element-specific and may range from virtually 100% for Group I metals like sodium, to less than 0.1% for readily hydrolysable metals such as plutonium.

For the essential elements the amounts in the body are normally controlled by physiological mechanisms, but for the non-essential, non-beneficial elements there are no such controls and the amounts in the body generally reflect the natural occurrence of the elements in food and water. For many such elements we may consider that there is a *base load* in the human body which reflects the natural intake of the elements in the diet. For some elements, industrial, mining or other human activities, may release metals into the environment. Such activities may result in a *civilization-related load* being added to the natural base load; in some circumstances this civilization-related load may be very much greater than the base load.

Supplying Elements to the body:

In order to keep healthy the body has specific daily requirements for essential and beneficial elements and these may be expressed as 'recom- mended daily amounts' (RDAs); Some RDAs are listed in Table **2.3.** Both essential and non-essential trace elements are taken into the body with foodstuffs, and some of the elements may be biologically incorporated into the food itself while, particularly with vegetables, another part may be taken into the body in the form of soil particles which adhere to the foodstuffs it has been estimated that an adult human may ingest as much as 100 g of topsoil per year. It is important to recognize these two sources of trace elements; first of all because the bio-availability of a trace element incorporated into a food material may be markedly different to that from a soil particle. Therefore a diet that, on the basis of a total elemental analysis, appears to provide adequate amounts of a particular element may in fact be quite inadequate because a large fraction of the metal is present in a highly insoluble and poorly available form in soil particles.

Table 2.3 *Typical recommendations for the daily intake of some inorganic nutrients by young adult males* (from Coultate, 1991). *The amounts absorbed from the gastrointestinal tract into the blood will generally be only a fraction of these quantities*

Element	*RDA*/mg	*Element*	*RDA*/mg
Calcium	500	Phosphorus	800
Magnesium	350	Zinc	15
Iron	10	Iodine	0.15
Copper	2	Selenium	0.05

Biological Significance:

It is most appropriate to classify metals of interest by their impact on health effects—nutritionally essential, nonessential with a possible beneficial effect, or nonessential with no beneficial effects. Table 2.4, below, lists the metals identified in the environmental chemistry paper as metals of concern; it also lists iron and magnesium, which are nutritionally essential.

Table 2.4. Classification of Metals Based on Characteristics of Health Effects

Nutritionally Essential Metals	Metals with Possible Beneficial Effects	Metals with No Known Beneficial Effects
Cobalt	Boron	Aluminum
Chromium III	Nickel	Antimony
Copper	Silicon	Arsenic
Iron	Vanadium	Barium
Manganese		Beryllium
Molybdenum		Cadmium
Selenium		Mercury
Zinc		Lead
		Strontium

The primary premise for this classification is that assessment of health risks for nutritionally essential metals requires its own approach or process: restrictive standards must allow sufficient exposure for the general population to prevent deficiencies, but nutritionally essential metals may cause adverse health effects at some levels below or beyond the level required for optimum nutrition.

Metals functions in metalloproteins:

Metal-Protein Interactions

Metals react with many different proteins in the body that may modify their toxicity and kinetics. An example is the interaction of lead with heme-synthesizing enzymes. Arsenic, cadmium, mercury, and lead interfere with enzymes involved with energy metabolism by substituting with essential metals. Many metals bind with albumin for purposes of transport in the circulatory system and across cell membranes and within cells. There are also several proteins that bind to specific metals.

Metallothioneins

Metallothioneins are a group of low-molecular-weight proteins (MW about 6,000 daltons), rich in sulfhydryl groups that serve as ligands for several essential and nonessential metals. In vitro studies have found that the highest affinity is for silver, then in descending order mercury, copper, bismuth, cadmium, lead, and zinc. However, studies of in vivo metallothioneins from various sources included zinc, copper, and cadmium. Metallothioneins have multiple binding sites that have different affinities for metals. Also, the types of metal bound to metallothioneins differ depending on the species, the organ, and previous exposures to metals, but most of them contain at least two different types of metals. For example, metallothioneins isolated from adult or fetal human livers contain mainly zinc and copper, while those from human kidneys contain cadmium, copper, and zinc.

In most cases the metallothioneins are inducible and perform a number of functions, including serving as a storage protein for zinc and copper in the liver, kidney, brain, and possibly skin and having an important protective role in cadmium toxicity.

There has been recent interest in the role of metallothionein as a modulator of immune response, and it is suggested that assessment of metallothionein status in peripheral blood monocytes may provide a non-invasive approach to assessing the risk of metal exposure to immunotoxicicty. While metallothioneins have an affinity for lead in vitro, in vivo binding to lead has not been demonstrated. Also, mercury may induce synthesis of metallothionein in vivo, but binding is only temporary regardless of the demonstrated in vitro affinity.

Transferrin

Transferrin is a glycoprotein that binds most of the ferric ion in plasma and has a role in transporting iron across cell membranes. This protein also transports aluminum and manganese.

Ferritin

Ferritin is primarily a storage protein for iron in reticuloendothelial cells of the liver, spleen, and bone. It plays an important role in turnover of iron. It has also been suggested that ferritin may serve as a general metal agonist since it binds a number of metals including cadmium, zinc, beryllium, and aluminum.

Ceruloplasmin

Ceruloplasmin is a copper-containing glycoprotein oxidase in plasma that converts ferrous to ferric iron, which then binds to transferrin.

Lead-binding protein(s)

Lead binds with a number of lead-binding proteins, but their identity or function is not as well

defined as that of other metal-specific proteins. The most studied lead-binding protein is the denatured lead-protein complex identified as the intracellular inclusion body occurring in cells, particularly in the liver and kidney in persons with high-level lead exposure. It has been suggested that lead-binding proteins may have a protective effect for lead.

Membrane carrier proteins

There are a number of recently discovered carrier proteins that transport metals across cell membranes. Many metals are transported as complexes with endogenous ligands; no transport systems are intended for the ligand itself. Many of these carrier proteins are multi-specific, accepting substrates that vary considerably but are recognized by the attached metal ion.

Metalloenzymes

Metalloenzymes are enzyme proteins containing metal ions (metal cofactors), which are directly bound to the protein or to enzyme-bound nonprotein components (prosthetic groups). About one-third of all enzymes known so far are metalloenzymes. Besides enzymes, other metalloproteins are involved in non-enzyme electron transfer reactions (cytochromes), may act as storage (e.g., ferritin for iron) or transport proteins (e.g., transferrin for iron). In the latter groups of proteins, the metal storage is reversible and the metal is a temporary component. Also ribozymes, i.e., RNA molecules with enzyme function may contain structurally and/or functionally important metal ions (mostly divalent metal ions such as Mg^{2+}) and may be therefore termed as metalloenzymes in a broader.

Biological Significance of Iron, Zinc, Copper, Molybdenum, Cobalt, Chromium, Vanadium, and Nickel

Living organisms store and transport transition metals both to give fitting concentrations of them to use in metalloproteins or cofactors and to ensure them against the harmful impacts of metal abundances; metalloproteins and metal cofactors are found in plants, creatures, and microorganisms. The ordinary fixation run for each metal in organic frameworks is limited, with the two lacks and abundances causing neurotic changes.

The transition metals and zinc are among the minimum rich metal ions in the ocean water from which contemporary organisms are thought to have advanced (Table 2.5). For a significant number of the metals, the fixation in human blood plasma enormously surpasses that in ocean water. Such information shows the significance of systems for gathering, storage, and transport of transition metals and zinc in living organisms.

Table 2.5 Concentrations of transition metals and zinc in sea water and human plasma

Element	Sea water (M) × 10^8	Human plasma (M) × 10^8
Fe	0.005–2	2230
Zn	8.0	1720
Cu	1.0	1650
Mo	10.0	1000
Co	0.7	0.0025
Cr	0.4	5.5
V	4.0	17.7
Mn	0.7	10.9
Ni	0.5	4.4

The metals are by and large discovered either bound specifically to proteins or in cofactors, for example, porphyrins or cobalamins, or in bunches that are in tum bound by the protein; the ligands are normally 0, N, S, or C. Proteins with which transition metals and zinc are most ordinarily related catalyze the intramolecular or intermolecular reworking of electrons. In spite of the fact that the redox properties of the metals are imperative in a considerable lot of the reactions, in others the metal seems to add to the structure of the dynamic state, e.g., zinc in the Cu-Zn dismutases and a portion of the iron in the photosynthetic response focus. Now and again comparable reactions are catalyzed by proteins with various metal focuses; the metal restricting destinations and proteins have developed independently for each sort of metal focus. Iron is the most well-known transition metal in science.

Biological Systems of Metal Storage, Transport, and Mineralization

Storage

The storage of iron

Three properties of iron can represent its broad use in earthly natural reactions:

- effortless redox reactions of iron ions;
- a broad collection of redox possibilities accessible by ligand substitution or modification (Table 4);
- Abundance and accessibility (Table 1) under conditions obviously surviving when earthly life started

The storage of zinc, copper, vanadium, chromium, molybdenum, cobalt, nickel, and manganese

Ions of nonferrous transition metals require a substantially less mind boggling organic storage framework, in light of the fact that the solubilities are considerably higher (210 - 8 M) than those for Fe 3+. Subsequently, the storage of nonferrous transition metals is more subtle, and information is more constrained. In addition, investigations are more troublesome than for iron, on the grounds that the sums in organic frameworks are so little. Basically nothing is known yet about the storage of vanadium, chromium, molybdenum, cobalt, nickel, and manganese, with the conceivable exception of accumulations of vanadium in the platelets of tunicates.

Zinc and copper, which are utilized as a part of the most elevated concentrations of any of the non- ferrous transition metals, are particularly bound by the protein metallothionein. Like the ferritins, the metallothioneins are a group of proteins, far reaching in nature and managed by the metals they tie. As opposed to ferritin, the measures of metal put away in metallothioneins are littler (up to twelve iotas for every atom), the measure of protein in cells is less, and the format (mRNA) isn't put away. Since the cell concentrations of the metallothioneins are generally low and the measure of metal required is moderately little, it has been hard to consider the organic destiny of copper and zinc in living organisms, and to find the common part of metallothioneins. Be that as it may, the regulation of metallothionein amalgamation by metals, hormones, and development factors bears witness to the organic significance of the proteins. The abnormal metal environments of metallothioneins have pulled in the attention of bioinorganic scientific experts.

Transport

Iron

The storage of iron in people and different warm blooded animals has been managed in the past section. Just a little fraction of the body's stock of iron is in travel at any minute. The transport of iron from storage destinations in cell ferritin or hemosiderin happens by means of the serum-transport protein transferrin.

The transferrins are a class of proteins that are bilobal, with every flap reversibly (and basically autonomously) restricting ferric ion. This complexation of the metal cation happens through earlier complexation of a synergistic anion that in vivo is bicarbonate (or carbonate). Serum transferrin is a monomeric glycoprotein of atomic weight 80 kDa. The precious stone structure of the related protein, lactoferrin, has been accounted for, and as of late the structure of a mammalian transferrin has been reasoned.

Ferritin is obviously an exceptionally old protein and is found in higher creatures, plants, and even microorganisms; in plants and creatures a typical ferritin ancestor is

demonstrated by arrangement conservation. Conversely, transferrin has been in ex" istence just generally as of late, since it is just discovered ia the phylum Chordata. In spite of the fact that the two iron-restricting locales of transferrin are adequately unique to be recognizable by dynamic and a couple of different investigations, their coordination environments have been known for quite a while to be very comparative. This was first found by different spectroscopies, and most as of late was affirmed by crystalstructure examination, which demonstrates that the environment includes two phenolateoxygens from tyrosine, two oxygens from the synergistic, bidentate bicarbonate anion, nitrogen from histidine, and (a shock at the season of precious stone structure investigation) an oxygen from a carboxylate gathering of an aspartate.

Zinc, copper, vanadium, chromium, molybdenum, and cobalt

One extremely intriguing late advancement has been the characterization of sequestering specialists created by plants which complex various metal ions, not simply ferric ions. A key compound, now all around portrayed, is mugeneic corrosive. The basic and chemical similitudes of mugeneic corrosive to ethylenediaminetetraacetic corrosive (EDTA) have been noted. Like EDTA, mugeneic corrosive structures a to a great degree solid .~omplex with ferric ion, yet in addition shapes very solid buildings with copper, zinc, and other transitionmetal ions. Like the siderophores delivered by microorganisms, the coordination environment obliged by mugeneic corrosive is basically octahedral. In spite of the fact that the coordination properties of this ligand are well laid out, and it has been demonstrated that divalent metal cations, for example, copper, aggressively hinder iron take-up by this ligand, the nitty gritty procedure of metal-ion conveyance by mugeneic corrosive and related mixes has not been explained.

Question	A	B	C	D	Answer
X-ray diffractometers are not used to identify the physical properties of which of the following?	Metals	Liquids	Polymeric materials	Solids	Liquids
X-ray diffractometers provide _____ information about the compounds present in a solid sample.	Quantitative	Qualitative	Quantitative and qualitative	Either quantitative or qualitative	Quantitative and qualitative
Which of the following is the most common instrument for photographic recording of diffraction patterns?	Debye-Scherrer powder camera	Gamma camera	Geiger tube	Scintillation counter	Debye-Scherrer powder camera
4. With the help of which of the following equations is the distance calculated from a known wavelength of the source and measured angle?	**Coolidge equation**	Bragg's equation	Debye equation	Scherrer equation	Bragg's equation
5. In Diffractometer, the identification of a component of the sample from its powder diffraction pattern is based upon the _____ of lines and their relative	Number, length	Number, intensity	Position, length	Position, intensity	Position, intensity

_____					
6. Diffractometers are similar to which of the following?	Optical grating spectrometer	Prism spectrometer	Photo multiplier	Photovoltaic cell	Optical grating spectrometer
7. Increasing the magnetic field?	a) produces less susceptibility artifacts	b) Reduces the risk of tissue heating	c) Increase the signal to noise	d) Reduces the danger from metallic projectiles	c) Increase the signal to noise
8. A major advantage of MRI is	a) the ease with which equipment is updated or replaced	its relatively low cost, compared to CT scans	c) dose not require specialized room	d) the ability to reposition the 'cross-section' through the body without repositioning the patient.	d) the ability to reposition the 'cross-section' through the body without repositioning the patient.
9. A growing application of MRI is "MRA", which stands for:	a) Magnetic Resonance Amplication	b) Magnetic Resonance Angiography	c) Minimal Radiology Applications	d) Medical Research Assistance	b) Magnetic Resonance Angiography
10. What does "MRI" stand for?	a) Magneto-Ray Idometry	b) Medical Radiometry Instrument	c) Magnetic Resonance Imaging	d) Maximal Radiology Imaging	c) Magnetic Resonance Imaging
11. What is a major health concern wth MRI?	a) Reaction to applied drugs	extrerme cold?	c) Radiation dose	d) localized burns due to metallic implants?	d) localized burns due to metallic implants?
12. Select one of the following objects that you think would alwasy be safe in the MRI suite.	a) A wheelchair	b) A stretcher	c) Scissors	d) None of the listed	d) None of the listed
13. Mass spectrometers are used to determine which of the following?	Composition in sample	Concentration of elements in sample	Relative mass of atoms	Properties of sample	Relative mass of atoms
14. Who invented mass spectrometers?	J.J Thompson	Goldstein	Nikola Tesla	Aston	J.J Thompson
15. In mass spectrometer, the sample that has to be analysed is bombarded with	Protons	Electrons	Neutrons	Alpha particles	Electrons

which of the following?					
16. Mass spectrometer separates ions on the basis of which of the following?	Mass	Charge	Molecular weight	Mass to charge ratio	Mass to charge ratio
17. In mass spectrometer, the ions are sorted out in which of the following ways?	By accelerating them through electric field	By accelerating them through magnetic field	By accelerating them through electric and magnetic field	By applying a high voltage	By accelerating them through electric and magnetic field
18. The procedure for mass spectroscopy starts with which of the following processes?	The sample is bombarded by electron beam	The ions are separated by passing them into electric and magnetic field	The sample is converted into gaseous state	The ions are detected	The sample is converted into gaseous state
19. In a mass spectrometer, the ion currents are measured using which of the following?	Scintillation counter	Ion counter	Electrometer tube	Electric fields	Electrometer tube
20. Which of the following ions pass through the slit and reach the collecting plate?	Negative ions of all masses	Positive ions of all masses	Negative ions of specific mass	Positive ions of specific mass	Positive ions of specific mass
21. Which of the following statements is not true about mass spectrometry?	Impurities of masses different from the one being analysed interferes	It has great sensitivity	It is suitable for data storage	It is suitable for library retrieval	Impurities of masses different from the one being analysed interferes
22. Light dependent stage can not be carried out without	a) Oxygen	b) carbon dioxide	c) water	d) all of these	c) water
23. Photolysis of six water molecules results in	a) 6 atoms of hydrogen	b) 12 atoms of hydrogen	c) 18 atoms of hydrogen	d) 24 atoms of hydrogen	b) 12 atoms of hydrogen
24. Photolysis is	a) light	b) light	c) dark stage	d) translocation	a) light

known to occur only in	dependent stage	independent stage			dependent stage
25. Which ion is kinetically inert?	a) Cr2+	b) Co3+	c) Co2+	d) Fe3+	Co3+
26. Which statement is correct?	a) A dissociative mechanism is a 2-step mechanism with the leaving group departing in the second step	b) An associative mechanism is a 2-step mechanism; the intermediate has a lower coordination number than the starting complex	c) In a dissociative interchange mechanism, bond breaking dominates over bond formation	d) In an associative interchange mechanism, the entering group associates with the substrate after the leaving group has departed	c) In a dissociative interchange mechanism, bond breaking dominates over bond formation
27. Which of the following cannot be obtained from an X-ray crystallography study?	a) A bond angle Si-O-Si in a mineral	b) The absolute configuration of a chiral natural product	c) The degree of folding of a Zn2Cl2 four-membered ring	d) The vibration frequency of a carbonyl group	d) The vibration frequency of a carbonyl group
28. What is meant by the 'phase problem' in X-ray crystallography?	a) The sample must be in the crystalline solid phase.	b) The phase of an X-ray wave changes when it is scattered by an atom	c) The relative phases of diffracted X-ray beams are lost when the diffraction pattern is recorded	d) Non-centrosymmetric crystal structures always give centrosymmetric diffraction patterns	c) The relative phases of diffracted X-ray beams are lost when the diffraction pattern is recorded
29. Which of the following statements about lattices and unit cells is correct?	a) Lattice points are chosen to lie on atoms.	b) Lattice points all have identical surroundings.	c) Lattices can be primitive or centred.	d) Unit cells are constructed by connecting adjacent lattice points to give the smallest possible repeat unit	b) Lattice points all have identical surroundings.
30. Consider the Bragg equation (1.3, page 20). If the value of the wavelength is	a) Bragg angles of reflections increase	b) The d spacings become smaller.	c) The diffraction pattern expands	d) Some previously accessible reflections can no longer be	d) Some previously accessible reflections can no longer be

doubled, which of the following is NOT true?				measured	measured
31. Calculation of Z (the number of molecules in the unit cell of a crystal structure) gives a value of 5. Which of the following cannot be an explanation of this unusual result?	a) An incorrect chemical formula for the compound	b) The presence of solvent of crystallization	c) An error in determining the unit cell parameters	d) An incorrect space group	d) An incorrect space group
32. The calculated value of Z is half what is expected for the probable space group of a crystal structure. On which of the following symmetry elements could the molecules lie to satisfy this result?	a) Inversion centres	b) Twofold screw axes	c) Glide plane	d) Fourfold rotation axes	a) Inversion centres
33. Calculation of Z from a crystal density measurement gives a value of 4.41 for an expected molecular mass of 451.1 daltons. What is the likely solvent of crystallization	a) Water H2O	b) Methanol CH3OH	c) Ethanol CH3CH2OH	d) Tetrahydrofuran C4H8O	c) Ethanol CH3CH2OH
34. The scattering contribution of one individual atom or ion to the total X-ray diffraction pattern of a crystal	a) The identity of the element	b) The oxidation state	c) The isotope	d) The position of the atom/ion in the unit cell	c) The isotope

structure depends on all but one of the following properties of the atom/ion. Which is the property that has no effect?					
35. For a centrosymmetric crystal structure, all diffracted X-ray beams have phases of either 0 or 180°; other phase values do not occur. What effect does this have on reverse Fourier transform calculations?	a) None	b) Half the reflections make no contribution	c) The different phase contributions can be ignored	d) The sine terms multiplied by the imaginary number i are all zero, so complex exponentials become cosines	d) The sine terms multiplied by the imaginary number i are all zero, so complex exponentials become cosines
36. Which of the following is not usually an advantage of synchrotron radiation compared with laboratory X-ray sources?	a) Intensity	b) Speed of data collection	c) Wavelength selection	d) Cost	d) Cost
37. What is the magnetic field outside a solenoid	Infinity	Half the value of the field inside	Double the value of the field inside	Zero	Zero
38. Which, among the following qualities, is not affected by the magnetic field	Moving charge	Change in magnetic flux	Current flowing in a conductor	Stationary charge	Stationary charge
39. When a charged particle moves at right angles to the magnetic field, the variable quantity is?	Momentum	Speed	Energy	Moment of inertia	Momentum
If the flow of electric current is	Zero	Infinity	Maximum	Half the original value	Zero

parallel to the magnetic field, the force will be					
41. The ratio of magnetic force to electric force on a charged particle getting undeflected in a field is?	1	0	5	3	1
42. What is the strength of magnetic field known as _____	Flux	Density	Magnetic strength	Magnetic flux density	Magnetic flux density
43. Weakest force in nature is?	Electric force	Gravitational force	Weak force	Magnetic force	Gravitational force
44. How can a magnetic field be produced?	Using a permanent magnet	Electric current	Using a temporary magnet	Using a permanent magnet or electric current	Using a permanent magnet or electric current
45. Can we see magnetic flux lines?	Yes	No	Depends on the strength of the field	Only when the field strength is very large	No
46. Magnetic Field lines move from _____	North to south	South to north	West to east	East to west	North to south
47. Which of the following have a non-crystalline structure?	Iron	Quartz	Silica glass	Tungsten	Silica glass
48. Which of the following have a non-crystalline structure?	Steel	Nickel	High density polythene	Low density polythene	Low density polythene
49. Which of the following is a characteristic of crystalline structure?	High density	Low density	Range of melting point	Varying structure	High density
50. Which of the following is characteristic of non-crystalline structures?	Long range of periodicity	Well defined structure and geometry	Low density	Sharp diffraction pattern	Low density
51. Which of the following factor is	Atomic packing has	Primary bonds are	Formation of 1-	Strong secondary bond	Strong secondary bond

not responsible for the formation of a non-crystalline structure?	open structure	absent	dimensional chain molecule		
52. A cubic unit cell satisfies which of the following equations	a=b=c, α=β=ϒ=90 degree	a≠b=c, α=β=ϒ=90 degree	a=b≠c, α=β=ϒ=90 degree	a=b=c, α≠β=ϒ=90 degree	a=b=c, α=β=ϒ=90 degree
53. A tetragon unit cell satisfies which of the following equations?	a=b=c, α=β=ϒ=90 degree	a≠b=c, α=β=ϒ=90 degree	a=b≠c, α=β=ϒ=90 degree	a=b=c, α≠β=ϒ=90 degree	a=b≠c, α=β=ϒ=90 degree
54. An Orthorhombic unit cell satisfies which of the following equations?	a=b=c, α=β=ϒ=90 degree	a≠b≠c, α=β=ϒ=90 degree	a=b≠c, α=β=ϒ=90 degree	a=b=c, α≠β=ϒ=90 degree	a≠b≠c, α=β=ϒ=90 degree
55. A Rhombohedra unit cell satisfies which of the following equations?	a=b=c, α=β=ϒ=90 degree	a≠b=c, α=β=ϒ=90 degree	a=b≠c, α=β=ϒ=90 degree	a=b=c, α=β=ϒ≠90 degree	a=b=c, α=β=ϒ≠90 degree
56. A Hexagonal unit cell satisfies which of the following equations?	a=b ≠ c, α=β=90 degree, ϒ=120 degree	a≠b=c, α=β=ϒ=90 degree	a=b≠c, α=β=ϒ=90 degree	a=b=c, α≠β=ϒ=90 degree	a=b ≠ c, α=β=90 degree, ϒ=120 degree
57. A Monoclinic unit cell satisfies which of the following equations?	a=b=c, α=β=90 degree ≠ ϒ	a≠b=c, α=β=ϒ=90 degree	a ≠ b≠c, α=β=90 degree ≠ ϒ	a=b=c, α≠β=ϒ=90 degree	a ≠ b≠c, α=β=90 degree ≠ ϒ
58. A Triclinic unit cell satisfies which of the following equations?	a=b=c, α=β=ϒ=90 degree	a≠b=c, α=β=ϒ=90 degree	a ≠ b ≠ c, α ≠ β ≠ ϒ ≠ 90 degree	a=b=c, α≠β=ϒ=90 degree	a ≠ b ≠ c, α ≠ β ≠ ϒ ≠ 90 degree
59. Which one of the following is most symmetrical?	Simple cubic cell	Hexagonal	Triclinic	Tetragonal	Simple cubic cell

60. Which one of the following is least symmetrical?	Tetragonal	Simple cubic	Monoclinic	Triclinic	Triclinic

UNIT-I

Physical Methods in Bioinorganic Chemistry: X-ray methods - magnetic resonance methods - mossbauer spectroscopy - magnetic measurements -other instrumental methods - atomic force microscopy - fast and time-resolved methods - stopped-flow kinetic methods - flash photolysis - time-resolved crystallography.

3.3 X-RAY CRYSTALLOGRAPHY

3.3.1 Introduction

X-ray crystallographic molecular structures of proteins have been available since the 1960s and 1970s when pioneering work by Kendrew[8] and Perutz[9] produced X-ray diffraction structures of myoglobin and hemoglobin. These oxygen carrying metalloproteins are discussed in Chapter 4. Since that time the introduction of sophisticated computer hardware and software has made the solution of protein structure in the solid state using X-ray crystallography more accurate and less time-consuming. The field continues to evolve as hardware and instrument design improvements are implemented and as crystallographers discover more powerful software algorithms for solving structures after the necessary data has been collected. At the time of this writing, 175+ X-ray crystallographic data sets were deposited in the Research Collaboratory for Structural Bioinformatics' Protein Data Bank (RCSB-PDB at http://www.rcsb.org/pdb/) for hemoglobin and hemoglobin mutants as well as 191+ data sets for myoglobin and myoglobin mutant species. Nuclear magnetic resonance protein structure determination in solution provides a complementary structural technique that does not require the production of single crystals necessary for X-ray diffraction studies. However, at this time, NMR solution structures are limited to smaller proteins of molecular weights less than 30,000. In contrast, X-ray crystallography can produce structures of proteins of up to 1×10^6 molecular weight. Recombinant DNA technology has aided the X-ray crystallographic study of proteins by allowing large amounts of a protein of interest to be produced through expression of its cloned gene in a microorganism. Site-directed mutagenesis of a selected protein's gene has allowed researchers to study three-dimensional structural changes brought about by amino acid replacement in the protein's primary amino acid sequence. These techniques are discussed in Sections 2.3.4 and 2.3.5. Much of the discussion in this section on X-ray crystallography has been taken from a recent text written by author and crystallographer

Jan Drenth.[10] Readers are referred to the Department of Crystallography site at Würzburg University (http://www.uni-wuerzburg.de/mineralogie/crystal/teaching/teaching.html) for tutorials on X-ray diffraction methodology. The site includes interactive tutorials describing basic examples, reciprocal space, the crystallographic phase problem, and diffuse scattering and defect structures. Tutorials on convolution theorem, modification of a structure, solving a simple structure, anomalous scattering, and powder diffraction are also found on this site.

If all the nuclei being detected in an NMR experiment (all protons in an organic ligand molecule, for instance) resonated at the same frequency, chemists would not be very interested because little information about structure, and so on, would be gained. However, when a magnetic field is applied during an NMR experiment, electrons surrounding nuclei in the molecules under study set up a secondary magnetic field. The secondary field opposes the main field, reducing the nuclear frequency. The magnitude of the frequency change is proportional to $\mathbf{B}_0$. This is important in that there will be larger separations between resonant frequencies at higher magnetic field strengths, allowing one to detect finer differences between the different protons in any liquid sample. The effect of electrons surrounding the nucleus on the nucleus in the applied magnetic field is termed screening (or shielding). Taking equation 3.30 and introducing the screening constant, σ, one finds equation 3.31:

$$\nu = \left(\frac{\gamma}{2\pi}\right)\mathbf{B}_0(1-\sigma) \qquad (3.31)$$

The screening constant, σ, is dimensionless and usually recorded in parts per million (ppm). Contributors to σ, opposite in sign, are σ_d (the diamagnetic term) and σ_p (the paramagnetic term). The diamagnetic term depends upon the density of circulating electrons. The paramagnetic effect in this context does *not* imply the presence of unpaired electrons (to be discussed below) but is substantial, and dominates, for heavier atoms with many electrons in outer orbitals involved in chemical bonding. Several factors affect σ_p:

1. The inverse of the energy separation, ΔE, between ground and excited electronic states of the molecule. This means that there will be a correlation between NMR spectra and absorption in the visible and ultraviolet spectral regions.
2. The relative electron density in p orbitals involved in bonding.
3. The value of $\langle 1/r^3 \rangle$, the average inverse cube distance from the nucleus to the electronic orbitals involved.

The paramagnetic screening constant becomes disproportionately larger for heavier elements; thus while ^{1}H, the proton, exhibits screening for its compounds within a range of 20 ppm, thallium (^{205}Tl) compound screening constants range over 5500 ppm. Changes in screening of each nucleus do not increase continuously with atomic number but are periodic, following the value of $\langle 1/r^3 \rangle$, increasing along each period and then falling markedly at the beginning of the next. Screening constants change in complex manners dependent upon a number of factors including charge density near the nucleus (^{14}N nucleus is 25 ppm more shielded in NH_3 than in NH_4^+), the influence of neighboring π systems, and oxidation states or coordination number of the nucleus being observed (^{31}P screening increases in the series $PCl_3 < PCl_4^+ < PCl_5 < PCl_6^-$). Usually, screening increases for substituted main group elements as the electronegativity of the substituent increases. The "normal" halogen effect, increased screening for the series $AlCl_4^- < AlBr_4^- < AlI_4^-$, is found to be a decreased screening effect for certain transition metals. The nephelauxetic effect (expansion of the electron cloud and increasing electron delocalization in ligand–metal bonding) changes the screening effect down the halogen group; thus while the difference between $AlCl_4^-$ and $AlBr_4^-$ is 22 ppm, that between $AlBr_4^-$ and AlI_4^- is 47 ppm.

Anisotropic magnets may be formed in chemical bonds within a molecule so that nuclei in the vicinity may be screened or descreened. Anisotropic behavior would be found in the vicinity of a carbonyl bond, for instance. The benzene ring exhibits ring current anisotropy, leading to large descreening (downfield shifts) of benzene protons. Molecules containing electric dipoles perturb molecular orbitals and therefore perturb the screening of a nuclei. The closer the nucleus is to the bond generating the electric field, the more they are descreened. In 1-chloropropane the descreening shifts, compared to CH_4, are α-CH_2 3.24 ppm, β-CH_2 1.58 ppm, and CH_3 0.83 ppm.

3.5.3 Spin–Spin Coupling

A nucleus under study by nuclear magnetic resonance techniques is affected by other nuclei in the same molecule. This phenomenon is known as *spin–spin coupling*. The effect arises (in adjacent nuclei) from the two electrons joining the nuclei in a covalent bond. Suppose the energy of states in which the electrons in the bond have opposing spins is lower than the state in which the electron spins are parallel. Then the ΔE between the two states (in this case a negative number) is called the coupling constant, J, expressed in frequency units, Hz. Internuclear

spin–spin coupling constants may be either positive or negative and depend on a number of factors:

1. The number and bond order of bonds intervening between the nuclei as well as the bond angles. Usually the interaction is observed only through one to four bonds, and the effect is attenuated (the J value becomes smaller) as the number of intervening bonds increases.
2. The magnetic moments of the two interacting nuclei. These are directly proportional to the product of the magnetogyric ratios $(\gamma_A\gamma_B)$ of the interacting nuclei.
3. The valence s electron density at the nucleus. This is affected by the s character of the bonding orbitals between the interacting nuclei.

Nuclei coupling to each other through spin–spin interactions may have very similar or very different chemical shifts. The difference or similarity will affect the appearance of the resonances associated with the coupled nuclei. Nuclei separated by small chemical shifts are denoted by the letters A, B, C while sets of nuclei separated by large chemical shifts are designated A, M, and X. The number of nuclei in each letter category is indicated by a subscript. Using the proton as an example, CH_3CH_2Cl (chloroethane) is an example of an A_3X_2 system while CH_2CHCl (vinyl chloride) is an example of an ABX system. When chemical shifts differences are large, coupling between protons on adjacent atoms will follow the simple $n + 1$ multiplicity rule for the number of peaks in a multiplet (the general rule is $2nI + 1$, where I is the nuclear spin). This is named a first-order pattern. The ABX system is almost first-order, but $A_aM_bX_x$ or $A_aB_bC_c$ systems exhibit complex spin-coupling multiplet patterns.

An example of spin–spin coupling between the ^{195}Pt nucleus ($I = 1/2$, abundance $= 33.8\%$) and the proton (^1H, $I = 1/2$, abundance $= 99.985\%$) is shown schematically in Figure 3.19 for the complex *trans*-$MeBrPt(PMe_2R)_2$ (where R is a 2,4-dimethoxyphenyl group).[19] The two major methyl proton resonances are indicated and are connected to the responsible peaks. The height of the central downfield methyl resonance indicates that it corresponds to the protons of four methyl groups attached to phosphorus, and thence to the magnetically inactive

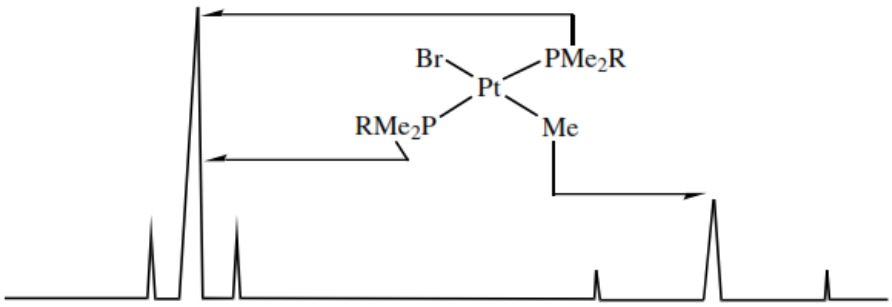


Figure 3.19 ^1H spectrum of the complex *trans*-$MeBrPt(PMe_2R)_2$. (Adapted with permission of Nelson Thornes Ltd. from Figure 3.13 of Akitt, J. W. *NMR and Chemistry*, 3rd ed., 1992.)

platinum nucleus. The two smaller satellite peaks at one-quarter intensity on either side of the major downfield peak originate from the same methyl protons coupled to the magnetically active ^{195}Pt nucleus. The longer coupling path from ^{195}Pt through ^{31}P to ^{1}H results in a weaker, smaller coupling constant (a so-called ^{3}J coupling) when compared to the upfield pattern for methyl protons of the methyl group directly attached to platinum. The 1 : 4 : 1 pattern for the upfield peak again indicates that the coupling corresponds to the 33.8% abundant platinum nucleus. The upfield resonance corresponds to the protons of the methyl group directly attached to the platinum atom, and thus the satellite peaks exhibit an appreciably stronger coupling and consequently a larger J value.

A more complete discussion of spin–spin coupling may be found in Chapter 3 of reference 19 and many instrumental chemistry texts.[2]

Mass bauer Spectroscopy

Mössbauer spectroscopy is a versatile technique used to study nuclear structure with the absorption and re-emission of gamma rays, part of the electromagnetic spectrum. The technique of Mössbauer spectroscopy is widely used in mineralogy to examine the valence state of iron, which is found in nature as Fe^{0}(metal), Fe^{2+}, and Fe^{3+}, as well as the type of coordination polyhedron occupied by iron atoms (trigonal, tetrahedral, octahedral, etc.). It is sometimes used to determine redox ratios in glasses and (less successfully) in rocks. Mössbauer spectroscopy is also used to assist in the identification of Fe oxide phases on the basis of their magnetic properties.

Fundamental Principles of Mössbauer Spectroscopy

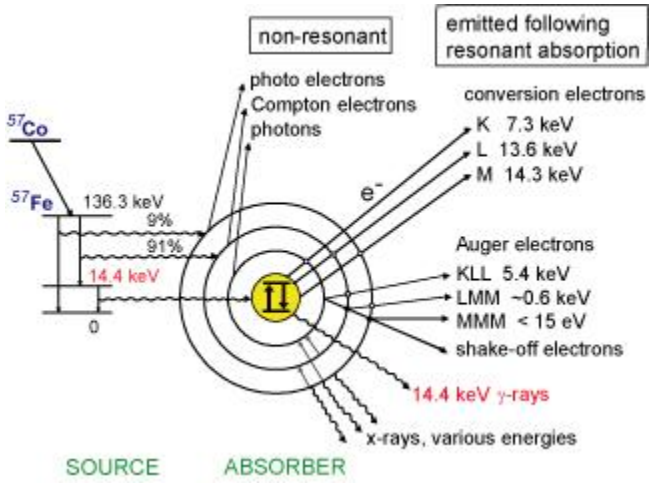


Figure 1. Details

The Mössbauer effect as generally applied to the study of minerals relies on the fact that ^{57}Fe, which is a decay product of ^{57}Co, is unstable. ^{57}Fe decays by giving off a gamma ray (γ-ray),

along with other types of energy. Figure 1 shows the nuclear decay scheme for $^{57}Co \rightarrow {}^{57}Fe$ and various backscattering processes for ^{57}Fe that can follow resonant absorption of an incident gamma photon, modified from DeGrave et al. (2005) and Dyar et al. (2006). If a nucleus gives off radiation or any other form of energy (in this case, in the form of a γ-ray), the nucleus must recoil (or move) with an equal and opposite momentum to preserve its energy (E), in the same way that a gun (by analogy, the nucleus) recoils when a bullet (the γ-ray) is fired out of it. We describe this general case in terms of energy by saying that:

$E_{\gamma\text{-rayemission}} = E_{transition} - E_R$,
where
$E_{\gamma\text{-rayemission}}$ =the energy of the emitted γ-ray
$E_{transition}$ =the energy of the nuclear transition
E_R = the energy of the recoil.

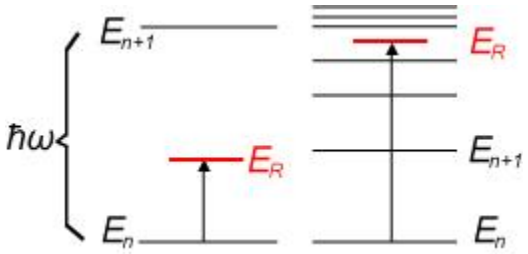


Figure 2. Details

Figure 2 shows a schematic of the vibrational energy levels in a solid. On the left, the recoil energy $E_R$of an emitted gamma photon is less than what is needed to reach the next higher energy level, so that excitation of a vibrational mode has low probability. The probability that no excitation will occur is given the symbol *f*, which represents the fraction of recoil-free events. A gamma ray would be emitted without losing energy to the solid, in what is called a zero-phonon transition. In other words, sometimes the nucleus absorbs the energy of the γ-ray and it doesn't recoil (instead, the entire structure, rather than just the nucleus, absorbs the energy). The variable *f* indicates the probability of this happening. This process of recoil-less emission forms the basis for Mössbauer spectroscopy. On the right, E_R is significantly greater in energy than the lowest excitation energy of the solid, which is E_{n+1}- E_n. Absorption of the recoil energy, E_R, by the solid thus becomes probable, and the photon emerges with energy reduced by E_R and with Doppler broadening. In the figure, ω represents frequency, and $\hbar$ is Planck's constant divided by 2π, and This figure is adapted from May (1971) and Dyar et al. (2006). The Mössbauer effect occurs because in solids, the value of *f* is high enough that recoil-free absorption is possible. Thus an atom of ^{57}Co can decay to ^{57}Fe, which gives off a γ-ray, and may be absorbed without recoil by a nearby ^{57}Fe, which happens to have just the right splitting between the energy levels in its nucleus to absorb it. This scenario will only happen if the decaying Co atom is surrounded by the same atoms as the absorbing Fe. If the receiving Fe atoms are in a different matrix (say, in a mineral) than in the emitter, then no absorption can occur.

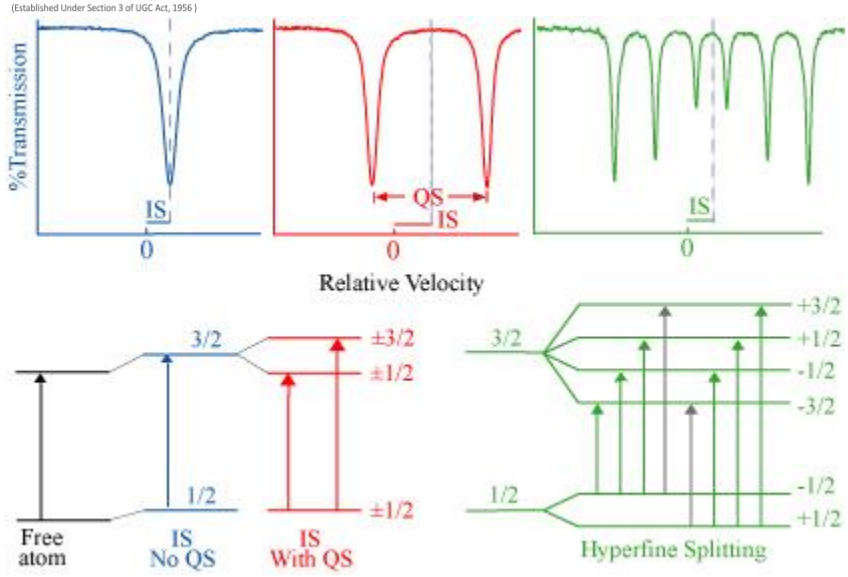


Figure 3. Details

When source and absorber atoms are in different local environments, their nuclear energy levels are different (Figure 3). At its simplest (blue), this appears in the transmission spectrum as a shift of the minimum away from zero velocity; this shift is generally called isomer shift (IS). The 1/2 and 3/2 labels represent the nuclear spin, or intrinsic angular moment, quantum numbers, I. Interaction of the nuclear quadrupole moment with the electric field gradient leads to splitting of the nuclear energy levels (red). For ^{57}Fe, this causes individual peaks in the transmission spectrum to split into doublets (red) having a quadrupole splitting of QS. When a magnetic field is present at the nucleus, Zeeman splitting takes place, yielding a sextet pattern (green); in the simplest case, the areas of the lines vary in the ratio of 3:2:1:1:2:3. For the spectrum shown, the outer lines have reduced intensity because of saturation effects. Two additional possible transitions shown in gray at lower right (m_I = -1/2 to +3/2 and m_I = +1/2 to -3/2) do not occur due to the selection rule, $|\Delta m_I| \leq 1$. Note that the lengths of the transition arrows have been greatly shortened to allow the splittings to be seen clearly. This figure is adapted from Dyar et al. (2006). So Mössbauer spectra are described using three parameters: isomer shift (δ), which arises from the difference in *s* electron density between the source and the absorber, quadrupole splitting (Δ which is a shift in nuclear energy levels that is induced by an electric field gradient caused by nearby electrons, and hyperfine splitting (for magnetic materials only). Graphically, quadrupole splitting is the separation between the two component peaks of a doublet, and isomer shift is the difference between the midpoint of the doublet and zero on the velocity scale (Figure 3). Mössbauer parameters are temperature-sensitive, and this characteristic is sometimes exploited by using lower temperatures to improve peak resolution and induce interesting magnetic phenomena.

If the electrons around the Fe atom create a magnetic field, as in the case of magnetite, then the energy levels in the Fe nucleus will split to allow six possible nuclear transitions, and a sextet (six-peak) spectrum results. The positions of the peaks in the sextet defines what is called the hyperfine splitting (Hint or BHf, depending on the units used) of the nuclear energy levels.

Iron atoms in different local environments and those having different oxidation states absorb at different, diagnostic energies. A typical Mössbauer spectrum thus consists of sets of peaks (usually doublets and sextets), with each set corresponding to an iron nucleus in a specific environment in the sample (an Fe nuclear site). Different sets of peaks appear depending on what the Fe nucleus "sees" in its environment. The nuclear environment depends on a number of factors including the number of electrons (Fe^0, Fe^{2+}, Fe^{3+}), the number of coordinating anions, the symmetry of the site, and the presence/absence of magnetic ordering (which may be temperature-dependent). Thus the spectrum of a given mineral may consist of a superposition of doublets and sextets.

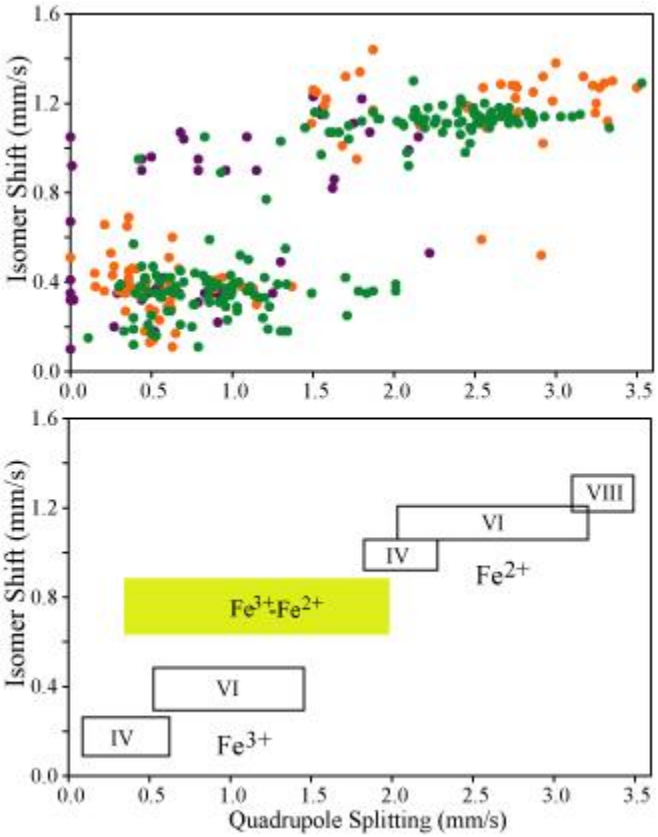


Figure 4. Details

The combination of isomer shift and quadrupole splitting parameters (along with the hyperfine field, in the case of magnetically ordered phases) is usually sufficient to identify the valence state and site occupancy of Fe in a given site and individual mineral (Figure 4). In minerals, these ranges have largely been determined empirically from Mössbauer spectra measured with use of spectrum-fitting routines commonly available to the geological community. Exact values of Mössbauer parameters are difficult to predict from theory because long-range interactions in complicated mineral structures are difficult to anticipate.

As seen in Figure 4, Fe atoms in minerals are predictably found in coordination polyhedra of

appropriate size based on radius ratios. The top half of Figure 4 plots the isomer shift and quadrupole splitting of several minerals whose iron valence state and coordination number are independently known (usually from single crystal X-ray diffraction), and the bottom of the figure shows the resultant groupings. Fe^{3+} occurs primarily in 4- or 6-coordination with oxygen, while Fe^{2+} may be rarely 4- or 5- coordinated, commonly 6-coordinated, and occasionally 8-coordinated with oxygen. Fe in 4-fold coordination with sulfur has subtly different parameters due to the effects of covalent bonding. Variations in Mössbauer parameters that are characteristic of each type of coordination polyhedron can be related to polyhedral site distortion; a thoughtful discussion of this topic can be found in Burns & Solberg (1988).

Mössbauer Spectroscopy Instrumentation - How Does It Work?

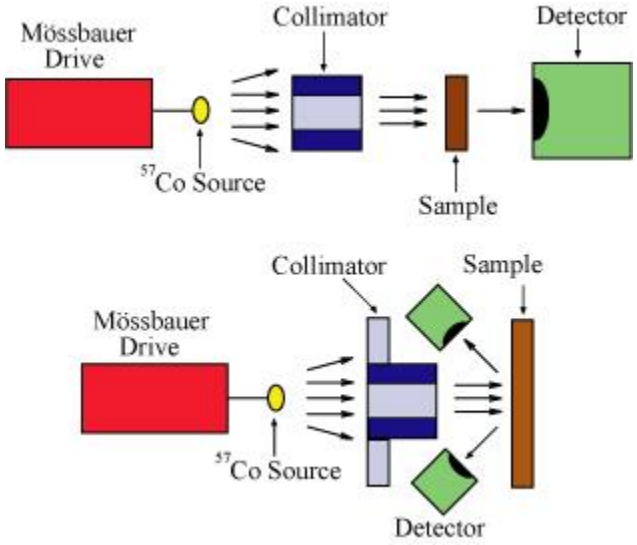


Figure 5. Details

The basic elements of a Mössbauer spectrometer are a source, sample, detector, and a drive to move the source or absorber. Most commonly, this is done by moving the source toward and away from the sample, while varying velocity linearly with time. For example, for ^{57}Fe, moving the source at a velocity of 1 mm/sec toward the sample increases the energy of the emitted photons by about ten natural linewidths. For simplicity, "mm/sec" is the conventional "energy" unit in Mössbauer spectroscopy. It is also possible to leave the source stationary and oscillate the sample, as is done with synchrotron Mössbauer. The location of the detector relative to the source and the sample defines the geometry of the experiment (Figure 5); most commonly, either transmission or backscatter modes are used.

Applications

The combination of isomer shift and quadrupole splitting (along with the hyperfine field, in the case of magnetic phases) is used to identify the valence state and site occupancy of Fe in a given site and individual mineral (Figure 4). If the phase is magnetically ordered, additional information

in the form of a value for the magnetic field (usually given in Teslas) can help with identification of some phases.

In some cases, Mössbauer spectrometers are also used to identify minerals. This application is limited, however, by the fact that many different minerals can have site geometries that are the same, such that their Mössbauer spectra and the resultant peak parameters will also be the same. For example, the spectra of amphibole and pyroxene group minerals are all very similar, so you could not tell these minerals apart by their Mössbauer spectra alone!

3.7 OTHER INSTRUMENTAL METHODS

3.7.1 Atomic Force Microscopy

Atomic force microscopy (AFM) is part of a range of emerging microscopic methods for chemists and biologists that offer the magnification range of both the light and electron microscope, but allow imaging under the "natural" conditions usually associated with the light microscope. AFM offers the prospect of high-resolution images of biological material, images of molecules and their interactions even under physiological conditions, and the study of molecular processes in living systems. Applications of AFM in the biosciences include analysis of (1) DNA and RNA, (2) protein–nucleic acid complexes, (3) chromosomes, (4) cellular membranes, (5) proteins and peptides, (6) molecular crystals, (7) biopolymers and biomaterials, and (8) ligand–receptor binding.

The atomic force microscope is one of about two dozen types of scanned-proximity probe microscopes. All of these microscopes work by measuring a local property—height, optical absorption, or magnetism—with a probe or "tip," typically made from Si_3N_4 or Si, placed very close to the sample. The small probe–sample separation (on the order of the instrument's resolution) makes it possible to take measurements over a small area. To acquire an image, the microscope raster-scans the probe over the sample while measuring the local property in question. The resulting image resembles an image on a television screen in that both consist of many rows or lines of information placed one above the other. Unlike traditional microscopes, scanned-probe systems do not use lenses, so the size of the probe rather than diffraction effects generally limit their resolution.

The concept of resolution in AFM is different from radiation-based microscopies because AFM imaging is a three-dimensional imaging technique. There is an important distinction between images resolved by wave optics and those resolved by scanning probe techniques. The former is limited by diffraction, whereas the latter is limited primarily by apical probe geometry and sample geometry. Usually the width of a DNA molecule is loosely used as a measure of resolution, because it has a known diameter of 2.0 nm in its B form.

Many biological processes—DNA replication, protein synthesis, drug interactions, and others—are largely governed by intermolecular forces. AFM has the ability to measure these forces, some of which may be in the nanonewton range. This makes it possible to quantify molecular interactions in biological systems such as important ligand–receptor interactions. The dynamics of many biological systems depends on the electrical properties of the sample surface, and AFM is able to image and quantify electrical surface charges. In addition to measuring binding and electrostatic forces, the atomic force microscope can also probe the micromechanical properties of biological samples. Specifically, the AFM can observe the elasticity and, in fact, the viscosity of samples ranging from live cells and membranes to bone and cartilage.

One area of significant progress for AFM has beam the imaging of nucleic acids. The ability to generate nanometer-resolved images of unmodified nucleic acids has broad biological applications. Chromosome mapping, transcription, translation, and small-molecule–DNA interactions such as intercalating mutagens provide exciting topics for high-resolution studies. The first highly reproducible AFM images of DNA were obtained only in 1991. Four major advances that have enabled clear resolution of nucleic acids are (1) control of the local imaging environment including sample modification, (2) TappingMode^{TM} scanning techniques, (3) improved AFM probes (such as standard silicon nitride probes modified by electron beam deposition and oxide-sharpened nanoprobes), and (4) compatible substrates (such as salinized mica and carbon coated mica).

There has been recent success in imaging individual proteins and other small molecules with the AFM. Smaller molecules that do not have a high affinity for common AFM substrates have been successfully imaged by employing selective affinity binding procedures. Thiol incorporation at both the 5′ and 3′ ends of short PCR (polymerase chain reaction, described in Section 2.3.5) products has been shown to confer a high affinity for ultraflat gold substrates and therefore improved AFM imaging.

It is informative to compare AFM with other techniques. The scanning tunneling microscope (STM) is considered the predecessor technique to AFM. The STM may have better resolution than the AFM but can only be applied to conducting samples while AFM can be applied to both conductors and insulators. Compared with the scanning electron microscope (SEM), the AFM provides extraordinary topographic contrast, direct height measurements, and unobscured views of surface features (no coating is necessary). Compared with transmission electron microscopes, three-dimensional AFM images are obtained without expensive sample preparation and yield far more complete information than the two-dimensional profiles available from cross-sectioned samples. New approaches in AFM have provided a solid foundation from which research is expanding into more complex analyses. Higher-resolution imaging of a variety of small molecules is improving at a rapid pace.

3.7.2 Fast and Time-Resolved Methods

3.7.2.1 Stopped-Flow Kinetic Methods. Enzyme kinetics happen on very fast time scales; for instance, it is known that the rate of reaction for copper–zinc superoxide dismutase (CuZnSOD), $\sim 1 \times 10^9\,M^{-1}\,s^{-1}$, approaches the diffusion-controlled rate. Chemists use various methods to study fast reactions. One of the most frequently used rapid kinetic techniques is that of stopped-flow in which the reactants (enzyme and substrate) are rapidly mixed. The lower practical limit for mixing to take place is about 0.2 ms. The stopped-flow principle of operation allows small volumes of solutions to be driven from high-performance syringes to a high-efficiency mixer just before passing into a measurement flow cell. As the solutions flow through, a steady-state equilibrium is established and the resultant solution is only a few ms old as it passes through the cell. The mixed solution then passes into a stopping syringe, which then allows the flow to be instantaneously stopped. Some of the resultant solution will be trapped in the flow cell and as the reaction proceeds, the kinetics can be followed using the appropriate measurement technique. The most common method of following the kinetics is by absorbance or fluorescence spectrometry, and in these cases the measurement cell is an appropriate spectrometer flow cell. Many commercially available absorbance and fluorescence spectrometers may be modified to accept stopped-flow accessories.

In order to use the stopped-flow technique, the reaction under study must have a convenient absorbance or fluorescence that can be measured spectrophotometrically. Another method, called rapid quench or quench-flow, operates for enzymatic systems having no component (reactant or product) that can be spectrally monitored in real time. The quench-flow is a very finely tuned, computer-controlled machine that is designed to mix enzyme and reactants very rapidly to start the enzymatic reaction, and then quench it after a defined time. The time course of the reaction can then be analyzed by electrophoretic methods. The reaction time currently ranges from about 5 ms to several seconds.

3.7.2.2 Flash Photolysis. Time-resolved spectroscopy techniques are a powerful means of studying materials, giving information about the nature of the excitations, energy transfer, molecular motion, and molecular environment, information that is not available from steady-state measurements. Flash photolysis is a rapidly advancing field with applications in many areas of science and technology. The technique allows one to follow a reaction using fast (nanosecond to microsecond) laser excitation pulses to cause absorption in the species of interest. Following the excitation, one must use fast electronic devices to measure the light emission of absorption by the species of interest. For instance, one laboratory uses a Yag laser (266-, 355-, and 532-nm excitations) or excimer (308-nm excitation) sources with transmission (10-ns resolution) or diffuse reflectance (200-ns resolution) detection. A necessary criteria for the use of flash photolysis methods is that the molecule under study must show a detectable change upon laser excitation.

One research group used a flash-photometric method to show photochemical NO displacement by CO in myoglobin.[32] Previous investigations of thermal and photochemical NO displacement by CO suggest that the local heme pocket around the ligand, although significantly altered (according to circular dichroism investigations), imposed a barrier against the outward diffusion of ligand (NO or CO) into the solvent. (Find a complete discussion of ligand attachments to hemes in myoglobin and hemoglobin in Sections 4.2, 4.3, and 4.9.) The researchers found in this case that nanosecond and picosecond flash photolysis in proteins at low pH showed an extremely efficient geminate recombination of the ligand—that is, reattachment of the ligand before its leaving the heme pocket. The process involved a four-coordinated species within the heme and took place through a single-exponential process. This occurred to a significantly larger extent for the case of NO-"chelated" protoheme (where no distal barrier for ligand is present) than for CO ligated under the same circumstances. At neutral pH, when the proximal histidine–Fe bond is intact, the geminate recombination for NO takes longer and displays multiexponential kinetics. Altogether, these results suggested that even though heme distal ligand and protein environment effects play a role in NO or CO ligation and deligation from the iron heme center, proximal ligand and protein environment effects make an important contribution in modulating ligand–iron bond formation in hemes.

3.7.2.3 Time-Resolved Crystallography. Time-resolved crystallography (TC) uses an intense synchrotron X-ray source and Laue data collection techniques to greatly reduce crystallographic exposure times. Normal time resolution for X-ray crystallography has been in the range of seconds or tens of seconds. TC has the potential to take snapshots of protein structural changes on a nanosecond time scale. Consequently, multiple exposures may be taken that capture the evolution of the crystallographic unit cell as it reacts over time. Traditionally, crystallographers have applied several techniques to obtain detailed structural information on reaction intermediates. The most common approach has been to design a series of stable structures that mimic normally short-lived intermediates. However, these structures

are stable precisely because they are not identical to the intermediates they seek to mimic, and key interactions are usually missing. Other experimental techniques and chemical intuition are called upon to supply the missing information, sometimes with only limited success. One successful attempt to understand how the attachment and release of carbon monoxide, and ultimately dioxygen, happens on a molecular scale is described in Section 4.9. In this case, Rodgers and Spiro studied the

and release of carbon monoxide, and ultimately dioxygen, happens on a molecular scale is described in Section 4.9. In this case, Rodgers and Spiro studied the nanosecond dynamics of the R to T transition in hemoglobin.[33] Using pulse-probe Raman spectroscopy, with probe excitation at 230 nm, these workers were able to model the R–T interconversion of the hemoglobin molecule as it moved from the R state (HbCO) to the T state (Hb).

Time-resolved crystallography, TC, now has the potential to offer detailed structural information on short-lived intermediates in macromolecular reactions under near-physiological, crystalline conditions, and this aids elucidation of the underlying molecular mechanisms. Interpretation of TC data has been hindered, in part due to the difficulty in extracting structural information on intermediates from time-resolved electron density maps. Under certain assumptions, these maps are weighted averages of the electron density maps of the different structural species present at the experimental time points. That is, these time-dependent electron density maps are structurally heterogeneous. Various researchers, most notably Krebs and Moffat, have proposed techniques for interpreting these maps.

In their 1996 *Science* article "Photolysis of the Carbon Monoxide Complex of Myoglobin: Nanosecond Time-Resolved Crystallography,"[34] Moffat, Wulff, and co-workers described the nanosecond time resolution of structural changes that occur in the carbon monoxide complex of myoglobin (MbCO) at room temperature on CO photodissociation by a nanosecond laser pulse. The Fe–CO bond was broken with a 10-ns laser pulse, and X-ray data sets were collected at different time delays between the laser flash and the X-ray pulse (4 ns, 1 μs, 7.5 μs, 50 μs, and 1.9 ms). Although the difference maps clearly showed release of the CO molecule from the heme, they also suggested that CO recombination in the crystal form contains a fast, geminate phase with a recombination rate comparable with or greater than the maximum photolysis rate applied by the laser pulse of $10^9\ s^{-1}$. This result confirmed that it is much more difficult to photolyze MbCO molecules in the crystal form than in solution. A second prominent feature of the X-ray difference maps arose from the motion of the iron atom out of the heme plane and toward the proximal histidine. A third feature indicated a transient "docking site" for the photodissociated CO; however, well-populated docking sites indicating CO exit from the binding pocket were not identified. A number of small electron density features indicated structural rearrangements of aa residues surrounding the heme, especially the residues of the E and F helices implicated by other methods in heme and protein relaxation effects, and in iron ion displacement in or out of the heme plane. Their data suggested that complete iron displacement and heme relaxation occurred in <4 ns, in agreement with other spectroscopic results.

Number of hydrogen bonds between adenine and thymine?	1	2	3	4	2
Number of hydrogen bonds between guanine and cytosine?	1	2	3	4	3
Which ratio is constant for DNA?	A + G / T + C	A + T / G + C	A + C / U + G	A + U / G + C	A + G / T + C
. According to Chargaff's rule, in a DNA molecule	The amount of adenine and thymine is equal to the amount of guanine and cytosine	The amount of adenine and guanine is equal to the amount of thymine and cytosine	The amount of adenine and uracil is equal to the amount of guanine and cytosine	The amount of adenine and guanine is equal to the amount of uracil and cytosine	The amount of adenine and thymine is equal to the amount of guanine and cytosine
Arrangement of nucleotides in DNA can be seen by	Ultracentrifuge	X-Ray crystallography	Light microscope	Electron microscope	X-Ray crystallography
Which of the following leads to disruption of nucleosomal structure?	Acetylation	Carboxylation	Phosphorylation	Methylation	Methylation
One of the following nucleic acids has a left handed helix	M-RNA	T-RNA	A-DNA	Z-DNA	Z-DNA
Which of the following statements is not true about RNA?	Does not have a double stranded structure	Thymine is present	Does not obey Chargaff's rule	The sugar contained in RNA is a ribose	Thymine is present
Which of the following is true about Z-DNA helix?	It has alternating GC sequences	It is a permanent conformation of DNA	It tends to be found at the 3' end of the genes	It has fewer base pairs per turn than B-DNA	It has alternating GC sequences
Which of the following statements is true?	The template strand matches the sequence of	The two strands of DNA run parallel to each other	G-C bonds are much more resistant to denaturation	The common form of DNA is left handed	G-C bonds are much more resistant to denaturation

	the RNA transcript		than A-T rich regions		than A-T rich regions
Identify the purine base of nucleic acids in the following	Cytosine	Thymine	Uracil	Adenine	Adenine
Which of the following are not the components of RNA?	Thymine	Adenine	Guanine	Cytosine	Thymine
Which of the following statements is true?	Sugar component of a nucleotide is ribose	Sugar component of a nucleotide is deoxyribose	The bases in nucleotides are attached to a pentose sugar moiety by a glycosidic linkage	The sugar molecule of the nucleotide is in L-configuration	The bases in nucleotides are attached to a pentose sugar moiety by a glycosidic linkage
What is the composition of nucleoside?	a sugar + a phosphate	a base + a sugar	a base + a phosphate	a base + a sugar + phosphate	a base + a sugar
What is the composition of nucleotide?	a sugar + a phosphate	a base + a sugar	a base + a phosphate	a base + a sugar + phosphate	a base + a sugar + phosphate
Group of adjacent nucleotides are joined by	Phosphodiester bond	Peptide bond	Ionic bond	Covalent bond	Phosphodiester bond
The sugar molecule in a nucleotide is	Pentose	Hexose	Tetrose	Triose	Pentose
Which of the following is true about phosphodiester linkage?	5'-phosphate group of one nucleotide unit is joined to the 3'-hydroxyl group of the next nucleotide	3'-phosphate group of one nucleotide unit is joined to the 5'-hydroxyl group of the next nucleotide	5'-phosphate group of one nucleotide unit is joined to the 5'-hydroxyl group of the next nucleotide	3'-phosphate group of one nucleotide unit is joined to the 3'-hydroxyl group of the next nucleotide	5'-phosphate group of one nucleotide unit is joined to the 3'-hydroxyl group of the next nucleotide
Which of the following is false about purine and pyrimidine bases?	They are hydrophobic and relatively insoluble in water at the near-neutral pH of the cell	At acidic or alkaline pH the bases become charged and their solubility in water increases	Purines have two rings in their structure, but pyrimidine bases have only one ring	At acidic or alkaline pH the bases become charged and their solubility in water	At acidic or alkaline pH the bases become charged and their solubility in water

				decreases	decreases
Building blocks of nucleic acids are	Nucleotides	Nucleosides	Amino acids	Histones	Nucleotides
Which organic molecule below is most closely related to lipids?	nucleotides	amino acids	CH2 chains ____		CH2 chains ____
Which organic molecule below is most closely related to nucleic acids?	nucleotides	sugars	CH2 chains		nucleotides
Nucleic acids include	a. glucose	glycogen	DNA and RNA	lipids and sugars.	DNA and RNA
A model of enzyme action is the	active site model	activator action model	induced fit model		induced fit model
Without enzymes, the chemical reactions in the body would	occur too slowly to support life processes	require a different pH	. occur at much the same rate as they do with enzymes		occur too slowly to support life processes
Enzymes that break down DNA catalyze the hydrolysis of the covalent bonds that join nucleotides together. What would happen to DNA molecules treated with these enzymes?	The phosphodiester bonds between deoxyribose sugars would be broken	The two strands of the double helix would separate	The pyrimidines would be separated from the deoxyribose sugars.		The phosphodiester bonds between deoxyribose sugars would be broken
Choose the pair of terms that correctly completes this sentence Catabolism is to anabolism as ____ is to ____.	exergonic; endergonic	work; energy	free energy; entropy		exergonic; endergonic

If an enzyme solution is saturated with substrate, the most effective way to obtain an even faster yield of products is to	add more of the enzyme	heat the solution to 90°C	add an allosteric inhibitor	add more of the enzyme
If an enzyme is added to a solution where its substrates and products are in equilibrium, what would occur	The reaction would change from endergonic to exergonic	Additional product would be formed	Nothing; the reaction would stay at equilibrium	Nothing; the reaction would stay at equilibrium
Which of these is a difference between DNA and RNA?	DNA contains thymine; RNA contains uracil.	. In DNA, adenine pairs with guanine; in RNA, adenine pairs with thymine	DNA consists of five different nucleotides; RNA consists of four different nucleotides	DNA contains thymine; RNA contains uracil.
What name is given to the reactants in an enzymatically catalyzed reaction	products	substrate	EA	substrate
Which of the following are nitrogenous bases of the pyrimidine type	thymine and guanine	cytosine and uracil	guanine and adenine	cytosine and uracil
Which of the following are nitrogenous bases of the purine type	guanine and adenine	uracil and cytosine	cytosine and guanine	guanine and adenine
A double-stranded DNA molecule contains a total of 120 purines and 120 pyrimidines.	240 adenine and 240 cytosine molecules	. 120 thymine and 120 adenine molecules	240 guanine and 240 thymine molecules	120 thymine and 120 adenine molecules

This DNA molecule could be comprised of					
. In the double helix structure of nucleic acids, cytosine hydrogen bonds to	ribose.	guanine	adenine		guanine
. The structural feature that allows DNA to replicate is the	twisting of the molecule to form an á helix	sugar-phosphate backbone	complementary pairing of the nitrogenous bases		complementary pairing of the nitrogenous bases
Which of the following describe(s) some aspect of metabolism?	synthesis of macromolecules	breakdown of macromolecules	control of enzyme activity	all the above	all the above
Which term most precisely describes the cellular process of breaking down large molecules into smaller ones?	catalysis	catabolism	anabolism		catabolism
. According to the first law of thermodynamics	the universe loses energy because of heat production	systems rich in energy are intrinsically unstable and will give up energy with time	energy can be neither created nor destroyed	A and B only	energy can be neither created nor destroyed
. How can one increase the rate of a chemical reaction?	Add a catalyst	Increase the entropy of the reactants	Decrease the concentration of the reactants		Add a catalyst
Which structural feature is shared by both uracil and thymine	Both contain two keto groups	Both contain one methyl group	Both contain a five-membered ring		Both contain two keto groups
Which	Both contain a	Both contain a	Both contain a pyrimidine		Both contain a

component is found in both adenosine and deoxycytidine	pyranose	1,1’-N-glycosidic bond			3’-OH group
Which property is shared by both GDP and AMP?	Both contain the same charge at neutral pH	Both contain the same number of phosphate groups.	Both contain the same purine.		Both contain the same furanose.
Which characteristic is shared by purines and pyrimidines?	Both contain two heterocyclic rings with aromatic character	Both can form multiple non-covalent hydrogen bonds.	Both exist in planar configurations with a hemiacetal linkage.	Both exist as neutral zwitterions under cellular conditions	Both can form multiple non-covalent hydrogen bonds.
Which property is found in nucleosides and nucleotides?	Both contain a nitrogenous base, a pentose, and at least one phosphate	Both contain a covalent phosphodister bond that is broken in strong acid	Both contain an anomeric carbon atom that is part of a β-N-glycosidic	Both contain an aldose with hydroxyl groups that can tautomerize	Both contain an anomeric carbon atom that is part of a β-N-glycosidic
Which characteristic is shared by both adenine and cytosine?	Both contain one methyl group	Both are anomeric	Both contain one keto group	Both are heterocyclic	Both are heterocyclic
Which component is found in both guanosine and uridine	Both contain an aldohexose	Both contain three hydroxyl groups	Both contain a 1’,9 - bond	Both contain a pyranose	Both contain three hydroxyl groups.
Which property is shared by both CTP and dTDP?	Both contain the same sugar.	Both contain the same charge at cellular pH	Both contain a planar six-membered ring	Both contain phosphodiester bonds	Both contain a planar six-membered ring
Which characteristic is found in both purines and pyrimidines?	They both have aromatic rings that undergo substantial tautomerization at nuetral pH	They both are weak bases that can be positively charged at neutral pH	They both have multiple pKa values that result in zwitterion form	They both can form stable N-glycosidic bonds with β D-ribofuranose.	They both can form stable N-glycosidic bonds with β D-ribofuranose.
Which is a general property of both nucleosides	Both contain a pentose in the form of a furanose	Both contain at least one 5’-phosphate group	Both contain a nitrogenous base that forms covalent H-bonds	Both contain a hemiacetal or hemiketal bond	Both contain a pentose in the form of a furanose

and nucleotides?					
Which structural feature is found in the single-stranded DNA molecule?	It can have a negatively-charged backbone composed of nitrogenous bases	Each 3',5'-phosphodiester bond will contain one phosphate group linking two deoxyribose sugars	It can have one end with a 5'-phosphate group while the other end has a 2'-hydroxyl group	Each purine and pyrimidine will be paired with a complementary base	Each 3',5'-phosphodiester bond will contain one phosphate group linking two deoxyribose sugars
Which is a possible sequence and structure for this DNA molecule?	If the single-stranded molecule has the sequence 5'-(ATGC)10, then it double-stranded form could assume a Z-DNA structure	If the single-stranded molecule has the sequence 5'-(GATC)10, then its double-stranded form could assume an H-DNA structure	If the single-stranded molecule has the sequence 5'-(CTGA)10, then its double-stranded form could assume a hairpin structure	If the single-stranded molecule has the sequence 5'-(TGAC)10, then its double-stranded form could assume a cruciform structure	If the single-stranded molecule has the sequence 5'-(ATGC)10, then its double-stranded form could assume a Z-DNA structure.
Which characteristic does this double-stranded molecule have when it forms a B- DNA structure?	The two strands will have parallel orientation and identical sequences.	The helix will be right-handed with 12 base-pairs per turn	Every base-pair will contain one purine and one pyrimidine	There are both covalent and non-covalent bonds between the two chains	Every base-pair will contain one purine and one pyrimidine
Which of the following double-stranded DNA molecules would denature at a lower temperature than the 40 base-pair double-stranded molecule described	a 40 base-pair molecule in which 25% of the bases are adenines	a 30 base-pair molecule in which 40% of the bases are guanines	a 20 base-pair molecule in which 10% of the bases are thymines	a 10 base-pair molecule in which 20 % of the bases are cytosines	a 10 base-pair molecule in which 20 % of the bases are cytosines
Which	Both will have	Both will	Both will be	Both will	Both will have

characteristic will this double-stranded DNA molecule share with a double-stranded RNA molecule of the same size?	secondary structure	contain inverted repeats	degraded by base	contain four types of base-pairs	secondary structure
Which will be a characteristic of this one single strand?	The single-stranded chain will contain both ribose and deoxyribose	The single-stranded chain will contain both purines and pyrimidines	The single-stranded chain will contain one 5’-end and one 3’-end		The single-stranded chain will contain one 5’-end and one 3’-end
Which of the following double-stranded DNA molecules would denature at aboutthe same temperature as the double-stranded molecule containing a 5’-(GA)20-3’ strand	a molecule which contains a (GC)20 strand	a molecule which contains a (TA)20 strand	a molecule which contains a (GACT)10 strand	a molecule which contains a (GGGA)10 strand	a molecule which contains a (GACT)10 strand
Which characteristic will be shared when comparing the single 5’-(GA)20-3’ strand to another single-stranded DNA molecule with the sequence 5’-(AT)20-3’?	Both contain a palindromic sequence.	Both can form the same secondary structures	Both could hybridize to the same RNA molecule	Both will have the same overall charge	Both will have the same overall charge
A new virus, virus X, is isolated and studied. Which molecule is	a linear DNA molecule containing 10,000 base-pairs	a linear RNA molecule containing plasmids	a circular DNA molecule containing nucleosomes	a circular RNA molecule with a molecular weight of 10 billion	a linear DNA molecule containing 10,000 base-pairs

most likely to					
Which are characteristics of bacterial genetic material?	It is double-stranded and supercoiled.	It is single-stranded and relaxed	It is circular and compacted into palindromes	It is linear and attached to a protein scaffold	It is double-stranded and supercoiled.

UNIT-I

Binding of Metal Ions and Complexes to Biomolecules: Nucleic acid structures - fundamental interactions with nucleic acids - binding interactions of tris(phenanthroline) metal complexes with DNA - techniques to monitor binding - applications of metal complexes that bind to nucleic acids -biopolymer promoted metal ligand interactions.

A. Nucleic-Acid Structures [1]

Figure 8.1 displays a single deoxyribonucleotide and the four different nucleic-acid bases. As may be evident, each mononucleotide along a nucleic-acid polymer contains a variety of sites for interactions with metal ions, from electrostatic interactions with the anionic phosphate backbone to soft nucleophilic interactions with the purine heterocycles. The different nucleic-acid bases furthermore offer a range of steric and electronic factors to exploit. Coordination of a metal complex to the N7 nitrogen atom of a purine, for example, would position other coordinated ligands on the metal center for close hydrogen bonding to the O6 oxygen atom of guanine, but would lead to clashes with the amine hydrogen atoms of adenine.

The monomeric units strung together in a polynucleotide furthermore provide an array of polymeric conformers. Figure 8.2A *(See color plate section, pages C-14, C-15.)* shows three crystallographically characterized structures of double-helical DNA oligonucleotides,[2–4] Figure 8.2B a schematic illustration of other conformations of DNA, and Figure 8.2C the crystal structure [5] of yeast $tRNA^{Phe}$. In double-helical DNA,[1] the two antiparallel polynucleotide strands

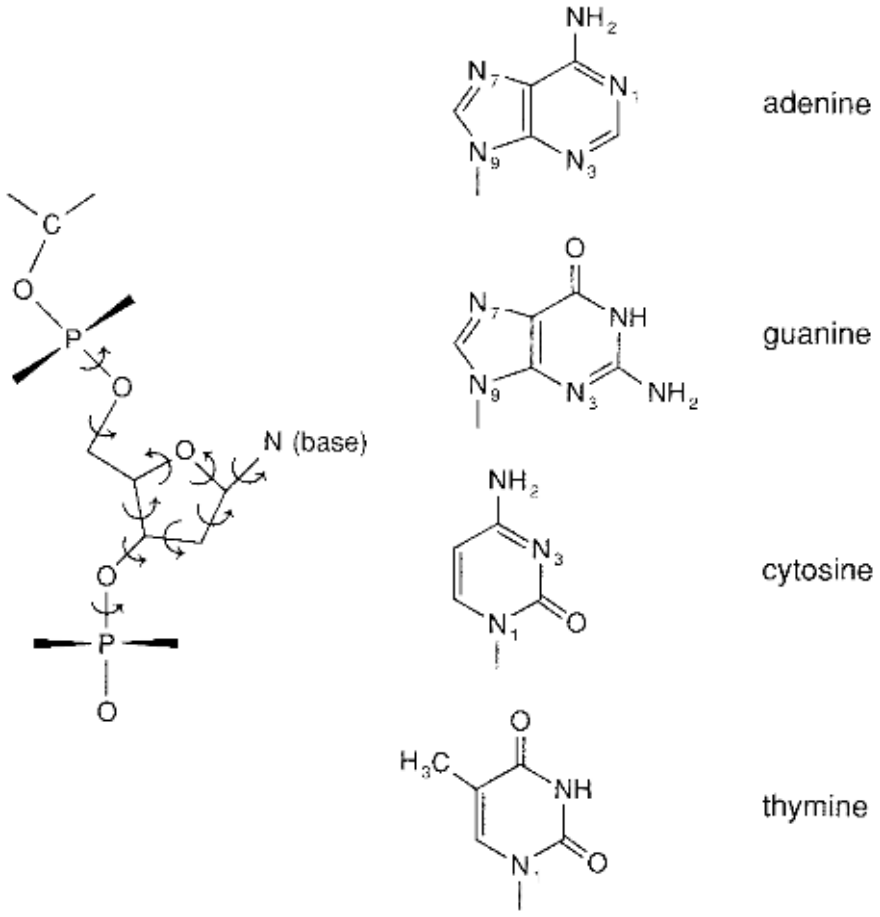


Figure 8.1
Illustration of a mononucleotide unit. Arrows indicate the various torsional angles within each unit that together generate the wide range of conformations available in the polymer. Also shown are the individual bases as well as the commonly employed numbering scheme.

are intertwined in a helix, stabilized through Watson-Crick hydrogen bonding between purines and pyrimidines, and through π-π stacking interactions among the bases arranged in the helical column. There are electrostatic repulsions between the anionic phosphate backbones of the polymer, causing a stiffening; each double-helical step has two formal negative charges. An atmosphere of metal ions condensed along the sugar-phosphate backbone serves partially to neutralize these electrostatic interactions. In the B-DNA conformation, the bases

are stacked essentially perpendicular to the helical axis, and the sugars are puckered in general, with a C2′-endo geometry (the C2′ carbon is to the same side as the C5′ position relative to a plane in the sugar ring defined by the C1′, C4′, and O atoms). This conformer yields a right-handed helix with two distinct, well-defined grooves, termed the major and minor. The A-form helix, while still right-handed, is distinctly different in structure. The sugar rings are puckered generally in the C3′-endo conformation, causing the bases to be pushed out from the center of the helix toward the minor groove, and tilted relative to the helix perpendicular by almost 20°. What results is a shorter and fatter helix than the B-form; the helical pitch is 28.2 Å in A-DNA for an 11-residue helix and 33.8 Å for a 10-residue helix in B-DNA. The A-form helical shape is best characterized by the very shallow minor groove surface; what was the major groove in the B-form has been pulled deeply into the interior of the A-conformer and is really not accessible to binding by small molecules in solution. Transitions to the A-conformation are promoted by hydrophobic solvents or solutions of high ionic strength. The Z-conformation is perhaps most distinctive, owing to its left-handed helicity.[4] The conformer was dubbed Z-DNA because of the zig-zag in the helix. Alternations both in sugar puckering, between C2′-endo and C3′-endo, and in the rotation of the base about the glycosidic bond, anti or syn relative to the sugar, are evident, and lead to a dinucleoside repeating unit versus a mononucleoside repeat in the A- and B-helices. Alternating purine-pyrimidine sequences have the highest propensity to undergo transitions into the Z-form. It is actually this syn conformation of purines that leads to the left-handed helicity of the polymer. But it is not only its left-handedness that distinguishes the Z-conformation. The polymer is long and slender (the pitch is 45 Å for a 12-residue helix), and the major groove is a shallow and wide, almost convex, surface, whereas the minor groove is narrowed into a sharp and small crevice.

Even less defined structurally are other conformations of DNA, some of which are illustrated schematically in Figure 8.2B (see color plate section, page C-15). Double-helical DNA can bend,[6] form loops and cruciforms,[7] and fold back on itself into intramolecular triple helices, termed H-DNA.[8] At the ends of chromosomes, four strands may even come together in a unique conforma-

of chromosomes, four strands may even come together in a unique conformation. These structures, characterized thus far by means of biochemical techniques, arise because of sequence and local torsional stress, or supercoiling. Many of these structures are stabilized by the binding of highly charged metal ions, probably because the highly charged metal center in a small volume can neutralize the electrostatic repulsions between polyanionic strands that are bundled together. Metal complexes can furthermore be extremely useful in targeting and characterizing these structures, as we will see. In chromosomes the DNA is packaged by histone proteins into even tighter bundles, with helical segments wrapped about the basic proteins to form superhelical nucleosomal units which are then arranged like beads on a string of more loosely packed DNA.[9]

This complexity in DNA structure is in fact small compared to that of RNA. Figure 8.2C (see color plate section, page C-15) shows the first crystallographically characterized structure[5] of an RNA polymer, yeast $tRNA^{Phe}$. Ostensibly single-stranded RNAs do not exist as random coils, but instead fold up into well-defined three-dimensional structures, much like proteins. The structural variety, of course, bears some resemblance to that found in DNAs. Double-helical regions in the tRNA are A-like in conformation; helices fold together as one might imagine to occur in cruciforms, and even triple-helical segments are evident where three strands fold together in the polymer. But overall our ability to characterize structures of RNA thus far is lower than that with DNAs. RNAs are less stable in solution than is DNA, and fewer chemical as well as enzymatic tools are available for structural characterization. Yet the recent discovery of ribozymes,[10] the finding that RNAs can indeed catalyze nucleolytic reactions, makes our need to understand these structures even greater. Again transition-metal chemistry may participate in stabilizing, promoting, and probing these structures.

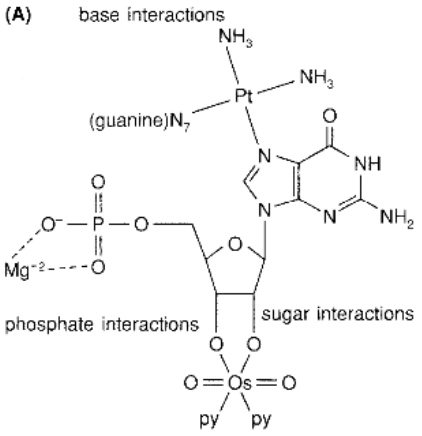
B. Fundamental Interactions with Nucleic Acids

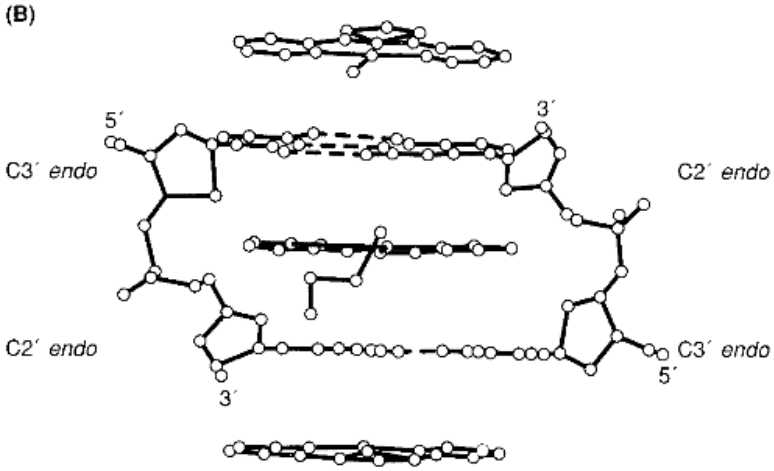
Metal ions and complexes associate with DNA and RNA in a variety of ways, as illustrated in Figure 8.3. Both strong covalent interactions and weak noncovalent complexes are observed.[11] Each may yield a significant perturbation in the nucleic acid and/or may be exploited to obtain a site-specific response. Clearly there are some general guidelines, based on principles of coordination chemistry, that may be helpful in sorting out these interactions.

1. Coordination

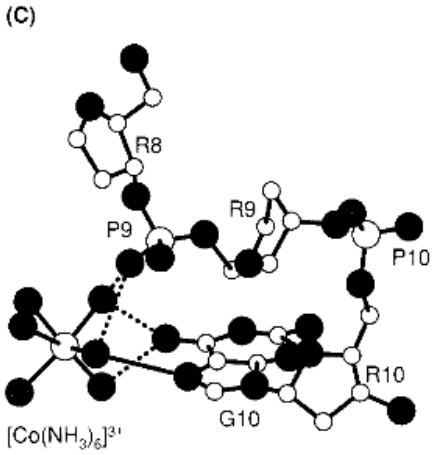
Most prevalent among covalent complexes with DNA are those involving coordination between soft metal ions and nucleophilic positions on the bases. The structure[12] of *cis*-$(NH_3)_2$Pt-dGpG is an example: its platinum center coordinates to the N7 position of the guanine bases. In terms of interactions with the full polynucleotide, it is likely that the *cis*-diammineplatinum center, with two coordination sites available, would yield an intrastrand crosslink between neighboring guanine residues on a strand (see Chapter 9). Other nucleophilic sites targeted by soft metal ions on the bases include the N7 position of adenine, the N3 position on cytosine, and the deprotonated N3 position on thymine and uracil.[12,13] Some additional covalent binding to the N1 positions of the purines has also been observed. Indeed, coordination by the metal to one site on the heterocyclic base lowers the pK_a and increases the metal-binding affinity to secondary sites. It is noteworthy, however, that in base-paired double-helical DNA only the N7 positions on the purines are easily accessible in the major groove of the helix. Base binding at the purine N7 position is, of course, not limited to soft metal ions such as Pt(II), Pd(II), and Ru(II). Coordination at these sites has been evident also with first-row transition-metal ions such as Cu(II) and Zn(II).[13] For these, as is consistent with basic coordination chemistry, the lability of complexes formed is higher.

Transition-metal ions with decreasing softness are capable of coordinating also to the phosphate oxygen atoms. The ionic versus covalent character of these complexes clearly depends on the metal ions involved. In a classic study, examining the melting temperature of double-helical DNA in the presence of dif-





intercalation



hydrogen bonding

2. Intercalation and hydrogen bonding

But important interactions of metal complexes with polynucleotides are not restricted to those involving direct coordination of the metal center to the polymer. Instead, an abundance of highly selective interactions arise from an ensemble of weaker noncovalent interactions between the ligands of coordinatively saturated metal complexes and the nucleic acid. Two primary examples of noncovalent association are given by metallointercalation and hydrogen-bonding interactions of coordinated ligands.[17,18] Planar aromatic heterocyclic ligands such as phenanthroline and terpyridine can stack in between the DNA base pairs, stabilized through dipole-dipole interactions. Here, depending on the complex and its extent of overlap with the base pairs, the free energy of stabilization can vary from ~2 to 10 kcal. Nonintercalative hydrophobic interactions of coordinated ligands in the DNA grooves also can occur, as we will see. Hydrogen-bonding interactions of coordinated ligands with the polynucleotide are quite common, and arise in particular with the phosphate oxygen atoms on the backbone. With cobalt hexaammine, for example, hydrogen bonding to an oligonucleotide occurs between the ammine hydrogens and both phosphate oxygen atoms and purine bases.[19]

A mix of covalent and noncovalent interactions is also possible. With *cis*-diammineplatinum(II) coordinated to the guanine N7 position, the ammine ligands are well-poised for hydrogen-bonding interactions with the phosphate backbone.[12] The steric constraints on the molecule must be considered, however. With $Pt(terpy)Cl^+$, both intercalation of the terpy ligand and direct coordination of the platinum center (after dissociation of the coordinated chloride) are available, but not simultaneously; coordination of the platinum to the base would likely position the terpyridyl ligand away from the base stack in the DNA major groove, precluding intercalation.[20] Sigel and coworkers[21] have studied the thermodynamics of noncovalent interactions coupled to direct coordination of simple first-row transition-metal complexes with mononucleotides, and these results illustrate well the interplay of weak noncovalent interactions and direct coordination in generating geometric specificity in complex formation.

III. A CASE STUDY: TRIS(PHENANTHROLINE) METAL COMPLEXES

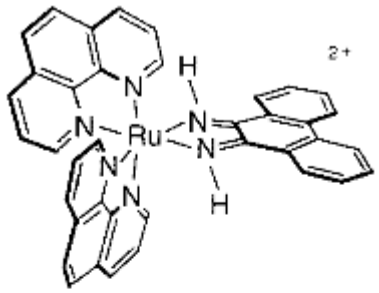
Now we may examine in detail the interaction of one class of metal complexes with nucleic acids, how these complexes bind to polynucleotides, the techniques used to explore these binding interactions, and various applications of the complexes to probe biological structure and function. Tris(phenanthroline) metal complexes represent quite simple, well-defined examples of coordination complexes that associate with nucleic acids. Their examination should offer a useful illustration of the range of binding modes, reactivity, techniques for study, and applications that are currently being exploited and explored. In addition, we may contrast these interactions with those of other transition-metal complexes, both derivatives of the tris(phenanthroline) family and also some complexes that differ substantially in structure or reactivity.

A. Binding Interactions with DNA

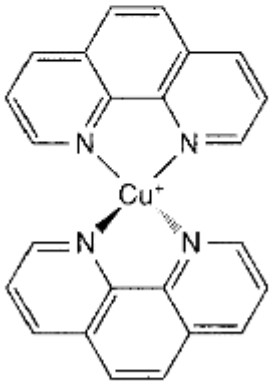
Tris(phenanthroline) complexes of ruthenium(II), cobalt(III), and rhodium(III) are octahedral, substitutionally inert complexes, and as a result of this coordinative saturation the complexes bind to double-helical DNA through a mixture of noncovalent interactions. Tris(phenanthroline) metal complexes bind to the double helix both by intercalation in the major groove and through hydrophobic association in the minor groove.[11b,40] Intercalation and minor groove-binding are, in fact, the two most common modes of noncovalent association of small molecules with nucleic acids. In addition, as with other small molecules, a nonspecific electrostatic interaction between the cationic complexes and the DNA polyanion serves to stabilize association. Overall binding of the tris(phenanthroline) complexes to DNA is moderate ($\log K = 4$).[41]

The tris(phenanthroline) complexes represented the first examples of ''three-dimensional intercalators'' and illustrated that octahedral metal complexes could also intercalate into the helix.[40,45,46] Here one can consider the partial intercalation of one ligand into the helix, providing the remaining ligands on the complex an opportunity to enhance specificity or reactivity at a given site.

Curiously, one unique and apparently general characteristic of metallointercalators is their preference for intercalation from the *major groove* of the helix. Most small molecules associate with DNA from the minor groove, but metallointercalators, both those that are square planar, such as (terpyridyl)platinum(II) complexes, and those that are octahedral, such as the tris(phenanthroline) metal complexes, appear to intercalate into the major groove. This then mimics quite well the association of much larger DNA-binding proteins with the helix; DNA regulatory proteins generally appear to target the major groove. The reason why metallointercalators favor major groove association is still unclear.



$[Ru(phen)_2\ phi]^{2+}$



$Cu(phen)_2^+$

Figure 8.8
Some metal complexes that bind DNA noncovalently primarily through intercalation (top) or binding in the minor groove (bottom). Some metalloporphyrins also primarily associate via intercalation.

The tris(phenanthroline) metal complexes themselves do not offer an illustration of hydrogen-bonding interactions with the helix, since these ligands lack hydrogen-bonding donors and acceptors, but as mentioned already, hydrogen bonding of coordinated ligands to the helix can add some measure of stabilization, comparable to, but likely no greater in magnitude than, that provided by intercalative stacking, hydrophobic, or dispersive interactions. Indeed, mixed-ligand derivatives of the phenanthroline complexes have been prepared that include hydrogen-bonding groups (amides, hydroxyls, and nitro substituents) on the ancillary phenanthroline ligands, and these have shown no greater avidity for double-helical DNA than their counterparts with hydrophobic substituents.[42] A large number of weak hydrogen-bonding interactions to DNA by one complex can be stabilizing, however, as with, for example, hexaamminecobalt(III) or hexaaquoterbium(III).

Tris(phenanthroline) metal complexes also do not offer an opportunity to explore covalent binding interactions with the helix in greater detail, but these interactions are, in fact, a major focus of Chapter 9, concerned with the mode of action of cisplatin. One derivative of the tris(phenanthroline) series, $Ru(phen)_2Cl_2$, has been shown to bind to DNA covalently.[48] In aqueous solution the dichlororuthenium(II) complex undergoes hydrolysis to form an equilibrium mixture of bis(phenanthroline) diaquo and chloroaquo species. These species bind covalently to DNA, with preferential reactivity at guanine sites. It is interesting that the same structural deformations in the DNA evident upon binding *cis*-diammineplatinum units become apparent upon coordination of bis(phenanthroline)ruthenium(II). It is also noteworthy that the chiral preference in coordination is for the Λ-isomer. As with groove binding, direct coordination to base positions requires a complementary symmetry, with the the Λ-isomer binding *against* the right-handed groove. This preference for the Λ-isomer reaffirms that, rather than noncovalent intercalation (which would favor the Δ-

isomer), covalent binding dominates the interaction. The energetic stabilization in direct coordination of the ruthenium(II) center is certainly more substantial than the weaker stabilization derived from intercalation. $Rh(phen)_2Cl_2^+$ and its derivatives have also been shown to bind covalently to DNA but only upon photoactivation, since light is needed to promote dissociation of the coordinated chloride and substitution of the nucleic acid base as a ligand.[49]

B. Techniques to Monitor Binding

Many of the same techniques employed in studying the basic chemistry of coordination complexes can be be used in following the binding of transition-metal complexes to nucleic acids, but biochemical methods, with their often exquisite sensitivity, become valuable aids as well in delineating specific binding interactions. Tris(phenanthroline) metal complexes are particularly useful to illustrate this point, since here the metal center in the complex is selected in terms of the technique used for examination.

Coordination complexes are often visibly colored, and these colorations provide a useful and sensitive spectroscopic handle in following fundamental reactions. This notion holds as well with tris(phenanthroline) metal complexes in their interactions with nucleic acids. $Ru(phen)_3^{2+}$ and its derivatives are highly colored because of an intense metal-to-ligand charge-transfer band ($\lambda_{max} = 447$ nm, $\epsilon = 1.9 \times 10^4\ M^{-1}cm^{-1}$). Furthermore, the complexes are highly photoluminescent ($\lambda_{em} = 610$ nm, $\tau = 0.6\ \mu s$ in aerated aqueous solution). On binding

to nucleic acids these transitions are perturbed. Hypochromism is observed in the charge-transfer band, and intercalation leads to an increase in lifetime of the charge-transfer excited state.[43,46] Indeed, single-photon counting experiments show a biexponential decay in emission from $Ru(phen)_3^{2+}$ bound to double-helical DNA. The longer-lived component ($\tau = 2\ \mu s$) has been assigned as the intercalated component and the shorter-lived 0.6 μs component has been attributed to a mixture of free and groove-bound species. These spectroscopic perturbations permit one to define equilibrium-binding affinities for the different components of the interaction as a function of metal-center chirality and under different solution conditions.[41] One can also follow the polarization of emitted

light from the complexes after excitation with polarized light, and these studies have been helpful in describing the dynamics of association of the complexes on the helix.[41,43] Mixed-ligand complexes of ruthenium(II) show similar spectroscopic perturbations, and these have been used to characterize binding affinities and chiral preferences, as well as the extent of intercalation versus groove binding as a function of ligand substitution on the metal center.[42] The spectroscopic handle of the metal center therefore affords a range of experiments to monitor and characterize the binding of the metal complexes to polynucleotides.

There are numerous other classic techniques of inorganic chemistry that have been or could be applied in studying the binding of metal complexes to nucleic acids. Coordination complexes have invariably been used in x-ray diffraction experiments because of the high electron density of the metal center. The tris(phenanthroline) metal complexes have not yet been applied in this context, but, as mentioned already, platinum metallointercalators were examined by fiber diffraction to delineate intercalation requirements. In fact, many nucleic-acid crystal structures have required specific metal ion additions for isomorphous heavy-metal derivatives to solve the structure. Such has certainly been true for the crystal structure of $tRNA^{Phe}$, where heavy-metal ions such as platinum, osmium, and mercury were targeted to specific base positions, and lanthanide ions were used to label phosphate positions around the periphery of the molecule.[53] Other techniques can also be exploited to monitor and characterize binding. A recent novel illustration is one from electrochemistry, which has been applied in monitoring the binding of $Co(phen)_3^{3+}$ to DNA.[54] Surely other techniques, from EXAFS to scanning tunneling microscopy, will be exploited in the future.

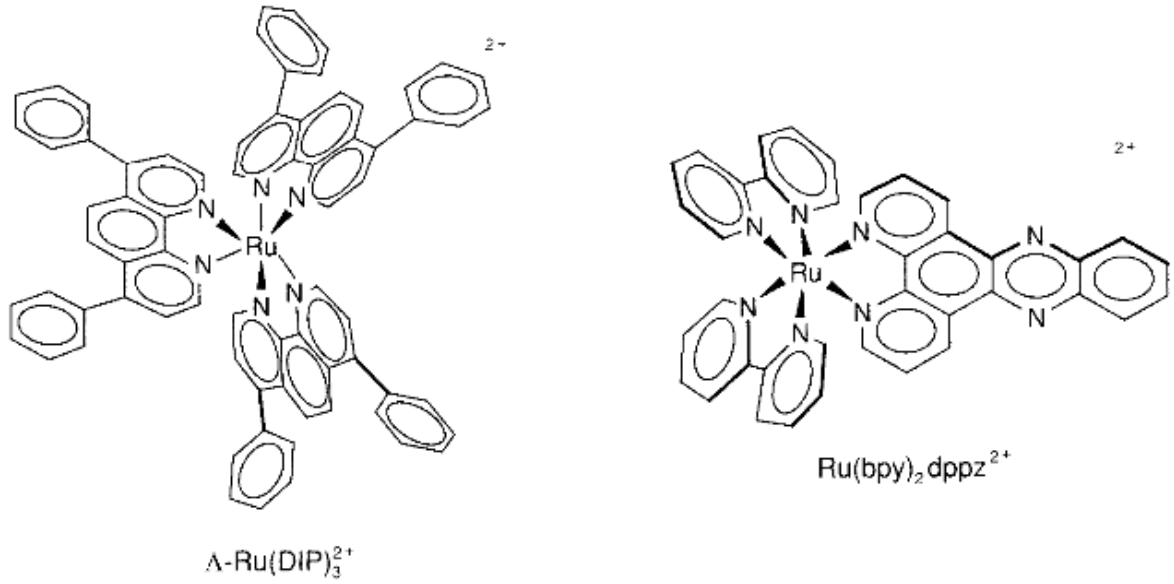
IV. APPLICATIONS OF DIFFERENT METAL COMPLEXES THAT BIND NUCLEIC ACIDS

Both the spectroscopy and the chemical reactivity of transition-metal complexes, coupled to biochemical assays, can therefore be exploited to obtain a wide range of useful reagents to probe nucleic acids. Here some specific applications are described.

A. Spectroscopic Probes

As discussed above, the tris(phenanthroline)ruthenium(II) complexes offer a novel spectroscopic probe of nucleic acids, since their luminescence is increased upon intercalation into the double helix. As a result the complexes provide a simple luminescent stain for DNA in fluorescent microscopy experiments. More interesting, perhaps, is the conformational selectivity of derivatives of tris(phenanthroline)ruthenium. $Ru(DIP)_3^{2+}$ (DIP = 4,7-diphenyl-1,10-phenanthroline) shows enantiospecificity in binding to B-form DNA.[40] Because of the steric bulk of the phenyl rings, detectable binding is seen only with the Δ-isomer in a right-handed helix; no binding is evident with the Λ-isomer. But with the left-handed Z-form helix, both isomers bind avidly.[40,58] The shallow left-handed major groove can accomodate the two enantiomers. A left-handed but more B-like helix shows selectivity instead for the Λ-isomer. Spectroscopic experiments that measure the chiral selectivity of $Ru(DIP)_3^{2+}$ isomers in binding to a given DNA then provide a novel probe for helical handedness. Indeed, Λ-$Ru(DIP)_3^{2+}$ was the first spectroscopic probe for Z-DNA (or other alternate conformations that are sufficiently unwound to permit binding by the bulky left-handed isomer).[58]

Both simpler bipyridyl and phenanthroline derivatives as well as dppz complexes of ruthenium are currently being tethered onto other DNA binding moieties, in particular onto oligonucleotides, so as to develop new, nonradioactive luminescent probes for DNA sequences. These transition-metal complexes may provide the basis for the development of new families of DNA diagnostic agents, and many industrial laboratories are currently exploring routes to accomplish these goals. Figure 8.12 illustrates Λ-$Ru(DIP)_3^{2+}$ and $Ru(bpy)_2dppz^{2+}$, two complexes whose luminescence properties can be employed to probe nucleic acids.



Λ-Ru(DIP)$_3^{2+}$ $Ru(bpy)_2dppz^{2+}$

Figure 8.12
Two spectroscopic probes of nucleic acids: Λ-Ru(DIP)$_3^{2+}$ and $Ru(bpy)_2dppz^{2+}$.

B. Metallofootprinting Reagents

Probably the most widespread application of metal nucleic-acid chemistry in the biology community has been the utilization of metal complexes for chemical footprinting. The footprinting technique (Figure 8.11) was developed by biologists[62] as a means of locating protein-binding sites on DNA.[32]P-end-labeled double-stranded DNA fragments could be digested with a nuclease, such as DNAse, in the presence or absence of DNA-binding protein. After electrophoresis of the denatured digests and autoradiography, one would find a ''footprint,'' that is, the inhibition of cleavage by DNAse, at the spot bound by protein, in comparison to a randomly cleaved pattern found on the DNA in the absence of binding protein. Although DNAse is still widely used, this footprinting reagent has some disadvantages: (i) the nuclease is not sequence-neutral in its cleavage, resulting in lots of noise in the footprinting background; and (ii) since the nuclease is itself a large protein, its ability to provide high-resolution footprinting patterns of smaller molecules is quite limited.

Inorganic photochemistry has also been applied in developing metal complexes as photofootprinting reagents. Uranyl acetate, for example, at high concentrations, upon photolysis, promotes DNA cleavage.[66] It is thought that the ions interact with the phosphates, generating some excited-state radical chemistry, although no detailed characterization of this chemistry has been undertaken.

C. Conformational Probes

Metal complexes are also finding wide application in probing the local variations in conformation that arise along nucleic-acid polymers. X-ray crystallography has been critical in establishing the basic conformational families of double-helical DNA, and to some extent how conformations might vary as a function of nucleic-acid sequence. Yet many conformations have still not been described to high resolution, and only a few oligonucleotides have been crystallized. Other techniques are therefore required to bridge the small set of oligonucleotide crystal structures that point to plausible structures and the large array of structures that arise as a function of sequence on long helical polymers. Furthermore, only a very small number of RNA polymers has been characterized crystallographically; hence other chemical methods have been needed to describe the folding patterns in these important biopolymers. Metal complexes, mainly through specific noncovalent interactions, appear to be uniquely useful in probing the structural variations in nucleic acids.

1. Nonspecific reactions of transition-metal complexes

Hydroxyl radical cleavage with $Fe(EDTA)^{2-}$ illustrates again how simple metal complexes can be used in characterizing nucleic acids. One example involves efforts to describe the local structural variations in ''bent'' DNA. Biochemists had found that DNA fragments containing runs of adenines, such as in the tract dAAAAAA, possessed unusual gel-electrophoretic mobilities. Indeed, kinetoplast DNA isolated from mitochondria of trypanosomes showed a remarkable lacework pattern of structure, with loops and circles of DNA; these structures were found to be governed by the placement of these $d(A)_6$ tracts. By constructing a series of oligonucleotides with adenine runs positioned either in or out of phase relative to one another, researchers found that the adenine tracts caused a local bending of the DNA toward the minor groove.[6] But what were the detailed characteristics of these bent sites? Using hydroxyl radical cleavage of DNA, generated with $Fe(EDTA)^{2-}$, Tullius and coworkers found a distinctive pattern of cleavage across the adenine tracts, consistent with a locally perturbed structure.[68] Here the notion again was that $Fe(EDTA)^{2-}$ in the presence of peroxide would generate hydroxyl radicals at a distance from the helix, and thus careful densitometric analysis of the cleavage across ^{32}P-end-labeled DNA fragments would reveal any differential accessibility of sugar residues to cleavage mediated by the radicals caused by the bending. The cleavage patterns suggested a smooth bending of the DNA across the tract and indicated furthermore an asymmetry in structure from the 5′- to 3′-end of the adenine run.

2. Transition-metal complexes as shape-selective probes

Transition-metal complexes have also been designed with three-dimensional structures that target complementary structures along the helical polymer. This recognition of DNA sites, based upon *shape selection*, has proved to be extremely useful both in demarcating and in characterizing structural variations along the polymer and in developing an understanding of those factors important to the recognition of specific polynucleotide sites. Complexes, basically derivatives of the tris(phenanthroline) metal series, have been designed that specifically target A- and Z-form helices, cruciforms, and even subtle variations such as differential propeller twisting within B-form DNA.[11c] By appropriate substitution of the metal at the center of the coordinatively saturated complex, complexes that cleave the DNA at the binding site are obtained. Figure 8.14 shows some of these shape-selective conformational probes.

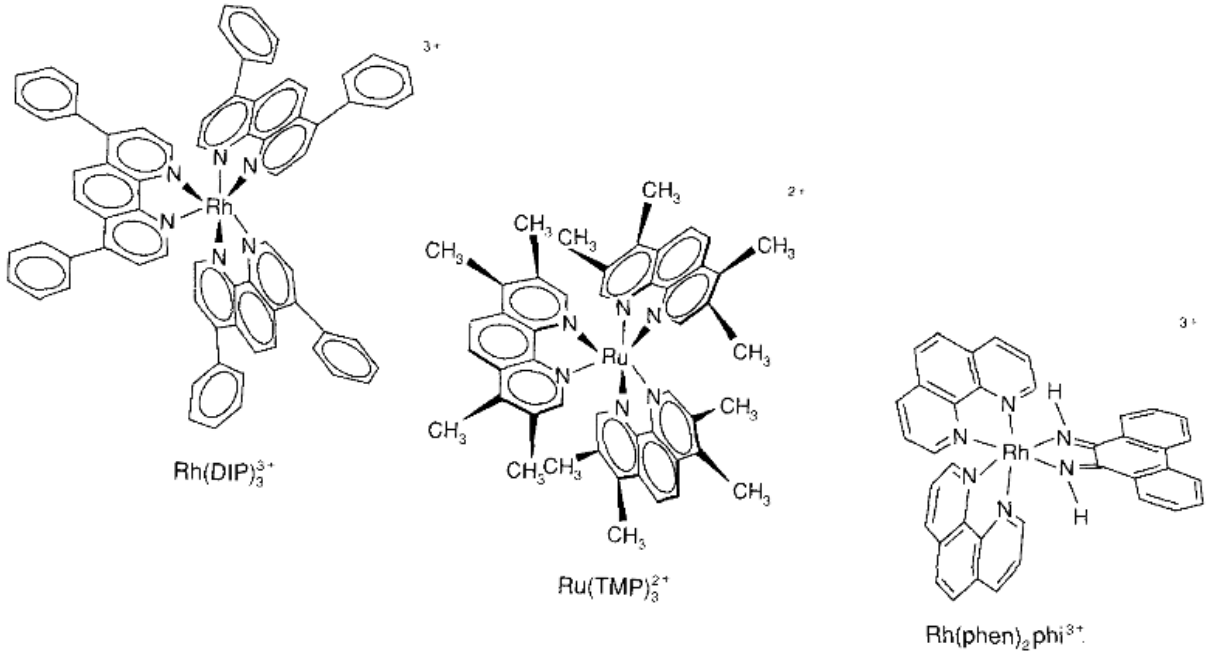


Figure 8.14
Shape-selective probes that target local DNA conformations. $Rh(DIP)_3^{3+}$, which with photoactivation promotes double-stranded cleavage at cruciform sites; $Ru(TMP)_3^{2+}$, a photoactivated probe for A-like conformations; and $Rh(phen)_2phi^{3+}$, which targets openings in the DNA major groove.

The most common oxidation state of lanthanides is	4	3	2	1	3
Paramagnetism is a property of	completely filled electronic sub-shells	unpaired electrons	non-transition elements	elements with noble gas configuration.	unpaired electrons
The 3d element show variable oxidation states. What is the maximum oxidation state shown by the element Mn?	6	7	4	5	7
Colour in transition metal compounds is attributed to	small size metal ions	absorption of light in UV region	Complete (n s) subshell	Incomplete (n - 1) d subshell.	Incomplete (n - 1) d subshell.
In the first transition series, the element with highest melting point is	Mn	Fe	Cr	Cu	Cr
Which of the following does not form coloured complexes?	Ni (II)	Cu (I)	Fe (II)	Cr (VI)	Cu (I)
Which of the following statements concerning transition elements is not true?	They are all metals	They easily form complexes	Compounds containing their ions are coloured	They show multiple oxidation states always differing by two units	They show multiple oxidation states always differing by two units
Among transition elements the elements the element with lowest melting point belongs to group	3	11	6	12	12
Which of the following triads	Ba, Sr, Ca	Rb, Cs, Fr	Sc, Ti, V	F, Cl, Br	Sc, Ti, V

is an example of transition elements?					
The 3d metals ions are generally paramagnetic in nature because	They form coloured salts	They have one or more unpaired d-electrons	They have one or more paired s-electrons	They are reducing agents	They have one or more unpaired d-electrons
Brass is an alloy of	Silver and copper	Copper and zinc	Copper and tin	Copper, zinc and tin	Cu and Zn
Which one of the following metals is used as a catalyst in the Habers process?	Tungsten	Molybdenum	Chromium	Iron containing molybdenum.	Iron containing molybdenum.
For the following system at equilibrium, what will cause the partial pressure of HF to increase? $UO_2(s) + 4\ HF(g)$ $UF_4(g) + 2\ H_2O(g)$	adding $UO_2(s)$	decreasing the volume	adding $UF_4(g)$	None	adding $UF_4(g)$
Which of the following is a strong acid?	HF	H_3PO_4	HCl	H_2CO_3	HCl
The following are strong bases EXCEPT	LiOH	$Al(OH)_3$	KOH	$Ba(OH)_2$	$Al(OH)_3$
For the following reaction, which of the following is a conjugate acid-base pair? $HC_2O_4^-(aq) + H_2O(l)$ $H_3O^+(aq) + C_2O_4^{2-}(aq)$	Equilibrium lies to the left, because H_2O is a stronger acid than $CH_3NH_3^+$	Equilibrium lies to the left, because OH^- is a stronger base than CH_3NH_2	Equilibrium lies to the right, because $CH_3NH_3^+$ is a stronger acid than H_2O	Equilibrium lies to the right, because OH^- is a stronger acid than H_2O	Equilibrium lies to the right, because $CH_3NH_3^+$ is a stronger acid than H_2O
A Brønsted-Lowry base is defined as a substance that	acts as a proton donor.	increases $[H^+]$ when placed in water	acts as a proton acceptor	decreases $[H^+]$ when placed in water	acts as a proton acceptor
Which of the following is true?	HF is a stronger acid than HI,	HF is a stronger acid than HI,	HF is a weaker acid than HI, because I is	HF is a weaker acid than HI, because the HF	HF is a weaker acid than HI,

	because F is more electronegative than I.	because the HF bond is weaker than the HI bond	more electronegative than F.	bond is stronger than the HI bond.	because the HF bond is stronger than the HI bond.
Which one of the following would be expected to change the value of the equilibrium constant?	adding reactant	adding product	adding a catalyst	changing the temperature	changing the temperature
What is the conjugate base of HCO3–?	OH–	H2CO3	CO32–	HCO_3^+	CO32–
A Brønsted-Lowry acid is defined as a substance that _____	decreases [H3O+] when dissolved in water	increases [OH–] when dissolved in water	acts as a proton donor	acts as a proton acceptor	acts as a proton donor
A substance that is capable of acting as both an acid and as a base is _____.	amphiprotic	conjugated	autosomal	binary acid-base	amphiprotic
The magnitude of Kw indicates that	water autoionizes very slowly	water autoionizes very quickly	water autoionizes only to a very small extent	the autoionization of water is exothermic	water autoionizes only to a very small extent
Which one of the following is the strongest acid?	HIO3	HIO2	HIO	IO_4^-	HIO3
Which one of the following binary acids is the strongest?	CH4	NH3	H2O	H2S	H2S
Which of these is not a Lewis acid?	AlCl3	C4H10	FeCl3	SO3	C4H10
Increasing the magnetic field?	produuces less susceptibility artifacts.	Reduces the risk of tissue heating.	Increase the signal to noise	Reduces the danger from metallic projectiles	Increase the signal to noise
A major advantage of MRI is:	the ease with which equipment is	its relatively low cost, compared to	dose not require specialized	the ability to reposition the 'cross-section'	the ability to reposition the 'cross-

	updated or replaced	CT scans	room	through the body without repositioning the patient.	section' through the body without repositioning the patient.
A growing application of MRI is "MRA", which stands for:	Magnetic Resonance Amplication	Magnetic Resonance Angiography	Minimal Radiology Applications	Medical Research Assistance	Magnetic Resonance Angiography
What does "MRI" stand for?	Magneto-Ray Idometry	Medical Radiometry Instrument	Magnetic Resonance Imaging	Maximal Radiology Imaging	Magnetic Resonance Imaging
True or False - T1 increases with magnetic field	FALSE	True			TRUE
What is a major health concern wth MRI?	Reaction to applied drugs	extrerme cold?	Radiation dose	localized burns due to metallic implants?	localized burns due to metallic implants?
Compare MRI to CT ("CAT scans"). Which is true?	Both methods use X-rays, but exposure is higher with CT.	CT reveals soft structures, while MRI is better at dense material, such as bone.	Both methods produce cross-sectional images at a specified plane through the body.		Both methods produce cross-sectional images at a specified plane through the body.
Select one of the following objects that you think would alwasy be safe in the MRI suite.	A wheelchair	A stretcher	Scissors	None of the listed	None of the listed
True or False - T1 relaxation is shorter than T2 relaxation.	False	TRUE			FALSE
What is the maximum stength of magnet approved for medical imaging of patient?	7.0 T	1.5 T	5.0 T	3.0 T	3.0 T
Which statement most correctly describes crystal	The theory considers covalent interactions	The theory considers electrostatic interactions	The theory rationalizes the non-degeneracy of	The theory rationalizes why the metal d orbitals are	The theory rationalizes the non-degeneracy

field theory for a d block complex of unspecified geometry?	between a metal centre and the surrounding ligands	between a metal ion and the surrounding ligands which are taken to be point charges	the metal d orbitals by considering the electrostatic repulsions between point charge ligands and electrons in the metal d orbitals	split into two levels	of the metal d orbitals by considering the electrostatic repulsions between point charge ligands and electrons in the metal d orbitals
Which of the following correctly places the ligands in their order in the spectrochemical series?	Br– < Cl– < NH3 < H2O	I– < Br– < H2O < [OH]–	F– < Cl– < H2O < NH3	I– < Cl– < H2O < en	I– < Cl– < H2O < en
Which of the following correctly places the metal centres in their order in the spectrochemical series?	Mn(II) < Fe(III) < Rh(III)	Co(III) < Co(II) < Rh(III)	Pt(IV) < Pd(II) < Ni(II)	Pd(II) < Ni(II) < Pt(IV)	Mn(II) < Fe(III) < Rh(III)
Which metal complex ion is expected to be subject to a Jahn-Teller distortion?	[Cr(OH2)6]3+	[Cr(NH3)6]2+	[Cr(CN)6]3–	[Cr(bpy)3]2+	[Cr(NH3)6]2+
Which of the following complex ions is tetrahedral?	[PdCl4]2–	[PtCl4]2–	$[NiCl_4]^{2-}$	[AuCl4]–	[NiCl4]2–
Match up the correct formula and magnetic property. Which pair is correct?	[Zn(OH2)6]2+; paramagnetic	[Co(NH3)6]3+ ; diamagnetic	[CoF6]3–; diamagnetic	[V(OH2)6]2+; diamagnetic	[Co(NH3)6]3+ ; diamagnetic
Which statement is incorrect about typical metal	They are likely to obey the 18-electron rule	They contain π-acceptor ligands	M is in a zero oxidation state	They are likely to be paramagnetic	They are likely to be paramagnetic

carbonyl complexes M(CO)n?					
Which of the following is a π-donor ligand?	Cl–	NH3	CO	PF3 [Co(CO)4]–	Cl–
Which of the following complexes does not obey the 18-electron rule?	[Fe(CO)4]2–	[Rh(CO)2I2]–	[Mn(CO)5]–		[Rh(CO)2I2]–
Which of the following statements is incorrect?	The electronic spectrum of [Ni(NH3)6]2+ contains 3 absorptions	Absorptions in the electronic spectrum of [Mn(OH2)6]2+ are extremely weak	For a tetrahedral d4 complex, 3 absorptions are expected in its electronic spectrum	The absorption in the electronic spectrum of [Ti(OH2)6]3+ is assigned to the Eg ← T2g transition	For a tetrahedral d4 complex, 3 absorptions are expected in its electronic spectrum
[Cr(CN)6]3– is expected to be:	paramagnetic with μeff ≈ 3.87 μB	diamagnetic	paramagnetic with μeff < 3.87 μB	paramagnetic with μeff > 3.87 μB	paramagnetic with μeff ≈ 3.87 μB
Which series correctly places the ligands in order of increasing nephelauxetic effect?	F– < Cl– < I–	I– < Cl– < F–	en < NH3 < H2O	I–< Br– < [CN]–	F– < Cl– < I–
For which pair of complexes is the order of values of Δoct correct?	[Rh(NH3)6]3+ > [Co(NH3)6]3+	[Fe(CN)6]4– > [Fe(CN)6]3–	[Cr(OH2)6]2+ > [Cr(OH2)6]3+	[CrF6]3– > [Cr(CN)6]3–	[Rh(NH3)6]3+ > [Co(NH3)6]3+
The CFSE for a high-spin d4 octahedral complex is:	–0.6Δoct	–1.8Δoct	–1.6Δoct + P	–1.2Δoct	–0.6Δoct
The visible spectra of salts of the following complexes are measured in aqueous solution. For which complex would the spectrum	[MnO4]–	[CoCl4]2–	[Co(OH2)6]2+	[Mn(OH2)6]2+	[MnO4]–

contain absorptions with thehighest εmax values?					
A d1 electron configuration corresponds to which of the following terms?	2D	^1D	2P	3P	2D
How many microstates are possible for a d2 configuration, including both weak and strong field limits?	15	50	10	90	45
The 'd-d' transitions in an octahedral [NiX6]2+ complex are:	Laporte forbidden but spin allowed	Laporte forbidden and spin forbidden	Laporte allowed and spin allowed	Laporte allowed but spin forbidden	Laporte forbidden but spin allowed
Which of the following is NOT a side effect of Digoxin toxicity?	Bradycardia	Yellow vision changes	Scooping of the T segment on ECG	Hypokalemia	Hypokalemia
Which of the following chelating agents is recommended for acute Lead poisoning with signs of encephalopathy ?	Succimer	Penicillamine	Calcium EDTA	Dimercaprol + Calcium EDTA	Dimercaprol + Calcium EDTA
In the complex [K(18-crown-6)]+, the number of 5-membered chelate rings that are formed is:	6	5	3	8	6
When [EDTA]4- coordinates to a metal ion, M2+, to give [M(EDTA)]2-, the	4	5	6	7	5

number of chelate rings formed is:					
Within the HSAB principle, a hard acid:	is not very polarizable	has a low charge density	shows a preference for soft bases		is not very polarizable

UNIT-I

Binding of Metal Ions and Complexes to Biomolecules: Nucleic acid structures - fundamental interactions with nucleic acids - binding interactions of tris(phenanthroline) metal complexes with DNA - techniques to monitor binding - applications of metal complexes that bind to nucleic acids -biopolymer promoted metal ligand interactions.

A. Nucleic-Acid Structures [1]

Figure 8.1 displays a single deoxyribonucleotide and the four different nucleic-acid bases. As may be evident, each mononucleotide along a nucleic-acid polymer contains a variety of sites for interactions with metal ions, from electrostatic interactions with the anionic phosphate backbone to soft nucleophilic interactions with the purine heterocycles. The different nucleic-acid bases furthermore offer a range of steric and electronic factors to exploit. Coordination of a metal complex to the N7 nitrogen atom of a purine, for example, would position other coordinated ligands on the metal center for close hydrogen bonding to the O6 oxygen atom of guanine, but would lead to clashes with the amine hydrogen atoms of adenine.

The monomeric units strung together in a polynucleotide furthermore provide an array of polymeric conformers. Figure 8.2A *(See color plate section, pages C-14, C-15.)* shows three crystallographically characterized structures of double-helical DNA oligonucleotides,[2–4] Figure 8.2B a schematic illustration of other conformations of DNA, and Figure 8.2C the crystal structure [5] of yeast $tRNA^{Phe}$. In double-helical DNA,[1] the two antiparallel polynucleotide strands

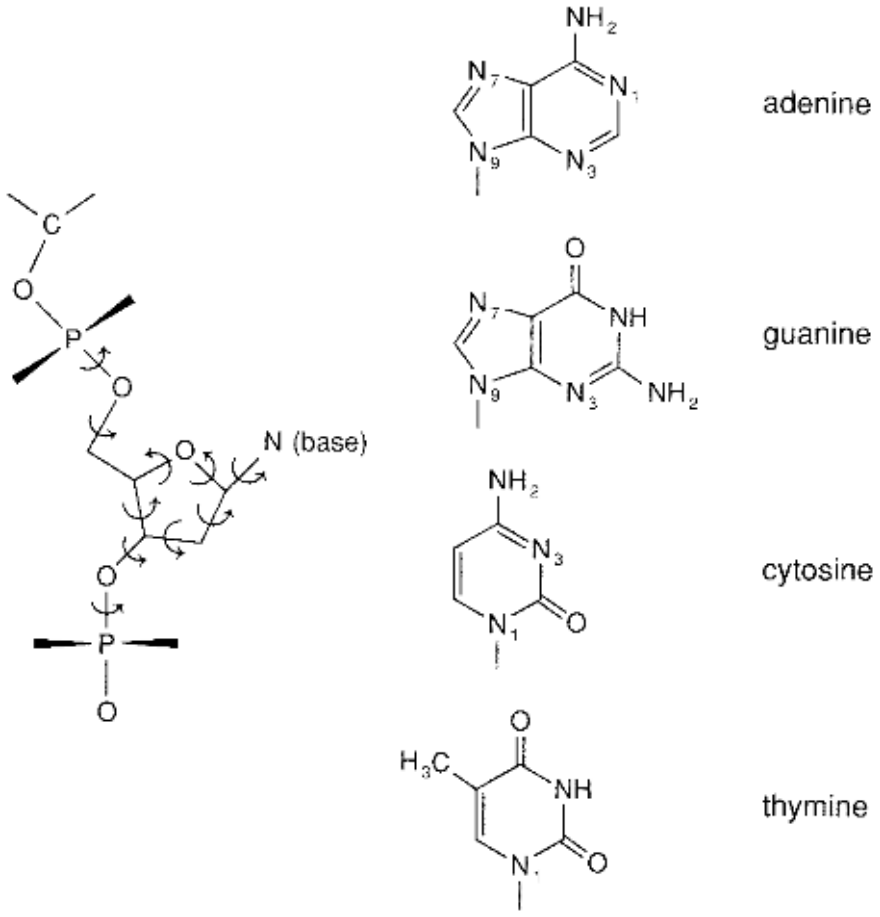


Figure 8.1
Illustration of a mononucleotide unit. Arrows indicate the various torsional angles within each unit that together generate the wide range of conformations available in the polymer. Also shown are the individual bases as well as the commonly employed numbering scheme.

are intertwined in a helix, stabilized through Watson-Crick hydrogen bonding between purines and pyrimidines, and through π-π stacking interactions among the bases arranged in the helical column. There are electrostatic repulsions between the anionic phosphate backbones of the polymer, causing a stiffening; each double-helical step has two formal negative charges. An atmosphere of metal ions condensed along the sugar-phosphate backbone serves partially to neutralize these electrostatic interactions. In the B-DNA conformation, the bases

are stacked essentially perpendicular to the helical axis, and the sugars are puckered in general, with a C2′-endo geometry (the C2′ carbon is to the same side as the C5′ position relative to a plane in the sugar ring defined by the C1′, C4′, and O atoms). This conformer yields a right-handed helix with two distinct, well-defined grooves, termed the major and minor. The A-form helix, while still right-handed, is distinctly different in structure. The sugar rings are puckered generally in the C3′-endo conformation, causing the bases to be pushed out from the center of the helix toward the minor groove, and tilted relative to the helix perpendicular by almost 20°. What results is a shorter and fatter helix than the B-form; the helical pitch is 28.2 Å in A-DNA for an 11-residue helix and 33.8 Å for a 10-residue helix in B-DNA. The A-form helical shape is best characterized by the very shallow minor groove surface; what was the major groove in the B-form has been pulled deeply into the interior of the A-conformer and is really not accessible to binding by small molecules in solution. Transitions to the A-conformation are promoted by hydrophobic solvents or solutions of high ionic strength. The Z-conformation is perhaps most distinctive, owing to its left-handed helicity.[4] The conformer was dubbed Z-DNA because of the zig-zag in the helix. Alternations both in sugar puckering, between C2′-endo and C3′-endo, and in the rotation of the base about the glycosidic bond, anti or syn relative to the sugar, are evident, and lead to a dinucleoside repeating unit versus a mononucleoside repeat in the A- and B-helices. Alternating purine-pyrimidine sequences have the highest propensity to undergo transitions into the Z-form. It is actually this syn conformation of purines that leads to the left-handed helicity of the polymer. But it is not only its left-handedness that distinguishes the Z-conformation. The polymer is long and slender (the pitch is 45 Å for a 12-residue helix), and the major groove is a shallow and wide, almost convex, surface, whereas the minor groove is narrowed into a sharp and small crevice.

Even less defined structurally are other conformations of DNA, some of which are illustrated schematically in Figure 8.2B (see color plate section, page C-15). Double-helical DNA can bend,[6] form loops and cruciforms,[7] and fold back on itself into intramolecular triple helices, termed H-DNA.[8] At the ends of chromosomes, four strands may even come together in a unique conforma-

of chromosomes, four strands may even come together in a unique conformation. These structures, characterized thus far by means of biochemical techniques, arise because of sequence and local torsional stress, or supercoiling. Many of these structures are stabilized by the binding of highly charged metal ions, probably because the highly charged metal center in a small volume can neutralize the electrostatic repulsions between polyanionic strands that are bundled together. Metal complexes can furthermore be extremely useful in targeting and characterizing these structures, as we will see. In chromosomes the DNA is packaged by histone proteins into even tighter bundles, with helical segments wrapped about the basic proteins to form superhelical nucleosomal units which are then arranged like beads on a string of more loosely packed DNA.[9]

This complexity in DNA structure is in fact small compared to that of RNA. Figure 8.2C (see color plate section, page C-15) shows the first crystallographically characterized structure[5] of an RNA polymer, yeast $tRNA^{Phe}$. Ostensibly single-stranded RNAs do not exist as random coils, but instead fold up into well-defined three-dimensional structures, much like proteins. The structural variety, of course, bears some resemblance to that found in DNAs. Double-helical regions in the tRNA are A-like in conformation; helices fold together as one might imagine to occur in cruciforms, and even triple-helical segments are evident where three strands fold together in the polymer. But overall our ability to characterize structures of RNA thus far is lower than that with DNAs. RNAs are less stable in solution than is DNA, and fewer chemical as well as enzymatic tools are available for structural characterization. Yet the recent discovery of ribozymes,[10] the finding that RNAs can indeed catalyze nucleolytic reactions, makes our need to understand these structures even greater. Again transition-metal chemistry may participate in stabilizing, promoting, and probing these structures.

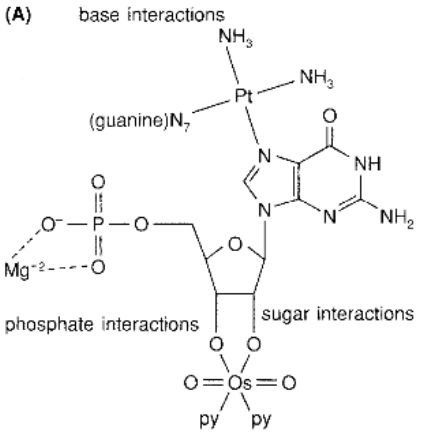
B. Fundamental Interactions with Nucleic Acids

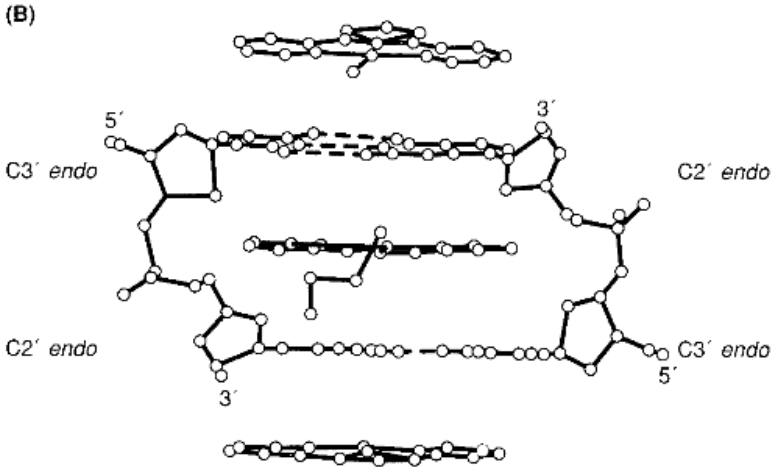
Metal ions and complexes associate with DNA and RNA in a variety of ways, as illustrated in Figure 8.3. Both strong covalent interactions and weak noncovalent complexes are observed.[11] Each may yield a significant perturbation in the nucleic acid and/or may be exploited to obtain a site-specific response. Clearly there are some general guidelines, based on principles of coordination chemistry, that may be helpful in sorting out these interactions.

1. Coordination

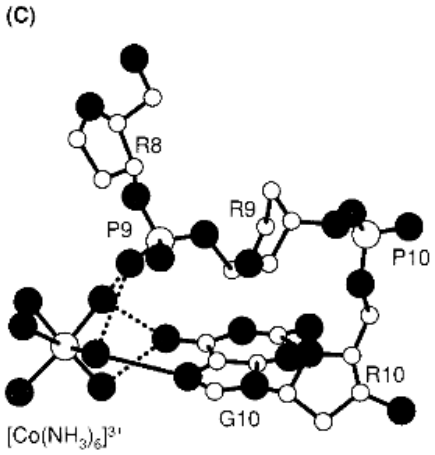
Most prevalent among covalent complexes with DNA are those involving coordination between soft metal ions and nucleophilic positions on the bases. The structure[12] of *cis*-$(NH_3)_2$Pt-dGpG is an example: its platinum center coordinates to the N7 position of the guanine bases. In terms of interactions with the full polynucleotide, it is likely that the *cis*-diammineplatinum center, with two coordination sites available, would yield an intrastrand crosslink between neighboring guanine residues on a strand (see Chapter 9). Other nucleophilic sites targeted by soft metal ions on the bases include the N7 position of adenine, the N3 position on cytosine, and the deprotonated N3 position on thymine and uracil.[12,13] Some additional covalent binding to the N1 positions of the purines has also been observed. Indeed, coordination by the metal to one site on the heterocyclic base lowers the pK_a and increases the metal-binding affinity to secondary sites. It is noteworthy, however, that in base-paired double-helical DNA only the N7 positions on the purines are easily accessible in the major groove of the helix. Base binding at the purine N7 position is, of course, not limited to soft metal ions such as Pt(II), Pd(II), and Ru(II). Coordination at these sites has been evident also with first-row transition-metal ions such as Cu(II) and Zn(II).[13] For these, as is consistent with basic coordination chemistry, the lability of complexes formed is higher.

Transition-metal ions with decreasing softness are capable of coordinating also to the phosphate oxygen atoms. The ionic versus covalent character of these complexes clearly depends on the metal ions involved. In a classic study, examining the melting temperature of double-helical DNA in the presence of dif-





intercalation



hydrogen bonding

2. Intercalation and hydrogen bonding

But important interactions of metal complexes with polynucleotides are not restricted to those involving direct coordination of the metal center to the polymer. Instead, an abundance of highly selective interactions arise from an ensemble of weaker noncovalent interactions between the ligands of coordinatively saturated metal complexes and the nucleic acid. Two primary examples of noncovalent association are given by metallointercalation and hydrogen-bonding interactions of coordinated ligands.[17,18] Planar aromatic heterocyclic ligands such as phenanthroline and terpyridine can stack in between the DNA base pairs, stabilized through dipole-dipole interactions. Here, depending on the complex and its extent of overlap with the base pairs, the free energy of stabilization can vary from ~2 to 10 kcal. Nonintercalative hydrophobic interactions of coordinated ligands in the DNA grooves also can occur, as we will see. Hydrogen-bonding interactions of coordinated ligands with the polynucleotide are quite common, and arise in particular with the phosphate oxygen atoms on the backbone. With cobalt hexaammine, for example, hydrogen bonding to an oligonucleotide occurs between the ammine hydrogens and both phosphate oxygen atoms and purine bases.[19]

A mix of covalent and noncovalent interactions is also possible. With *cis*-diammineplatinum(II) coordinated to the guanine N7 position, the ammine ligands are well-poised for hydrogen-bonding interactions with the phosphate backbone.[12] The steric constraints on the molecule must be considered, however. With $Pt(terpy)Cl^+$, both intercalation of the terpy ligand and direct coordination of the platinum center (after dissociation of the coordinated chloride) are available, but not simultaneously; coordination of the platinum to the base would likely position the terpyridyl ligand away from the base stack in the DNA major groove, precluding intercalation.[20] Sigel and coworkers[21] have studied the thermodynamics of noncovalent interactions coupled to direct coordination of simple first-row transition-metal complexes with mononucleotides, and these results illustrate well the interplay of weak noncovalent interactions and direct coordination in generating geometric specificity in complex formation.

III. A CASE STUDY: TRIS(PHENANTHROLINE) METAL COMPLEXES

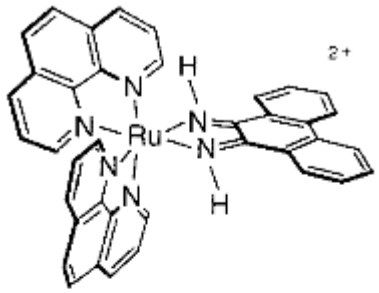
Now we may examine in detail the interaction of one class of metal complexes with nucleic acids, how these complexes bind to polynucleotides, the techniques used to explore these binding interactions, and various applications of the complexes to probe biological structure and function. Tris(phenanthroline) metal complexes represent quite simple, well-defined examples of coordination complexes that associate with nucleic acids. Their examination should offer a useful illustration of the range of binding modes, reactivity, techniques for study, and applications that are currently being exploited and explored. In addition, we may contrast these interactions with those of other transition-metal complexes, both derivatives of the tris(phenanthroline) family and also some complexes that differ substantially in structure or reactivity.

A. Binding Interactions with DNA

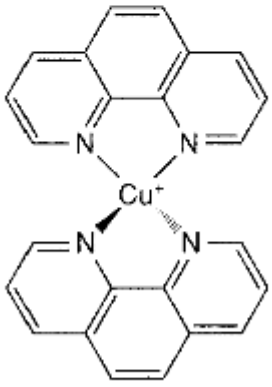
Tris(phenanthroline) complexes of ruthenium(II), cobalt(III), and rhodium(III) are octahedral, substitutionally inert complexes, and as a result of this coordinative saturation the complexes bind to double-helical DNA through a mixture of noncovalent interactions. Tris(phenanthroline) metal complexes bind to the double helix both by intercalation in the major groove and through hydrophobic association in the minor groove.[11b,40] Intercalation and minor groove-binding are, in fact, the two most common modes of noncovalent association of small molecules with nucleic acids. In addition, as with other small molecules, a nonspecific electrostatic interaction between the cationic complexes and the DNA polyanion serves to stabilize association. Overall binding of the tris(phenanthroline) complexes to DNA is moderate ($\log K = 4$).[41]

The tris(phenanthroline) complexes represented the first examples of ''three-dimensional intercalators'' and illustrated that octahedral metal complexes could also intercalate into the helix.[40,45,46] Here one can consider the partial intercalation of one ligand into the helix, providing the remaining ligands on the complex an opportunity to enhance specificity or reactivity at a given site.

Curiously, one unique and apparently general characteristic of metallointercalators is their preference for intercalation from the *major groove* of the helix. Most small molecules associate with DNA from the minor groove, but metallointercalators, both those that are square planar, such as (terpyridyl)platinum(II) complexes, and those that are octahedral, such as the tris(phenanthroline) metal complexes, appear to intercalate into the major groove. This then mimics quite well the association of much larger DNA-binding proteins with the helix; DNA regulatory proteins generally appear to target the major groove. The reason why metallointercalators favor major groove association is still unclear.



$[Ru(phen)_2\ phi]^{2+}$



$Cu(phen)_2^+$

Figure 8.8
Some metal complexes that bind DNA noncovalently primarily through intercalation (top) or binding in the minor groove (bottom). Some metalloporphyrins also primarily associate via intercalation.

The tris(phenanthroline) metal complexes themselves do not offer an illustration of hydrogen-bonding interactions with the helix, since these ligands lack hydrogen-bonding donors and acceptors, but as mentioned already, hydrogen bonding of coordinated ligands to the helix can add some measure of stabilization, comparable to, but likely no greater in magnitude than, that provided by intercalative stacking, hydrophobic, or dispersive interactions. Indeed, mixed-ligand derivatives of the phenanthroline complexes have been prepared that include hydrogen-bonding groups (amides, hydroxyls, and nitro substituents) on the ancillary phenanthroline ligands, and these have shown no greater avidity for double-helical DNA than their counterparts with hydrophobic substituents.[42] A large number of weak hydrogen-bonding interactions to DNA by one complex can be stabilizing, however, as with, for example, hexaamminecobalt(III) or hexaaquoterbium(III).

Tris(phenanthroline) metal complexes also do not offer an opportunity to explore covalent binding interactions with the helix in greater detail, but these interactions are, in fact, a major focus of Chapter 9, concerned with the mode of action of cisplatin. One derivative of the tris(phenanthroline) series, $Ru(phen)_2Cl_2$, has been shown to bind to DNA covalently.[48] In aqueous solution the dichlororuthenium(II) complex undergoes hydrolysis to form an equilibrium mixture of bis(phenanthroline) diaquo and chloroaquo species. These species bind covalently to DNA, with preferential reactivity at guanine sites. It is interesting that the same structural deformations in the DNA evident upon binding *cis*-diammineplatinum units become apparent upon coordination of bis(phenanthroline)ruthenium(II). It is also noteworthy that the chiral preference in coordination is for the Λ-isomer. As with groove binding, direct coordination to base positions requires a complementary symmetry, with the the Λ-isomer binding *against* the right-handed groove. This preference for the Λ-isomer reaffirms that, rather than noncovalent intercalation (which would favor the Δ-

isomer), covalent binding dominates the interaction. The energetic stabilization in direct coordination of the ruthenium(II) center is certainly more substantial than the weaker stabilization derived from intercalation. $Rh(phen)_2Cl_2^+$ and its derivatives have also been shown to bind covalently to DNA but only upon photoactivation, since light is needed to promote dissociation of the coordinated chloride and substitution of the nucleic acid base as a ligand.[49]

B. Techniques to Monitor Binding

Many of the same techniques employed in studying the basic chemistry of coordination complexes can be be used in following the binding of transition-metal complexes to nucleic acids, but biochemical methods, with their often exquisite sensitivity, become valuable aids as well in delineating specific binding interactions. Tris(phenanthroline) metal complexes are particularly useful to illustrate this point, since here the metal center in the complex is selected in terms of the technique used for examination.

Coordination complexes are often visibly colored, and these colorations provide a useful and sensitive spectroscopic handle in following fundamental reactions. This notion holds as well with tris(phenanthroline) metal complexes in their interactions with nucleic acids. $Ru(phen)_3^{2+}$ and its derivatives are highly colored because of an intense metal-to-ligand charge-transfer band ($\lambda_{max} = 447$ nm, $\epsilon = 1.9 \times 10^4\ M^{-1}cm^{-1}$). Furthermore, the complexes are highly photoluminescent ($\lambda_{em} = 610$ nm, $\tau = 0.6\ \mu s$ in aerated aqueous solution). On binding

to nucleic acids these transitions are perturbed. Hypochromism is observed in the charge-transfer band, and intercalation leads to an increase in lifetime of the charge-transfer excited state.[43,46] Indeed, single-photon counting experiments show a biexponential decay in emission from $Ru(phen)_3^{2+}$ bound to double-helical DNA. The longer-lived component ($\tau = 2\ \mu s$) has been assigned as the intercalated component and the shorter-lived 0.6 μs component has been attributed to a mixture of free and groove-bound species. These spectroscopic perturbations permit one to define equilibrium-binding affinities for the different components of the interaction as a function of metal-center chirality and under different solution conditions.[41] One can also follow the polarization of emitted

light from the complexes after excitation with polarized light, and these studies have been helpful in describing the dynamics of association of the complexes on the helix.[41,43] Mixed-ligand complexes of ruthenium(II) show similar spectroscopic perturbations, and these have been used to characterize binding affinities and chiral preferences, as well as the extent of intercalation versus groove binding as a function of ligand substitution on the metal center.[42] The spectroscopic handle of the metal center therefore affords a range of experiments to monitor and characterize the binding of the metal complexes to polynucleotides.

There are numerous other classic techniques of inorganic chemistry that have been or could be applied in studying the binding of metal complexes to nucleic acids. Coordination complexes have invariably been used in x-ray diffraction experiments because of the high electron density of the metal center. The tris(phenanthroline) metal complexes have not yet been applied in this context, but, as mentioned already, platinum metallointercalators were examined by fiber diffraction to delineate intercalation requirements. In fact, many nucleic-acid crystal structures have required specific metal ion additions for isomorphous heavy-metal derivatives to solve the structure. Such has certainly been true for the crystal structure of $tRNA^{Phe}$, where heavy-metal ions such as platinum, osmium, and mercury were targeted to specific base positions, and lanthanide ions were used to label phosphate positions around the periphery of the molecule.[53] Other techniques can also be exploited to monitor and characterize binding. A recent novel illustration is one from electrochemistry, which has been applied in monitoring the binding of $Co(phen)_3^{3+}$ to DNA.[54] Surely other techniques, from EXAFS to scanning tunneling microscopy, will be exploited in the future.

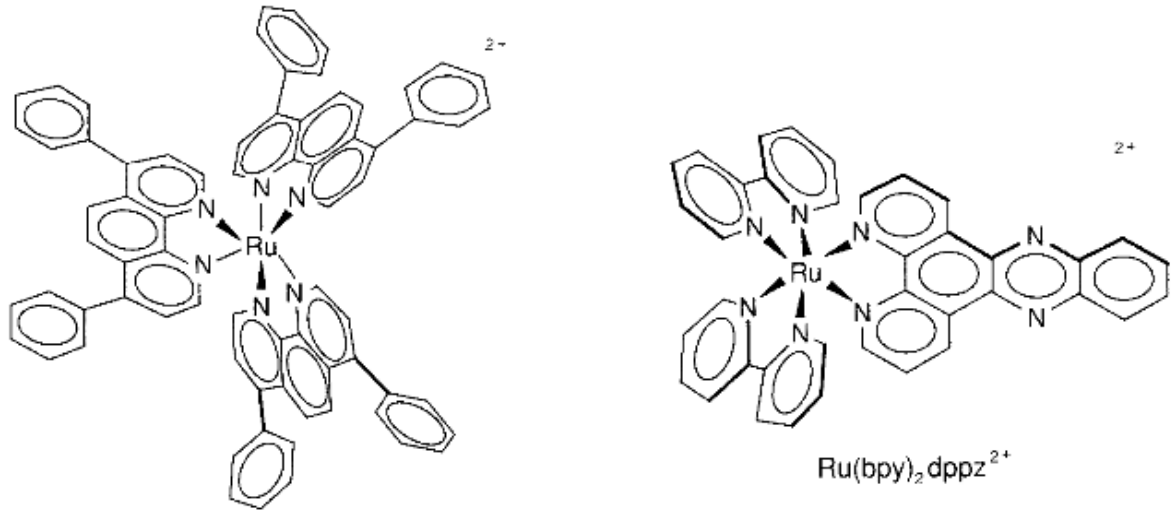
IV. APPLICATIONS OF DIFFERENT METAL COMPLEXES THAT BIND NUCLEIC ACIDS

Both the spectroscopy and the chemical reactivity of transition-metal complexes, coupled to biochemical assays, can therefore be exploited to obtain a wide range of useful reagents to probe nucleic acids. Here some specific applications are described.

A. Spectroscopic Probes

As discussed above, the tris(phenanthroline)ruthenium(II) complexes offer a novel spectroscopic probe of nucleic acids, since their luminescence is increased upon intercalation into the double helix. As a result the complexes provide a simple luminescent stain for DNA in fluorescent microscopy experiments. More interesting, perhaps, is the conformational selectivity of derivatives of tris(phenanthroline)ruthenium. $Ru(DIP)_3^{2+}$ (DIP = 4,7-diphenyl-1,10-phenanthroline) shows enantiospecificity in binding to B-form DNA.[40] Because of the steric bulk of the phenyl rings, detectable binding is seen only with the Δ-isomer in a right-handed helix; no binding is evident with the Λ-isomer. But with the left-handed Z-form helix, both isomers bind avidly.[40,58] The shallow left-handed major groove can accomodate the two enantiomers. A left-handed but more B-like helix shows selectivity instead for the Λ-isomer. Spectroscopic experiments that measure the chiral selectivity of $Ru(DIP)_3^{2+}$ isomers in binding to a given DNA then provide a novel probe for helical handedness. Indeed, Λ-$Ru(DIP)_3^{2+}$ was the first spectroscopic probe for Z-DNA (or other alternate conformations that are sufficiently unwound to permit binding by the bulky left-handed isomer).[58]

Both simpler bipyridyl and phenanthroline derivatives as well as dppz complexes of ruthenium are currently being tethered onto other DNA binding moieties, in particular onto oligonucleotides, so as to develop new, nonradioactive luminescent probes for DNA sequences. These transition-metal complexes may provide the basis for the development of new families of DNA diagnostic agents, and many industrial laboratories are currently exploring routes to accomplish these goals. Figure 8.12 illustrates Λ-$Ru(DIP)_3^{2+}$ and $Ru(bpy)_2dppz^{2+}$, two complexes whose luminescence properties can be employed to probe nucleic acids.



Λ-Ru(DIP)$_3^{2+}$ $Ru(bpy)_2dppz^{2+}$

Figure 8.12
Two spectroscopic probes of nucleic acids: Λ-Ru(DIP)$_3^{2+}$ and $Ru(bpy)_2dppz^{2+}$.

B. Metallofootprinting Reagents

Probably the most widespread application of metal nucleic-acid chemistry in the biology community has been the utilization of metal complexes for chemical footprinting. The footprinting technique (Figure 8.11) was developed by biologists[62] as a means of locating protein-binding sites on DNA. ^{32}P-end-labeled double-stranded DNA fragments could be digested with a nuclease, such as DNAse, in the presence or absence of DNA-binding protein. After electrophoresis of the denatured digests and autoradiography, one would find a ''footprint,'' that is, the inhibition of cleavage by DNAse, at the spot bound by protein, in comparison to a randomly cleaved pattern found on the DNA in the absence of binding protein. Although DNAse is still widely used, this footprinting reagent has some disadvantages: (i) the nuclease is not sequence-neutral in its cleavage, resulting in lots of noise in the footprinting background; and (ii) since the nuclease is itself a large protein, its ability to provide high-resolution footprinting patterns of smaller molecules is quite limited.

Inorganic photochemistry has also been applied in developing metal complexes as photofootprinting reagents. Uranyl acetate, for example, at high concentrations, upon photolysis, promotes DNA cleavage.[66] It is thought that the ions interact with the phosphates, generating some excited-state radical chemistry, although no detailed characterization of this chemistry has been undertaken.

C. Conformational Probes

Metal complexes are also finding wide application in probing the local variations in conformation that arise along nucleic-acid polymers. X-ray crystallography has been critical in establishing the basic conformational families of double-helical DNA, and to some extent how conformations might vary as a function of nucleic-acid sequence. Yet many conformations have still not been described to high resolution, and only a few oligonucleotides have been crystallized. Other techniques are therefore required to bridge the small set of oligonucleotide crystal structures that point to plausible structures and the large array of structures that arise as a function of sequence on long helical polymers. Furthermore, only a very small number of RNA polymers has been characterized crystallographically; hence other chemical methods have been needed to describe the folding patterns in these important biopolymers. Metal complexes, mainly through specific noncovalent interactions, appear to be uniquely useful in probing the structural variations in nucleic acids.

1. Nonspecific reactions of transition-metal complexes

Hydroxyl radical cleavage with $Fe(EDTA)^{2-}$ illustrates again how simple metal complexes can be used in characterizing nucleic acids. One example involves efforts to describe the local structural variations in ''bent'' DNA. Biochemists had found that DNA fragments containing runs of adenines, such as in the tract dAAAAAA, possessed unusual gel-electrophoretic mobilities. Indeed, kinetoplast DNA isolated from mitochondria of trypanosomes showed a remarkable lacework pattern of structure, with loops and circles of DNA; these structures were found to be governed by the placement of these $d(A)_6$ tracts. By constructing a series of oligonucleotides with adenine runs positioned either in or out of phase relative to one another, researchers found that the adenine tracts caused a local bending of the DNA toward the minor groove.[6] But what were the detailed characteristics of these bent sites? Using hydroxyl radical cleavage of DNA, generated with $Fe(EDTA)^{2-}$, Tullius and coworkers found a distinctive pattern of cleavage across the adenine tracts, consistent with a locally perturbed structure.[68] Here the notion again was that $Fe(EDTA)^{2-}$ in the presence of peroxide would generate hydroxyl radicals at a distance from the helix, and thus careful densitometric analysis of the cleavage across ^{32}P-end-labeled DNA fragments would reveal any differential accessibility of sugar residues to cleavage mediated by the radicals caused by the bending. The cleavage patterns suggested a smooth bending of the DNA across the tract and indicated furthermore an asymmetry in structure from the 5′- to 3′-end of the adenine run.

2. Transition-metal complexes as shape-selective probes

Transition-metal complexes have also been designed with three-dimensional structures that target complementary structures along the helical polymer. This recognition of DNA sites, based upon *shape selection*, has proved to be extremely useful both in demarcating and in characterizing structural variations along the polymer and in developing an understanding of those factors important to the recognition of specific polynucleotide sites. Complexes, basically derivatives of the tris(phenanthroline) metal series, have been designed that specifically target A- and Z-form helices, cruciforms, and even subtle variations such as differential propeller twisting within B-form DNA.[11c] By appropriate substitution of the metal at the center of the coordinatively saturated complex, complexes that cleave the DNA at the binding site are obtained. Figure 8.14 shows some of these shape-selective conformational probes.

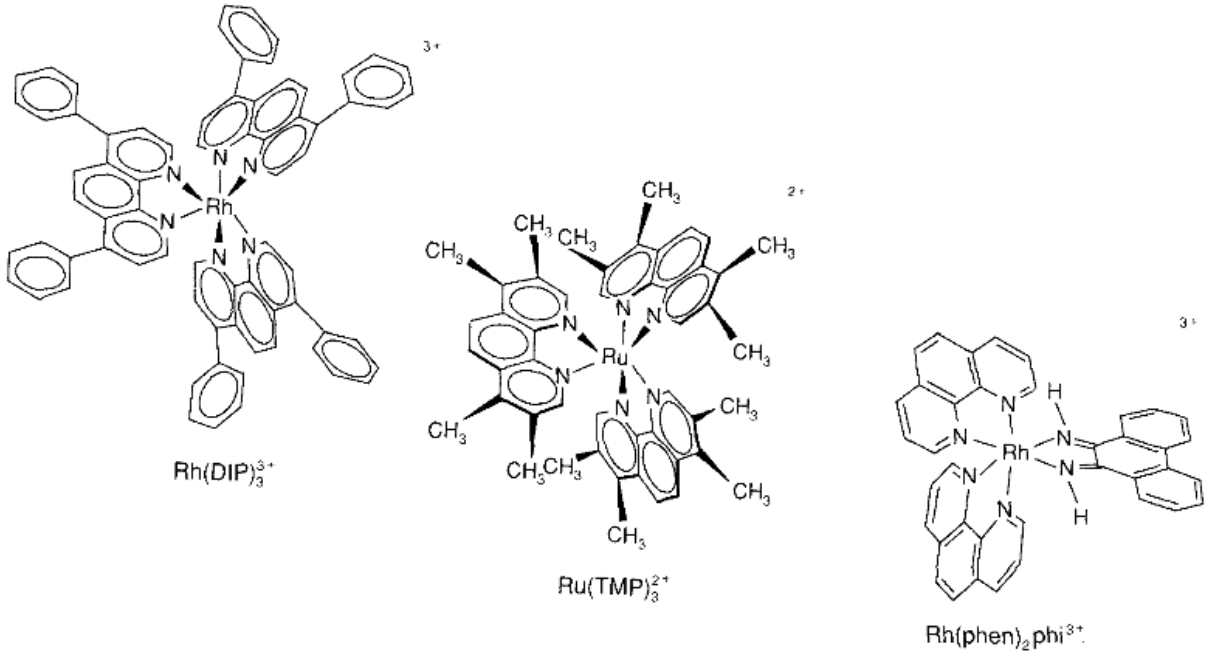


Figure 8.14
Shape-selective probes that target local DNA conformations. $Rh(DIP)_3^{3+}$, which with photoactivation promotes double-stranded cleavage at cruciform sites; $Ru(TMP)_3^{2+}$, a photoactivated probe for A-like conformations; and $Rh(phen)_2phi^{3+}$, which targets openings in the DNA major groove.

Which of the following agents used in drug combination regimens to treat testicular carcinoma is mot likely to cause nephrotoxicity? of the following agents used in drug combination regimens to treat testicular carcinoma is mot likely to cause nephrotoxicity?	Bleomycin	Cisplatin	Etoposide	Leuprolide		Cisplatin
The following anticancer drug has high emetogenic potentia	a) Vincristine	Chlorambucil	6-Mercaptopurine	Cisplatin		Cisplatin
The most effective antiemetic for controlling cisplatin	(a) Prochlorperazine	Ondansetron	Metoclopramide	Promethazine		Ondansetron

induced vomiting is						
A patient undergoing cancer chemotherapy is vomiting frequently. A drug that might help in this situation is	(a) Bromocriptine	Cimetidine	Ketanserin	Ondansetron		Ondansetron
Which one of the following statements about the mechanisms of action of drugs used in cancer chemotherapy is least accurate ?	Alkylatine agents commonly attack the nucleophilic N-7 position in guanine	Anthracyclines intercalate with base pairs to block nucleic acid synthesis	In steady doses, leuprolide inhibits the release of pituitary gonadotropins	Mercaptopurine is an irreversible inhibitor of HGPRT ase		Mercaptopurine is an irreversible inhibitor of HGPRT ase
Which of the following drugs or drug groups is not useful in the prevention of nausea	a) Dexamethasone	Dronabinol	Ketaserin	Ondansetron		Ketaserin

and vomiting included by cancer chemotherapy ?					
Cancer chemotherapy induced vomiting that is not controlled by metoclopramide alone can be suppressed by combining it with	(a) Amphetamine	Dexamethasone	Hyoscine	Cyclizine	Dexamethasone
Dose-response curves are used for drug evaluation in the animal laboratory and in the clinic, Quantal dose-response curves are often	Used for determining the therapeutic index of a drug	Used for determining the maximal efficacy of a drug	Invalid in the presence of inhibitors of the drug being studied	Obtained from the study of intact subject but not from isolated tissue preparations	Used for determining the therapeutic index of a drug
'Drug efficacy' refers to	The range of diseases in which the drug is beneficia	The maximal intensity of response that can be produced by the drug	The therapeutic dose range of the drug	The therapeutic index of the drug	The maximal intensity of response that can be produced by the drug
The therapeutic index	a) Safety	Potency	Efficacy	Dose variability	Safety

of a drug is a measure of its					
Following statement is true regarding therapeutic index	It is based on animal toxicity data	It reflects forms of toxicity that are important clinically	It takes into account idiosyncratic toxic reaction	All the above	It is based on animal toxicity data
When a drug has a low therapeutic index, that drug should be	Used mostly orally	Used mostly intravenously	Considered a potentially toxic substance	Given only in submilligram doses	Considered a potentially toxic substance
Which statement reagading phasespecific chemotherapeutic agents is correct? They	Are most effective in one phase of the cell cycle	Are effective in all phases of the cell cycle	Are only effective in G0 phase	Include the alkylating agents	Are most effective in one phase of the cell cycle
Chemotherapeutic index is defined as	a) LD50 (patient)/LD50 (pathogen)	LD50 (pathogen)/LD50 (patient)	LC50 (patient)/LC50 (pathogen)	LC50 (pathogen)/LC50 (patient)	a) LD50 (patient)/LD50 (pathogen)
Which of the following is always true	A more potent drug is more efficacious	A more potent drug is safer	A more potent drug is clinically superior	A more potent drug can produce the same response at lower doses	A more potent drug can produce the same response at lower doses
Which of the following	a) The study of which functional groups are important to the chemical	b) The study of the physicochemical properties that are important to the	c) The study of the structural features of a drug that are important to its biological	d) The study of the structural features of a drug that are important to its chemical stability	c) The study of the structural features of a drug that are important to its biological

statements best describes structure-activity relationships (SAR)?	reactivity of the drug	absorption of a drug into the blood supply	activity		activity
QSAR method involves	Target structure	Target properties	Ligand x-ray structure	Ligand	Ligand
One of the following is not used in QSAR	Molecular connectivity index	Molecular similarity index	Topological polar surface are	Partition coeffient	Molecular similarity index
One of the following is a quantum chemical parameter	STERIMOL	Taft constant	Highest occupied molecular orbital	Hammett constant	Highest occupied molecular orbital
Which of the following is a QSAR technique performed manually?	Hansch approach	Fujita Ban approach	Free Wilson approach	Topliss approach	Topliss approach
In 3D QSAR, blue regions indicate favourabl	Bulky groups	smaller groups	electron-rich groups	electron-difficient groups	electron-difficient groups

e points for					
In 3D QSAR, green regions indicate favourable points for	Bulky groups	smaller groups	electron-rich groups	electron-difficient groups	Bulky groups
In 3D QSAR, red regions indicate favourable points for	Bulky groups	smaller groups	electron-rich groups	electron-difficient groups	electron-rich groups
In 3D QSAR, yellow regions indicate favourable points for	Bulky groups	smaller groups	electron-rich groups	electron-difficient groups	smaller groups
The first step in the drug discovery process is	lead modification	lead identification	lead validation	lead optimization	lead identification
The safety of the candidate drug in human are studied in	phase I	phase II	phase III	phase IV	phase II
What is the purpose of Phase 1 clinical trials?	To select a lead compound from a lead series	To identify a target population.	To establish the safety of administration to humans	To test whether the proposed drug actually works	To establish the safety of administration to humans

Clinical development represents the shift from_____ to _____.	Laboratory science to patented research and manufacturing technology	) Project management responsibilities needed to manage human trials to technology	Lead compound to patented therapeutic research	Laboratory science to project management responsibilities needed to manage human trials	Laboratory science to project management responsibilities needed to manage human trials
What is the purpose of pre-clinical testing?	A To verify that a drug is sufficiently safe and effective to be tested in humans	To undergo preliminary testing in healthy humans to monitor the effects of the drug	To create a basic outline for the larger scale future tests on a widespread population	A and B	A To verify that a drug is sufficiently safe and effective to be tested in humans
On what does Phase 2 clinical trials test?	Animals	Healthy human volunteers	Widespread differentiated population	People with the target disease/condition	People with the target disease/condition
What is the primary focus of Phase 3 Clinical testing?	How to manage costs	The collection and analysis of highly specific efficacy end-point data	The optimal range of effective dosage.	The analysis of data results from the small-subset target population.	The collection and analysis of highly specific efficacy end-point data
Which phase in clinical development is the largest investment of both time and money?	phase I	phase II	phase III	phase IV	phase III
On what does Phase 3	Animals	Healthy human volunteers	Widespread differentiated population	Large-scale tests in people with the target	Large-scale tests in people with the target

trials test?				disease/populati on	disease/populat ion
Patent is a form of	Tangible Property	Intellectual Property	Industrial property	both b and c	both b and c
Patent protects	Discovery	Invention	New invention	Both (a) and (b)	New invention
Patent right is	Exclusive right	Natural right	Property right	Both (a) and (c)	Exclusive right
Patent right is	Limited period right	Territorial right	) Absolute right	Both (a) and (b)	Limited period right
Patentab ility criteria includes	Novelty	Inventive step	Capable of Industrial application	All the above	All the above
IPC means	Indian Patent Classification	International Panel Code	) International Patent Classification	International Postal Code	) International Patent Classification
Carbopla tin is a	platinum based anti-cancer drug	platinum based diabetics drug	plutonium based anticancer drug	plutonium based diabetics drug	platinum based anti-cancer drug
The drug carboplat in inhibits the synthesis of	RNA	DNA	PLATIN	ALL the above	ALL the above
Cisplatin is a	platinum based anti-cancer drug	platinum based diabetics drug	plutonium based anticancer drug	plutonium based diabetics drug	platinum based anti-cancer drug
Chemical ly cisplatin called as	cis-diamminedichl oridoplatinum(I)	cis-diamminedichlo ridoplatinum(II)	cis-diphenyldichlor idoplatinum(II)	cis-diamminediphen ylplatinum(I)	cis-diamminedichlo ridoplatinum(II)
What is meant by a drug's 'specifica tions'?	The molecular dimensions of a molecule	The physical properties of a drug	The purity tests and purity standards required of a drug	The functional groups on a drug that are important to its activity	The purity tests and purity standards required of a drug
Which of the following is not a priority in	Optimising the overall yield of a drug	Optimising the activity of a drug	Developing a cheap synthetic route	Optimising the purity of a drug	Optimising the activity of a drug

chemical development?					
What term is used to signify a preparation that appears identical to the preparation of an active drug but which has no biological activity?	Dummy drug	Peptidomimetic	Placebo	Gazebo	Placebo
Which of the following would NOT normally be considered as a 'costs of quality'?	prevention costs	warranty costs	marketing costs	inspection costs	marketing costs
Which one of the following would normally be considered as one of the 'costs of quality'?	transaction costs	transport costs	appraisal costs	marketing costs	appraisal costs
Process control is carried out	before production	during production	after production control	All of them	during production

Arrange the steps of QA in ascending order?	Customer needs, material control, design development, process control, marketing	Material control, process control, customer need, design development, finished product	Customer needs, design development, material control, process control, finished product	Material control, servicing, process control, material control, design development	Customer needs, design development, material control, process control, finished product
Which of the following DNA binding proteins interacts with DNA in a sequence specific manner?	Histone H3	DNA polymerase	NF-Kb	RNA polymerase	NF-kB
Which of the following is an equilibrium method that can be used to accurately determine DNA-protein dissociation constants?	Site directed mutagenesis	Chromatin Immunoprecipitatio	EMSA	Footprinting	Footprinting
Of the methods described in Chapter 5, which can be used to	EMSA and ChIP	EMSA only	ChIP only	footprinting	EMSA and ChIP

differentiate between individual DNA binding proteins or a protein complex that recognize the same DNA sequence					
What is the primary purpose of chromatin sonication when performing a ChIP experiment?	Reduce chromatin size	Melt double stranded DNA to single stranded	Reduce viscosity of the sample	Remove proteins binding to DNA	Reduce chromatin size
Of the methods described in Chapter 5, which can be used to detect in vivo DNA-protein interaction?	ChIP and ChIP-seq	Footprinting	EMSA		ChIP and ChIP-seq
In an EMSA experiment free DNA is	Charge	) Molecular weight	DNA digestion with DNAse	Antibody immunoprecipitation	Molecular weight

separated from protein-DNA complexes in a native gel by which following principle?					
DNA binding by proteins with the helix-turn-helix (HTH) motif does not involve	interactions with base pairs in the major groove of DNA	interactions with the sugar-phosphate backbone of DNA	hydrogen bonds, salt bridges, and van der Waals contacts.	melting of the DNA at the center of symmetry	melting of the DNA at the center of symmetry
A promoter is	a manager for a sports team	a specific sequence of DNA to which RNA polymerase binds	a specific sequence of DNA to which a catabolic repressor binds	a specific DNA sequence to which a restriction endonuclease binds.	a specific sequence of DNA to which RNA polymerase binds
The binding of lac repressor to DNA is analogous to	competitive inhibition of an enzyme	allosteric effects in enzyme regulation	uncompetitive inhibition of an enzyme	None of the above	competitive inhibition of an enzyme
The enhancer sites of eukaryotic promoters	are binding sites for activating transcription factors	can act at distances of <1000 base pairs	are found in eukaryotic viruses	All the above	All the above

UNIT-I

Drug Discovery and Design: Outline- therapeutic index, chemotherapeutic index, structure- activity relationship (SAR) and quantitative structure-activity relationship (QSAR)-Factors governing drug design- computer aided drug design-cancer chemotherapy-bioinorganic chemistry (DNA binding) of platinum anticancer drugs (cisplatin and carboplatin)-mechanism of action studies-clinical trials and their significance- production and quality control- patent protection.

In the past few decades there has been a hiatus in the momentum of research and discovery of **'novel medicinal compounds'.** This particular trend in drug development perhaps is augmented due to **two** vital factors, namely : *first*, strict empirical and rational approach to drug design ; and *secondly*, high standards of safety and therapeutic efficacy together with tremendous increased costs of research and development and finally the clinical trials.

'**Drug design**' or **'tailor-made compound'** aims at developing a drug with high degree of chemotherapeutic index and specific action. It is a logical effort to design a drug on as much a rational basis as possible thus reducing to the minimum the trial and error approach. It essentially involves the study of biodynamics of a drug besides the interaction between drug molecules and molecules composing the biological objects.

Drug design seeks to explain :

(*a*) Effects of biological compounds on the basis of molecular interaction in terms of molecular structures or precisely the physico-chemical properties of the molecules involved.

(*b*) Various processes by which the drugs usually produce their pharmacological effects.

(*c*) How the drugs specifically react with the protoplasm to elicit a particular pharmacological response.

(*d*) How the drugs usually get modified or detoxicated, metabolized or eliminated by the organism.

(*e*) Probable relationship between biological activity with chemical structure.

In short, **drug design** may be considered as an integrated whole approach which essentially involves various steps, namely : chemical synthesis, evaluation for activity-spectrum, toxicological studies, metabolism of the drug, *i.e.*, **biotransformation** and the study of the various metabolites formed, assay procedures, and lastly galenical formulation and biopharmaceutics.

The **'drug design'** in a broader sense implies random evaluation of synthetic as well as natural products in bioassay systems, creation of newer drug molecules based on biologically-active-prototypes derived from either plant or animal kingdom, synthesis of congeners displaying interesting biological actions, the basic concept of isosterism and bioisosterism, and finally precise design of a drug to enable it to interact with a receptor site efficaciously.

In the recent past, another terminology **'prodrugs'** has been introduced to make a clear distinction from the widely used term **'analogues'**. **Prodrugs** are frequently used to improve pharmacological or biological properties. **Analogues** are primarily employed to increase potency and to achieve specificity of action.

2. ANALOGUES AND PRODRUGS

In the course of **drug design** the *two* major types of chemical modifications are achieved through the formation of **analogues** and **prodrugs.**

An **analogue** is normally accepted as being that modification which brings about a carbon-skeletal transformation or substituent synthesis. *Examples :* **oxytetracycline, demclocycline, chlortetracycline, trans-diethylstilbesterol** with regard to **oestradiol.**

The term **prodrug** is applied to either an appropriate derivative of a drug that undergoes *in vivo* hydrolysis to the parent drug, *e.g.,* **testosterone propionate, chloramphenicol palmitate** and the like ; or an analogue which is metabolically transformed to a **biologically active drug,** for instance : **phenylbutazone** undergoes *in vivo* hydroxylation to **oxyphenbutazone.**

3. CONCEPT OF 'LEAD'

Another school of thought views **'drug design'** as the vital process of envisioning and preparing specific new molecules that can lead more efficiently to useful drug discovery. This may be considered broadly in terms of two types of investigational activities. These include :

(*a*) **Exploration of Leads,** which involves the search for a new lead ; and

(*b*) **Exploitation of Leads,** that requires the assessment, improvement and extension of the lead.

From the practical view-point it is the latter area wherein rational approaches to drug design have been mostly productive with fruitful results.

3.1 Examples

It is worthwhile to look into the right perspective of a few typical and classical examples of **drug design** as detailed below :

(*i*) Narcotic Analgesics

In the year 1939, Schaumann first identified and recognized the presence of a quaternary-carbon-atom in the morphine molecule, which eventually formed an altogether new basis and opened up a new horizon in the field of **drug design** of narcotic analgesics. Intensive research further led to the evolution of **pethidine** (**meperidine**) which incidentally combines both the properties of **morphine** and **atropine**. It possesses a quaternary carbon-atom and quite astonishingly a much simpler chemical structure to that of **morphine**.

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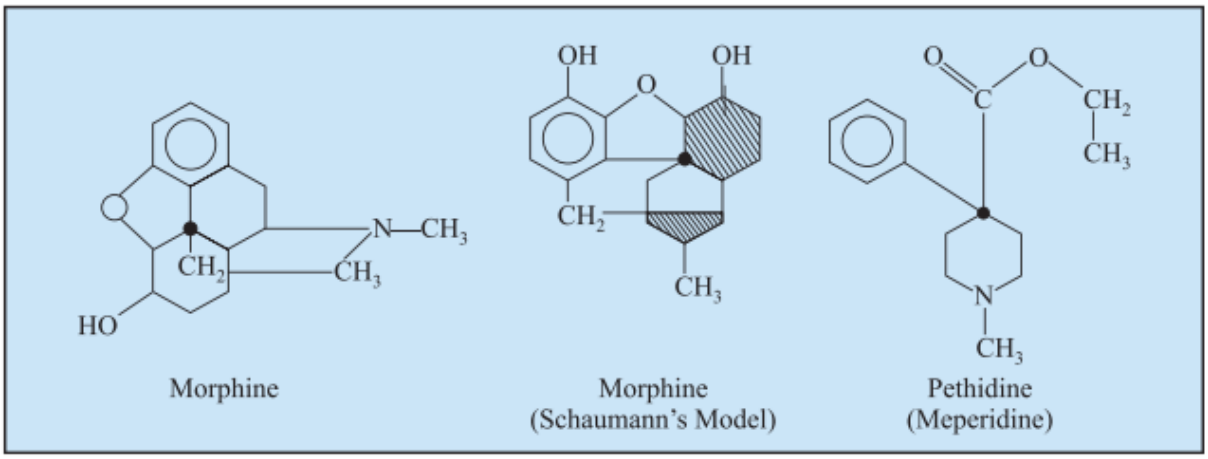
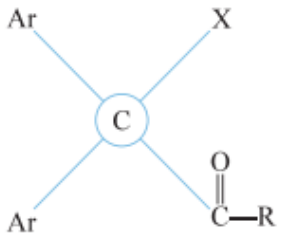


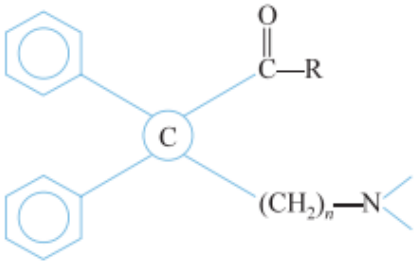
Morphine | Morphine (Schaumann's Model) | Pethidine (Meperidine)

Ehrhardt suggested a **general formula** relevant to the analgesic activity in 1949 as stated below :



where, Ar is the aromatic ring, X the basic side chain and (—C—) carbonyl function in the form of an ester, ketone or an amide.

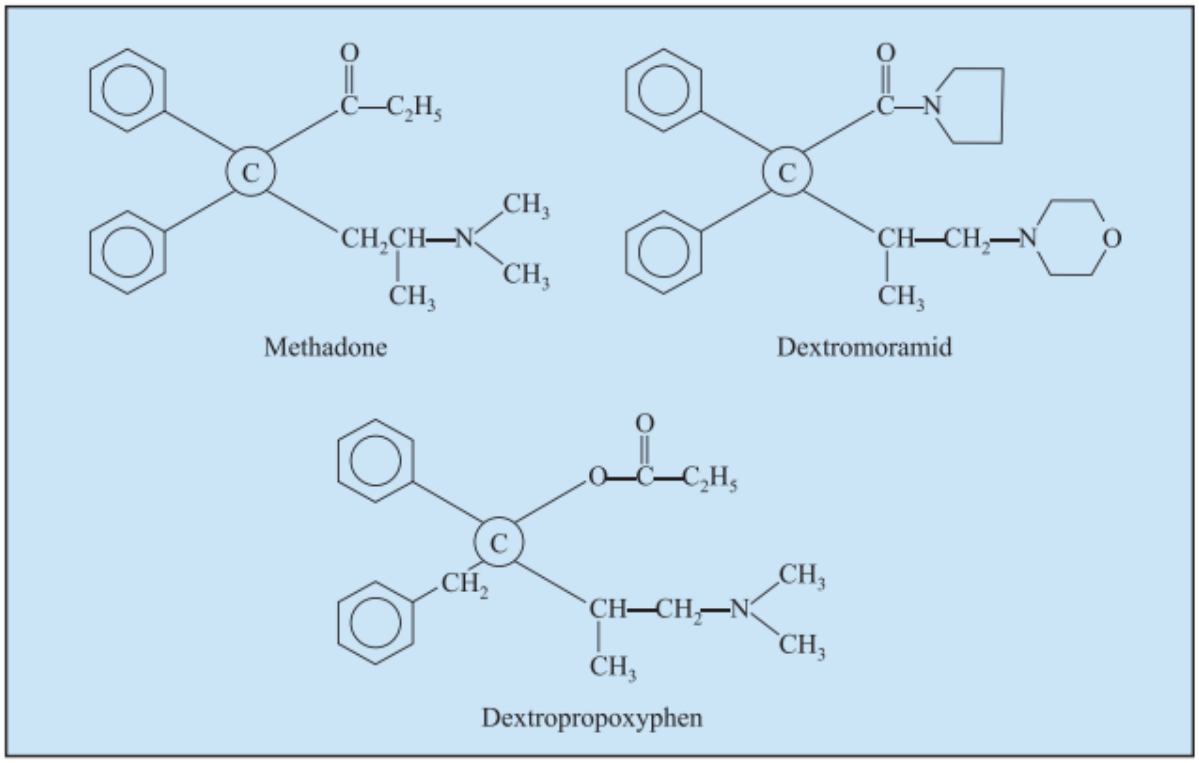
Later on, the above general formula was modified slightly as follows :



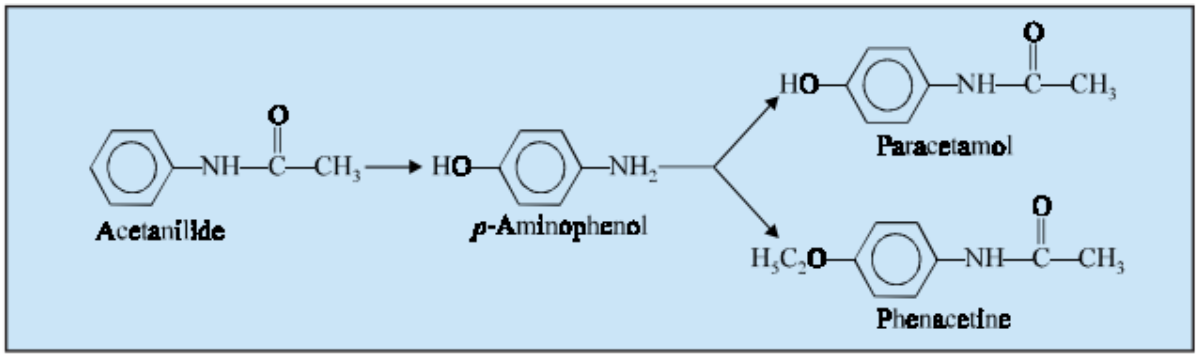
which successfully led to the development of the following *three* **narcotic analgesics,** namely : **methadone, dextromoramid** and **dextropropoxyphen.**

(*ii*) Antipyretic Analgesics

Another fruitful approach in **drug design** is the meticulous screening of the metabolite for probable pharmacological activity. The most interesting example is the bio-oxidation of acetanilide into *para*-aminophenol which subsequently on **chemical manipulation** has yielded better tolerated antipyretic-analgesics like **paracetamol** and **phenacetine.**

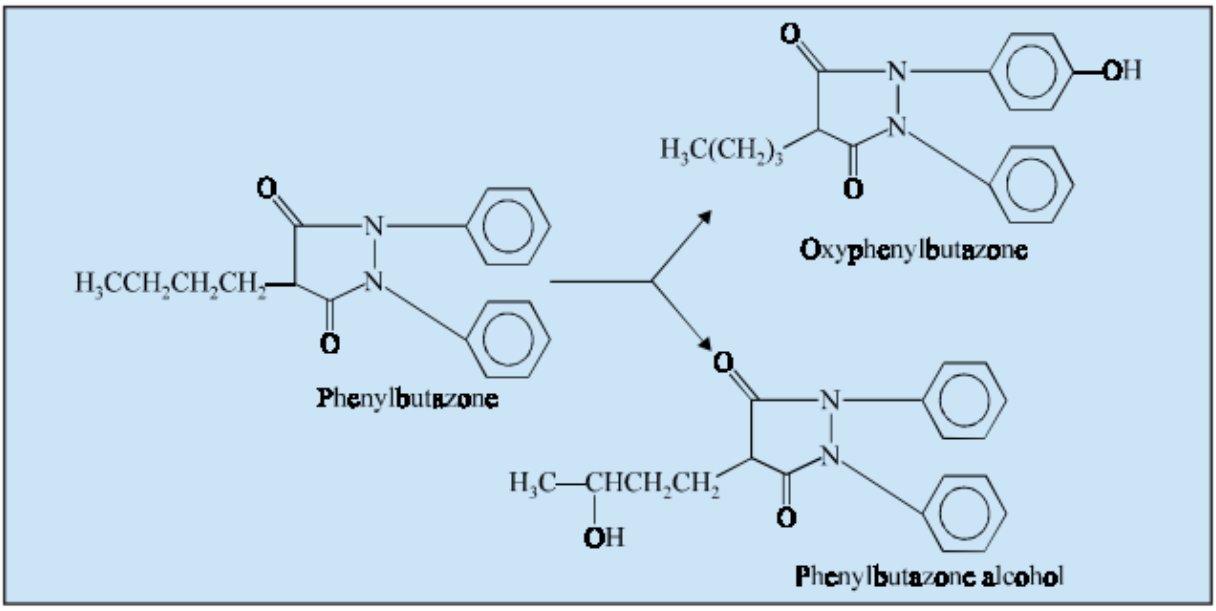


Quite recently **phenacetine** has been withdrawn completely because of its toxic after effects, though it dominated the therapeutic field for over 30 years as a potent antipyretic analgesics.



(*iii*) Antirheumatic Drugs

The study of the metabolite conversion of the antirheumatic drug phenylbutazone resulted in the introduction of a better tolerated drug **oxyphenylbutazone** as an **antirheumatic drug** and **phenylbutazone** alcohol as an **uricosuric agent.**



4. FACTORS GOVERNING DRUG-DESIGN

A few cardinal factors governing the efficacy towards the evaluation of **drug design** include :

(*a*) The smaller the expenditure of human and material resources involved to evolve a new drug of a particular value, the more viable is the design of the programme.

(*b*) Experimental animal and clinical screening operations of the new drugs.

(*c*) Relationships between chemical features and biolgoical properties need to be established retrospectively.

(*d*) **Quantitative structure-activity relationships (QSARs)** vary to an appreciable extent in depth and sophistication based on the nature of evaluation of structure or activity. A purposeful relation of structural variables must include steric factors, electronic features of component functional groups and, in general, the molecule as a whole.

(*e*) The trend to synthesize a huge number of newer medicinal compounds indiscriminately for exploratory evaluation still prevails which exclusively reflects the creative genuineness and conceptual functions of a highly individualized expression of novelty by a medicinal chemist.

(*f*) Introduction of functional groups in a molecule that need not essentially resemble metabolites, but are capable of undergoing bonding interactions with important functional groups of biochemical components of living organisms affords an important basis for exploration.

(*g*) Disease etiologies and various biochemical processes involved prove useful.

In the recent past a tremendous aggresive thrust has been observed in the enormous development of computer-based **adsorption, distribution, metabolism, and elimination (ADME)** of molecular models. Interestingly, a plethora of **predictive ADME molecular models** are heavily dependent upon the extensive and intensive application of **QSAR****. In short, one may have a significant and appreciable insight into the design of **chemical libraries for an elaborative biological evaluation** that could be entirely based upon the ensuing spatial arrangements and descriptors which prove to be absolutely essential and necessary for various **drug-like molecules** still under detailed investigative procedures.

The **pharmaceutical scientiests** of today are adequately equipped with highly advanced and most sophisticated methodologies based upon several latest **molecular modeling software** that would certainly and legitimately help them to attain perfection in the modification of the various structural characteristic features of a '**potential-drug candidate**' **in silico**. In true sense, such **predictions** with regard to the **physicochemical properties** of the **potential-drug candidate** prior to the actual laboratory synthesis invariably prove to be of immense help and guidance to the on-going, time-consuming, and money-churning research undertakings.

Computer-based techniques do offer enough strength and power to accomplish difficult and intricate problems with appreciable convenience. Thus, it is quite evident that the **computer-generated molecular models** (*i.e.*, of '**newer drugs**') should be accurate and precise enough to muster **enough confidence** amalgamated with a reasonably **high-degree of success rate***** amongst its users (*i.e.*, **medicinal chemists**). In other words, one ought to get the procedural steps duly validated, irresective of the wisdom and intellectual calibre of **CADD**, with respect to the **known-drug substances** so as to restore and gain confidence in a plethora of circumstances when similar techniques shall be applied to the **unknown-drug substances** *i.e.*, the newly designed molecules. Thus, the stark reality in terms of the distinct apparent differences between a **computer-generated model** and **reality of a known-drug** must always be borne in mind while making use of **computer simulations** in **drug-design**.

Nevertheless, the fundamental objective of **computer assisted drug design (CADD)** is to generate, and subsequently understand meticulously the most complex and intricate prevailing relationships at the molecular level between a skilfully designed **drug-like molecule** and a **disease-producing target** (*i.e.*, a **macromolecule**) in order to enable a medicinal chemist to make a fairly reliable and trustworthy prediction to increase molecular interactions with utmost accuracy.

There are a plethora of very critical and most vital **pharmacokinetic** characteristic properties so as to obtain a highly specific and effective therapeutic drug substance. Lipinski *et. al.* (1997)* postulated that the *three* **major physical variables** *viz.*, **potency**, **solubility** and **permeability** may be carefully adapted to increase the overall activity of **potential oral drug** substances **predominantly**. They also observed that relatively poor permeation (*i.e.*, absorption) is commonly attributed by the following characteristic features either inducted alone or more than one right into the proposed drug molecule :

Singh *et al.* (2003)** put forward a more latest predictive model (design) for the **cytochrome P-450 (CYP) 3A4** metabolism. This method exclusively rests upon the **primary lateral sclerosis (PLS)**, however, one of the descriptors is totally based on acute myocardial infarction (AMI)-calculated H-atom abstraction process.

In fact, there are several important assumptions, namely :

(1) **CYP-3A4 :** its greater susceptibility is a determining factor of the electronic atmosphere surrounding the specific H-atom undergoing abstraction phenomenon,

(2) **Abstraction** of the particular H-atom designates the '**rate-determining step**', and

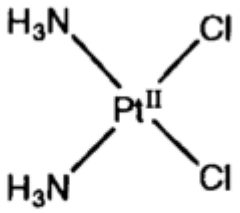
(3) '**Drug**' undergoing the process of metabolism enjoys almost a free access in the '**active-site**', of the specific enzyme till such time the '**most active H-atom**' is avilable abundantly.

AMI-H-atom Abstraction : The AMI-calculations essentially makes use of a procedure to explain the fact that '**unpaired electrons**' are involved, which eventually interacted on a series of known drug substances. It may be modified duly according to the availability of **chemical descriptors**.

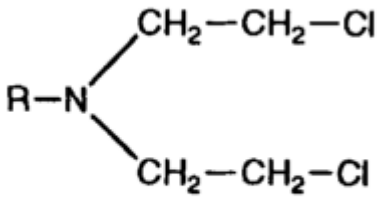
By the year 2020, there lies a tremendous scope for the phenomenal advancement and increment of both **toxicity predictions** and **in-silico characteristic feature predictions**. The **dependability**, **versatility**, and **reliability** of the **predictive ADME** procedures and methodologies would overwhelmingly incorporate and legitimately include its dire and intimate presence in practically each and every initial molecular modeling drug-design process rather than at a stage when the drug has already conceived literally.

CHELATION AND ANTICANCER ACTIVITY

The serendipitous discovery by Professor Barnett Rosenberg in the 1960s that the presence of a platinum wire in a culture caused severe growth disturbance of micro-organisms led to other revelations that coordination compounds of Periodic Table Group VIII in the d-block of the transition series possess anticancer (cytotoxic) activity. The difficulty encountered that such complexes tend to hydrolyse rapidly was suppressed by focussing on platinum, one of the more inert complexing metal ions. The first agent was *cis*-dichlorodiammineplatinum(II), which loses two chloride ions to form a platinum chelate with two nitrogens in the pyrimidine and purine bases of the DNA chain in the cell nucleus to form an intrastrand link that interferes with the copying of the DNA chain when the cell next attempts to divide. Other long-established cytotoxic agents, such as the nitrogen mustards, are known to form similar cross-links, but between bases on each of the two strands of the DNA molecule, *i.e.* interstrand links.



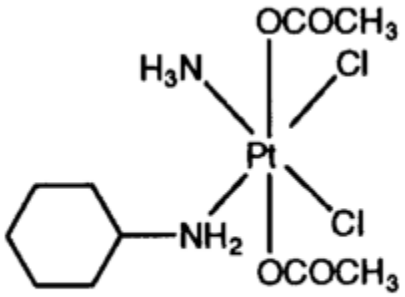
Cis-dichlorodiammine platinum(II) (Cisplatin®)



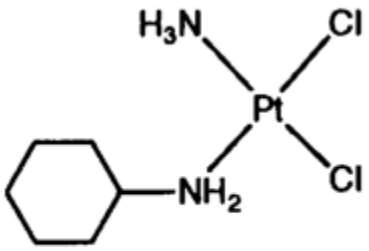
Nitrogen mustards

The spatial separation of the chloride ions in the *cis*-platinum complexes, 0.33 nm, and those of the chlorides at the ends of the chloroethyl arms of the nitrogen mustard, 0.80 nm, suit the formation of intra- and interstrand bridges, respectively. The cell-killing effect results from an inability, or very much decreased ability, of tumour cells to repair the intrastrand breaks.

Cis-dichlorodiammineplatinum(II) (Cisplatin®) was introduced clinically in the UK in 1979, when it was claimed to be the first heavy metal compound marketed for use in cancer treatment. Cisplatin has proved to be a very effective agent, either used alone or in combination with vinblastine and/or other cytotoxic drugs, in the treatment of ovarian and testicular cancer and also lung cancer. However, the agent suffers from serious disadvantages: first, it must be infused intravenously, and, secondly, it is extremely toxic, causing nausea and vomiting, as well as leukopaenia and renal dysfunction. Further, some tumours develop resistance to the drug. Recent research has concentrated on overcoming these defects; attaching a 1,1–dicarboxycyclobutane molecule to the diammineplatinum(II) to produce the *cis*-1,1–dicarboxycyclobutane-diammineplatinum(II) derivative, Carboplatin®, has removed some of the disadvantages to produce a second generation agent which is now in clinical use.



JM216



JM118

More recently, an orally active platinum drug [bis-acetatoammine-dichloro(cyclohexylamine)platinum(IV)] (JM216) has been developed. This compound is completely metabolized in the body to six metabolites. The main plasma metabolite is the platinum(II) reduction product [ammine(cyclohexylamine)-dichloroplatinum(II)] (JM118) and this compound appears likely to be the active cytotoxic moiety. JM216 is now undergoing clinical trials and may well enter clinical practice by the mid-1990s. A further interesting development, that is still in the research phase, is the compound [transammine(cyclohexylamine)-dichlorodihydroxoplatinum(IV)]. This is the first *trans*-platinum compound to show any selective antitumour activity *in vivo*, and in contrast to Cisplatin it appears to form interstrand cross-links in DNA.

Mechanism of action Studies

Charles River uses its adenoviral technology to elucidate the mechanism of action of small molecules. By combining the compound of interest with knockdown or overexpression of candidate gene in the same phenotypic assay, we can start to elucidate the mechanism of action of the compound(s).

In addition, we are able to access our other technologies to perform mode of action studies:

- Use of our in-house chemogenomics database to predict candidate targets
- Cross-screening of compounds of interest through panels of biochemical and cell-based assays linked by candidate pathways or candidate gene families
- Biophysical assays to look for compound binding to candidate target proteins – SPR, size exclusion chromatography, X-ray crystallography