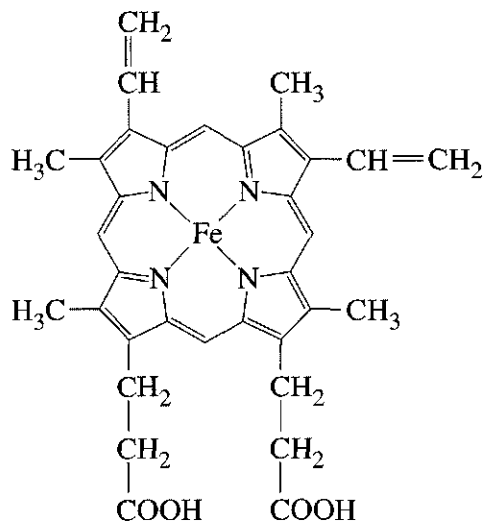


CHAPTER

16

Bioinorganic and
Environmental
Chemistry

Inorganic compounds of many types have biological action—for example, as toxins or medicines when ingested, as part of the body's normal functioning, or enabling essential processes in plants. The list of such compounds is far too long to cover with any degree of completeness in a short chapter. The approach here is to give only a few representative examples of bioinorganic compounds and their actions, along with some examples of the environmental effects of both metals and nonmetals.

Many biochemical reactions depend on the presence of metal ions. These ions may be present in specific coordination complexes or may act to facilitate or inhibit reactions in solution. In the first part of this chapter, we describe a few of these compounds and reactions, together with the biochemistry of NO, which has many functions that have only recently been discovered.

Many metals are essential to plant and animal life, although in many cases their role is uncertain. The list includes all the first-row transition metals except scandium and titanium, but only molybdenum and perhaps tungsten from the heavier transition metals.¹ Table 16-1 lists several that are important in mammalian biochemistry. The importance of iron is obvious from the number of roles it plays, from oxygen carrier in hemoglobin and myoglobin to electron carrier in the cytochromes to detoxifying agent in catalase and peroxidase.

How do inorganic compounds and ions help cause biochemical reactions? A partial list of their actions is given here, most related to metal ion complexes.

1. Promotion of reactions by providing appropriate geometry for breaking or forming bonds. Although many coordination sites in bioinorganic molecules are approximately tetrahedral, octahedral, or square-planar, they have subtle variations that provide for unusual reactions. An organic ligand may provide a pocket that is slightly too large or small for a particular reactant, or may have angles that make other sites on the metal more reactive. The binding of small molecules can also create reactive species by forcing them to adopt unusual angles or bond distances.

¹E. Frieden, *J. Chem. Educ.*, 1985, 65, 917.

TABLE 16-1
Metal-containing Enzymes and Proteins

<i>Metal</i>	<i>Compounds and Actions</i>
Fe (heme)	Hemoglobin, peroxidase, catalase, cytochrome P-450, tryptophan dioxygenase, cytochrome <i>c</i> , nitrite reductase
Fe (non-heme)	Pyrocatechase, ferredoxin, hemerythrin, transferrin, aconitase, nitrogenase
Cu	Tyrosinase, amine oxidases, laccase, ascorbate oxidase, ceruloplasmin, superoxide dismutase, plastocyanin, nitrite reductase
Co (B ₁₂ coenzyme)	Glutamate mutase, dioldehydrase, methionine synthetase
Co(II) (non-corrin)	Dipeptidase
Zn(II)	Carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase, DNA polymerase
Mg(II)	Activates phosphotransferases and phosphohydrolases, DNA polymerase
K(I)	Activates pyruvate phosphokinase and K-specific ATPase
Na(I)	Activates Na-specific ATPase
Mo	Nitrogenase, nitrate reductase, xanthine oxidase, formate dehydrogenase, sulfite oxidase, DMSO reductase
W	Aldehyde ferredoxin oxidoreductase

- Changes in acid-base activity. Water bound to a metal ion frequently is more acidic than free water, and coordination to proteins enhances this effect still more. This results in M—OH species that can then react with other substrates. Mg²⁺ and Zn²⁺ are common metal ions that serve this function.
- Changes in redox potentials. Coordination by different ligands changes redox potentials, making some reactions easier and some more difficult, and provides pathways for electron transfer.
- Some ions (Na⁺, K⁺, Ca²⁺, Cl⁻) act as specific charge carriers, with concentration gradients maintained and modified by membrane ion pumps and trigger mechanisms. Sudden changes in these concentration gradients are signals for nerve or muscle action.
- Organometallic reactions can create species that are otherwise not attainable. Cobalamin enzymes are particular examples of these catalysts.
- Inorganic ions, both cationic and anionic, are used as structural units to form bone and other hard structures. Maintenance of cell membranes and DNA structure also depends on the presence of cations to balance charges in the organic portions.
- A few small molecules have specific effects that do not fit easily into any of the categories above. Perhaps the most obvious is NO, which has many functions, primarily related to control of blood flow, neurotransmission, learning, memory, and, at higher concentrations, as a defensive cytotoxin against tumor cells and pathogens.

A relatively new feature of the study of bioinorganic molecules is the use of molecular orbital calculations to guide research into their mechanism of action. This is similar to calculations of minimum energies and transition states for other reactions, but requires either careful design of models to include the essential features of the large protein and nucleic acid molecules or their inclusion in the calculation, at considerable cost in complexity and time. Although such calculations can uncover new possibilities and show others as unlikely, they are not yet at the stage where they can prove any mechanism to be the true description. The results frequently depend on the design of the model, the complexity of the data sets used, and the computation methods; the environment around the active site may also be very important to the results.

16-1 PORPHYRINS AND RELATED COMPLEXES

One of the most important groups of compounds is the **porphyrins**, in which a metal ion is surrounded by the four nitrogens of a porphine ring in a square-planar geometry and the axial sites are available for other ligands. Different side chains, metal ions, and surrounding species result in very different reactions and roles for these compounds. The parent porphine ring and some specific porphyrin compounds are shown in Figure 16-1.

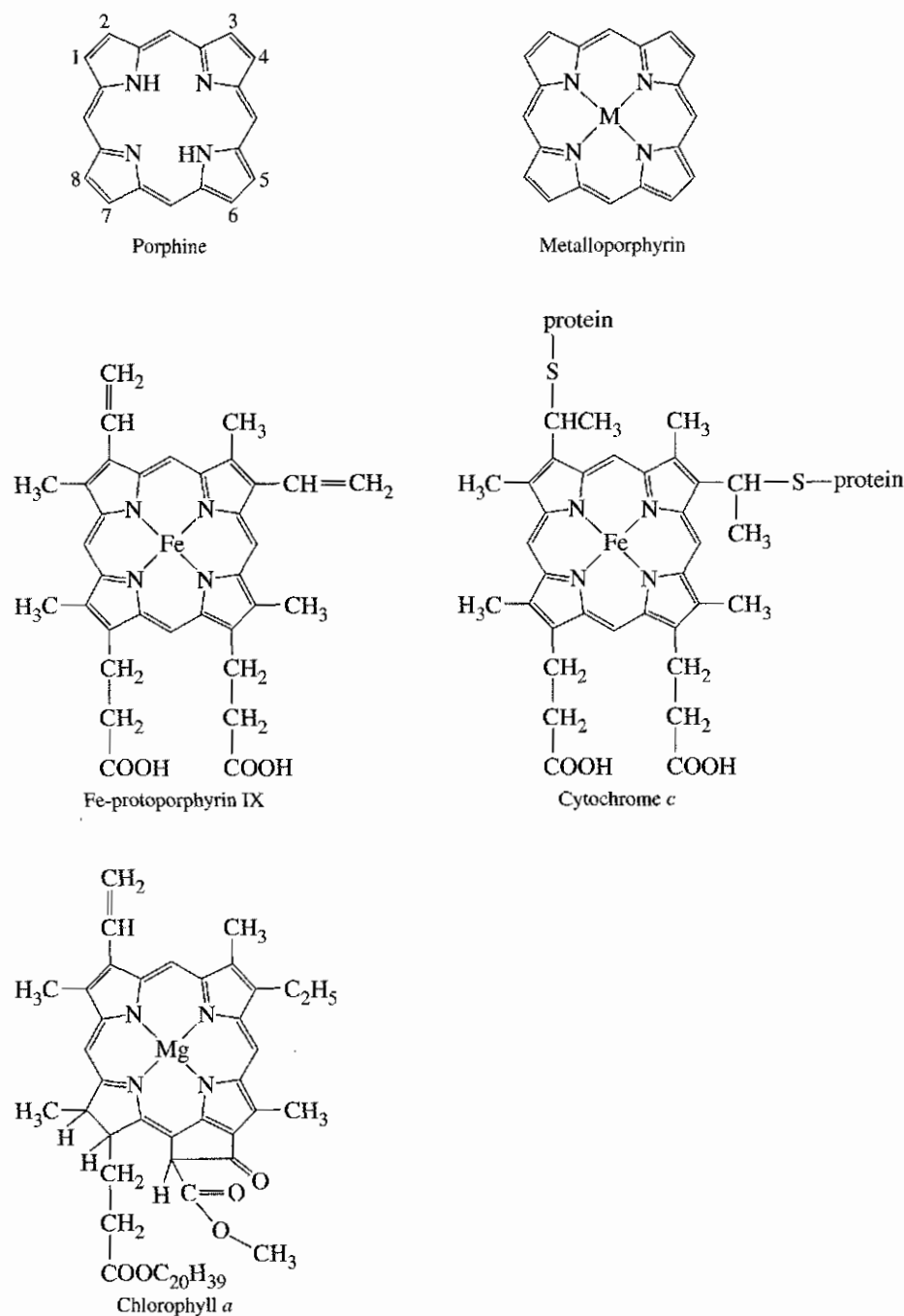
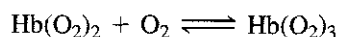
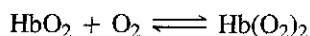


FIGURE 16-1 Porphine, Porphyrin, and Related Compounds.

16-1-1 IRON PORPHYRINS

Hemoglobin and myoglobin

The best known iron porphyrin compounds are hemoglobin and myoglobin, oxygen transfer and storage agents in the blood and muscle tissue, respectively. Each of us has nearly 1 kg of hemoglobin in our body, picking up molecular oxygen in the lungs and delivering it to the rest of the body. Each hemoglobin molecule is made up of four globin protein subunits, two α and two β . In each of these, the protein molecule partially encloses the heme group, bonding to one of the axial positions through an imidazole nitrogen, as shown in Figure 16-2. The other axial position is vacant or has water bound to it (the imidazole ring from histidine E7 is too far from the iron atom to bond). When dissolved oxygen is present, it can occupy this position, and subtle changes in the conformation of the proteins result. As one iron binds an oxygen molecule, the molecular shape changes to make binding of additional oxygen molecules easier. The four irons can each carry one O_2 , with generally increasing equilibrium constants:



The equilibrium constants increase, with the fourth constant many times larger (depending on the biological species from which the hemoglobin came) than the first; in the absence of the structural changes, K_4 would be much smaller than K_1 . As a result, as soon as some oxygen has been bound to the molecule, all four irons are readily oxygenated. In a similar fashion, initial removal of oxygen triggers the release of the remainder and the

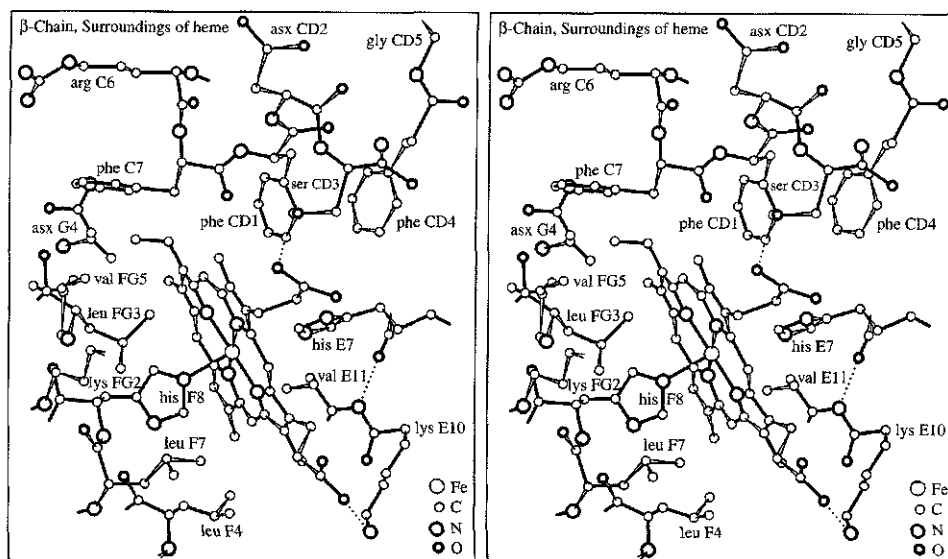
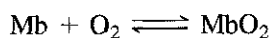


FIGURE 16-2 Heme Group Binding in Hemoglobin. Illustrated here is a stereo drawing of the surroundings of the heme in the β chain of hemoglobin. Broken lines indicate hydrogen bonds. (Reprinted by permission from J. F. Perutz and H. Lehmann, *Nature*, 1968, 219, 902. © 1968 Macmillan Magazines Limited.)

entire load of oxygen is delivered at the required site. The structural changes accompanying oxygenation have been described thoroughly by Baldwin and Chothia² and by Dickerson and Geis.³ This effect is also favored by pH changes caused by increased CO₂ concentration in the capillaries. As the concentration of CO₂ increases, formation of bicarbonate ($2\text{H}_2\text{O} + \text{CO}_2 \rightleftharpoons \text{HCO}_3^- + \text{H}_3\text{O}^+$) causes the pH to decrease and the increased acidity favors release of O₂ from the oxyhemoglobin, called the Bohr effect.

Myoglobin has only one heme group per molecule and serves as an oxygen storage molecule in the muscles. The myoglobin molecule is similar to a single subunit of hemoglobin. Bonding between the iron and the oxygen molecule is similar to that in hemoglobin, but the equilibrium is simpler because only one oxygen molecule is bound:



When hemoglobin releases oxygen to the muscle tissue, myoglobin picks it up and stores it until it is needed. The Bohr effect and the cooperation of the four hemoglobin binding sites make the transfer more complete when the oxygen concentration is low and the carbon dioxide concentration is high; the opposite conditions in the lungs promote the transfer of oxygen to hemoglobin and the transfer of CO₂ to the gas phase in the lungs. As shown in Figure 16-3, myoglobin binds O₂ more strongly than the first O₂ of hemoglobin. However, the fourth equilibrium constant of hemoglobin is larger than that for myoglobin by a factor of about 50.

In hemoglobin, the iron is formally Fe(II) and bonding to oxygen does not oxidize it to Fe(III). However, when the heme group is removed from the protein, exposure to oxygen oxidizes the iron quickly to a μ -oxo dimer containing two Fe(III) ions. The presence of hydrophilic protein around the heme seems to prevent oxidation of Fe(II) in hemoglobin, but the presence of water alone allows oxidation of the free heme. In a test of this hypothesis, Wang⁴ embedded a heme derivative saturated with CO in a polystyrene matrix and studied its equilibrium with CO and O₂. He was able to cycle the material between the oxygenated form, the CO-bound form, and the free heme with no oxidation to Fe(III). From this evidence, he concluded that a nonaqueous environment is required for reversible O₂ or CO binding. In hemoglobin, the protein surrounding the heme groups provides this nonaqueous environment and prevents oxidation. Others

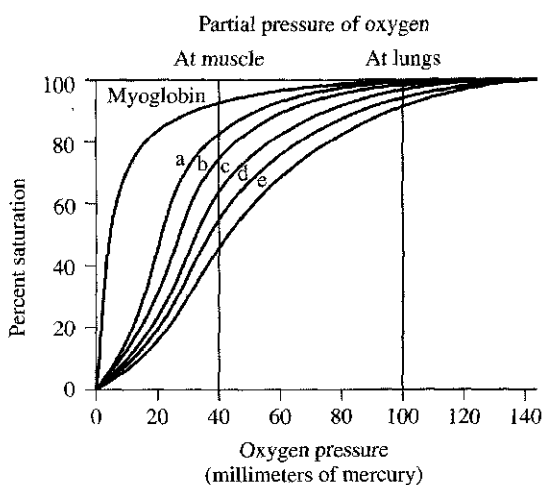


FIGURE 16-3 Myoglobin and Hemoglobin Binding Curves. Myoglobin, and hemoglobin at five different pH values: (a) 7.6. (b) 7.4, (c) 7.2, (d) 7.0, (e) 6.8. (Reproduced with permission from R. E. Dickerson and I. Geis, *Hemoglobin*, Benjamin/Cummings, Menlo Park, CA, 1983, p. 24.) (Irving Geis rights owned by Howard Hughes Medical Institute. Not to be reproduced without permission.)

²J. Baldwin and C. Chothia, *J. Mol. Biol.*, **1979**, *129*, 175.

³R. E. Dickerson and I. Geis, *Hemoglobin*, Benjamin/Cummings, Menlo Park, CA, 1983.

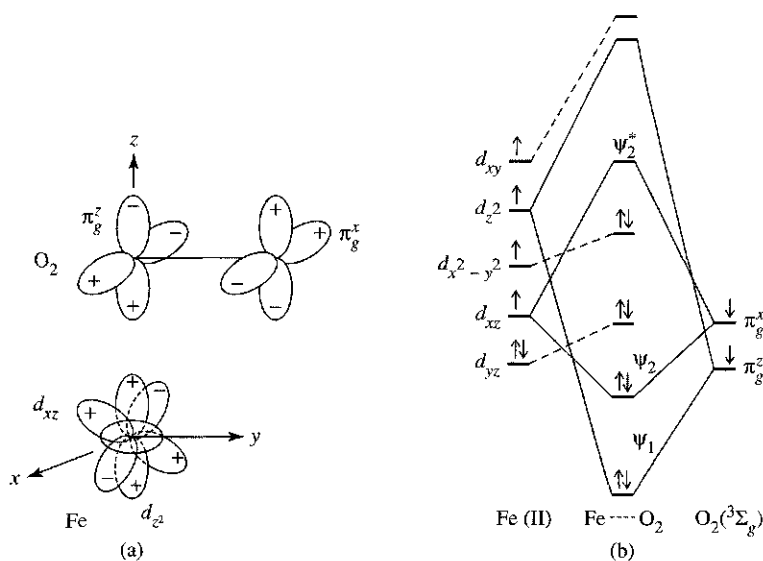
⁴J. H. Wang, *J. Am. Chem. Soc.*, **1958**, *80*, 3168.

have argued that oxidation results from one oxygen molecule simultaneously bonding to two hemes, which is effectively prevented by Wang's polystyrene matrix or the globin of native hemoglobin.

One method of studying hemoglobin (and many other complex biological systems) is through model compounds such as those used by Wang. Many heme derivatives have been synthesized and tested for oxygen binding, with a more complete understanding of the process as a goal.⁵ These compounds have been designed to protect the heme from the approach of another heme to prevent oxidation of the iron(II) by a cooperative reaction between two heme irons bridged by O_2 . In addition, some of the model compounds have an imidazole or pyridine nitrogen linked to the heme to hold it in a convenient location for binding to the iron. Some compounds have been tested as synthetic hemoglobin substitutes in human blood. Current candidates⁶ include modified bovine or human hemoglobin (polymerized to reduce loss by decomposition in the kidneys and to reduce its osmotic effect in the blood) and perfluorocarbons. The hemoglobin products still have problems with vasoconstriction, perhaps because the hemoglobins scavenge NO from the lining of the blood vessels, preventing its relaxing effect. They have much the same oxygen uptake curves as natural hemoglobin in red blood cells; the perfluorocarbons have nearly linear uptake curves, but have been useful in the treatment of premature infants with severe respiratory distress.

In hemoglobin, the Fe(II) is about 70 pm out of the plane of the porphyrin nitrogens in the direction of the imidazole nitrogen bonding to the axial position (Figure 16-2) and is a typical high-spin d^6 ion. When oxygen or carbon monoxide bond to the sixth position, the iron becomes coplanar with the porphyrin and the resulting compound is diamagnetic. Carbon monoxide is a strong enough ligand to force spin pairing and the resulting back π bonding stabilizes the complex. Oxygen bonds at an angle of approximately 130° , also with considerable back π bonding. Some have described the bonding as nearly that of $Fe(III) - O_2^-$, with enough metal to ligand electron transfer to result in a simple double bond between the oxygens. A structure, shown in Figure 16-4, has been proposed in which the triplet O_2 and the high-spin Fe(II) combine to form a spin-paired compound. The stronger σ interaction is between the d_{z^2} and π_g^z (antibonding π^*) orbitals. The weaker π interaction is between d_{xz} and π_g^x (antibonding π^*).

FIGURE 16-4 Electronic Structure of Oxyhemoglobin. (a) The most likely interaction between O_2 in the ground state (${}^3\Sigma_g^-$) and Fe(II)-heme in the high-spin state; x and y axes bisect the angle $N-Fe-N$. The signs on the oxygen orbitals are appropriate for the π^* orbitals. (b) The interaction between O_2 and Fe(II)-heme expressed in an energy level diagram. Fe in the high-spin state is located a little out of the porphyrin plane and, as the reaction proceeds, it is thought to move to the center in the plane. This effect is shown by the broken lines. (Reprinted with permission from E.-I. Ochiai, *J. Inorg. Nucl. Chem.*, **1974**, 36, 2129. © 1974, Pergamon Press PLC.)



⁵K. S. Suslick and T. J. Reinert, *J. Chem. Educ.*, **1985**, 62, 974-983.

⁶J. E. Squires, *Science*, **2002**, 295, 1004.

The increased ligand field results in pairing of the electrons and a weakened O—O bond. In hemoglobin, CO also forms bent bonds to Fe, probably because surrounding groups in the hemoglobin force it out of the linear form. This reduces the formation constant for CO—Hb. Without this reduction, normal amounts of CO in the body would be enough to interfere with oxygen transport.

Cytochromes, peroxidases, and catalases

Other heme compounds are also active biochemically. Cytochrome P-450 catalyzes oxidation reactions in the liver and adrenal cortex, helping to detoxify some substances by adding hydroxyl groups that make the compounds more water-soluble and more susceptible to further reactions. Unfortunately, at times this process has the reverse effect because some relatively safe molecules are converted into potent carcinogens. Peroxidases and catalases are Fe(III)-heme compounds that decompose hydrogen peroxide and organic peroxides. The reactions seem to proceed through Fe(IV) compounds with another unpaired electron on the porphyrin, which becomes a radical cation. Similar intermediates are also known in simpler porphyrin molecules.⁷

A model compound that decomposes hydrogen peroxide rapidly has been made from Fe(III) and triethylenetetramine (trien).⁸ Although the rate is not as high as that for catalase (Table 16-2), it is many times faster than that for hydrated iron oxide, which seems to have a large surface effect. The proposed mechanism for the $[\text{Fe}(\text{trien})]^{3+}$ reaction is shown in Figure 16-5. Tracer studies using ^{18}O -labeled water have shown that the reaction produces oxygen gas in which all of the oxygen atoms come from the peroxide; as a result, the steps forming O_2 must involve removal of hydrogen from H_2O_2 . Formation of water as the other product requires breakage of the oxygen-oxygen bond.

A group of cytochromes (labeled *a*, *b*, and *c*, depending on their spectra) serve as oxidation-reduction agents, converting the energy of the oxidation process into the synthesis of adenosine triphosphate (ATP), which makes the energy more available to other reactions. Copper is also involved in these reactions. The copper cycles between Cu(II) and Cu(I) and the iron cycles between Fe(III) and Fe(II) during the reactions. Details of the reactions are available in other sources.^{9,10}

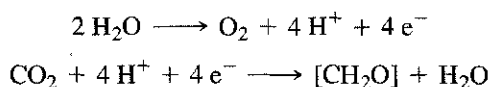
TABLE 16-2
Rates of Hydrogen Peroxide Decomposition

Catalyst	Relative Rate
Catalase	10^8
$[\text{Fe}(\text{trien})]^{3+}$	10^4
Methemoglobin [Fe(III) Hb]	1

16-1-2 SIMILAR RING COMPOUNDS

Chlorophylls

A porphine ring with one double bond reduced is called a chlorin. The chlorophylls (Figure 16-1) are examples of compounds containing this ring. They are green pigments found in plants, contain magnesium, and start the process of photosynthesis. They absorb light at the red end of the visible spectrum, transfer an electron to adjacent compounds and, by a series of complex reactions, finally transfer the energy of the light to the metabolic processes of the plant. The overall process can be summarized in the two reactions



⁷D. L. Hickman, A. Nanthakumar, and H. M. Goff, *J. Am. Chem. Soc.*, **1988**, *110*, 6384.

⁸J. H. Wang, *J. Am. Chem. Soc.*, **1955**, *77*, 822, 4715; *Acc. Chem. Res.*, **1970**, *3*, 90; R. C. Jarnagin and J. H. Wang, *J. Am. Chem. Soc.*, **1958**, *80*, 786.

⁹J. T. Groves, *J. Chem. Educ.*, **1988**, *11*, 928.

¹⁰E.-I. Ochiai, *Bioinorganic Chemistry*, Allyn and Bacon, Boston, 1977, pp. 150–165; T. E. Meyer and M. D. Kamen, *Adv. Protein Chem.*, **1982**, *35*, 105; G. R. Moore, C. G. S. Eley, and G. Williams, *Adv. Inorg. Bioinorg. Mech.*, **1984**, *3*, 1.

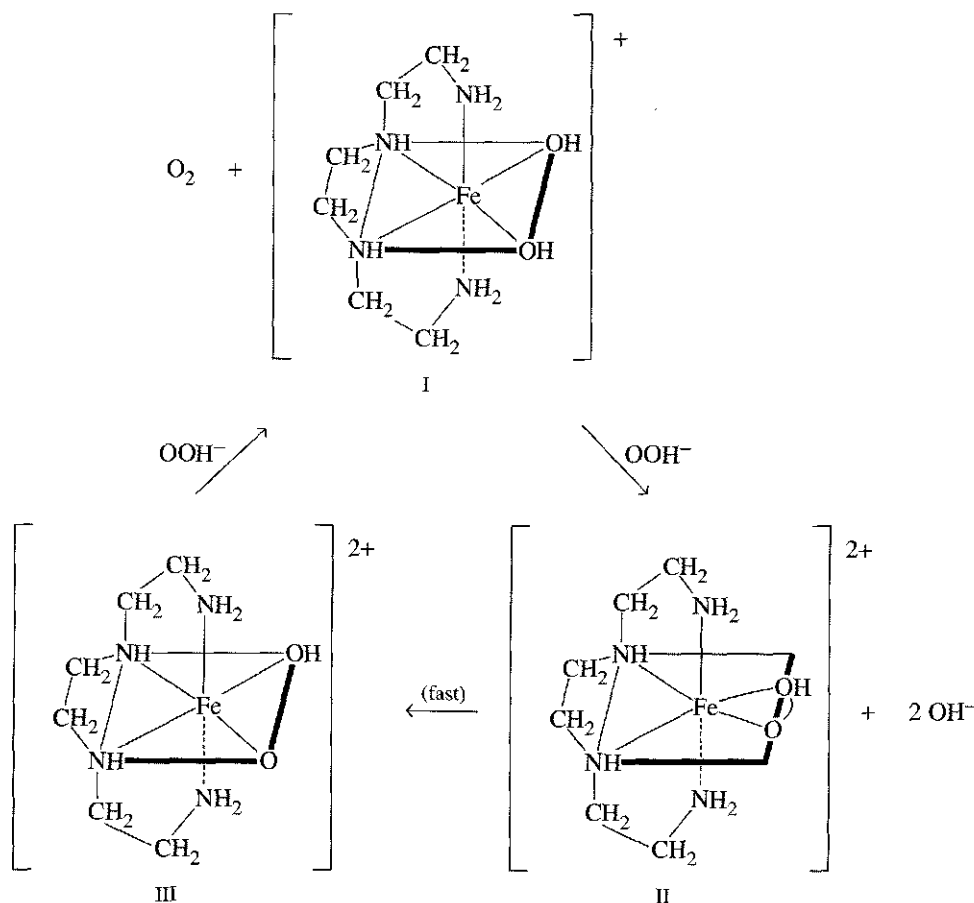


FIGURE 16-5 Mechanism of the $[Fe(trien)]^{3+}-H_2O_2$ Reaction. (Reproduced with permission from J. H. Wang, *J. Am. Chem. Soc.*, **1955**, 77, 4715. © 1955 American Chemical Society.)

where $[CH_2O]$ represents sugars, carbohydrates, and cellulose synthesized in the plant. In effect, this process also reverses the oxidation process that produces the energy for animal life, in which the $[CH_2O]$ compounds are converted back to water and carbon dioxide. The entire process is very complicated and is far from being completely understood, but includes a vital role for manganese in the first reaction.

Other compounds containing metal ions, such as the ferredoxins,¹¹ are involved in electron-transfer reactions, part of the photosynthetic pathway in plants, and in electron-transfer chains linked to hydroxylation and other reactions in mammals and bacteria. Ferredoxins are iron-sulfur compounds, many of which have active sites sometimes abbreviated as $Fe_2S_2(cys)_4$ (cys = cysteine, $HSCH_2NH_2CHCOOH$). The structures vary, but seem to have Fe(II) and Fe(III) in tetrahedral sites bridged by sulfide ions and bound into the protein by Fe—S bonds to cysteine. There are also other more complex ferredoxins that contain Fe_4S_4 or Fe_3S_4 units, again with tetrahedral iron and sulfide bridges. A similar structure has been suggested for a more uncertain Fe_6S_6 compound. Proposed structures for the Fe—S active sites of some of these compounds are shown in Figure 16-6.

¹¹C. R. Crossnoe, J. P. Germanas, P. LeMagueres, B. Mustata, and K. L. Krause, *J. Mol. Biol.*, **2002**, 318, 503; R. Morales, M. Frey, and J.-M. Mousesca, *J. Am. Chem. Soc.*, **2002**, 124, 6714.

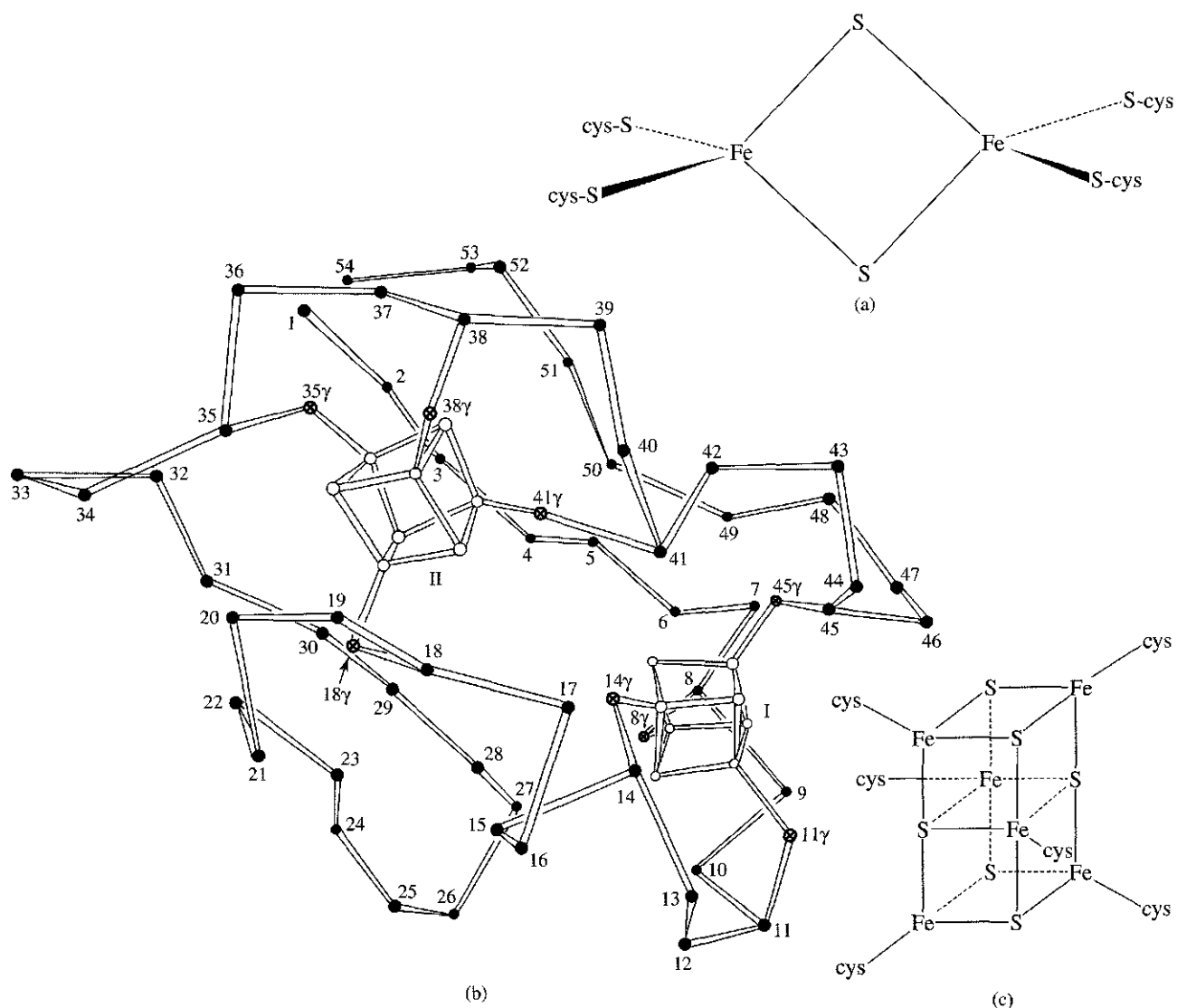
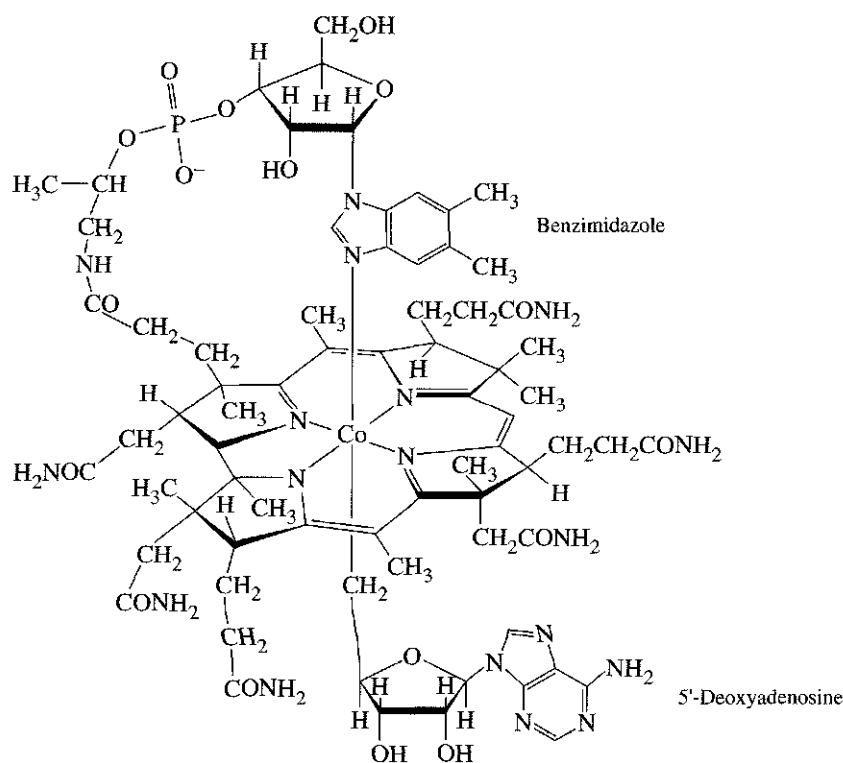


FIGURE 16-6 Structures of Fe—S Protein-Active Sites. (a) Ferredoxin. (From E.-I. Ochiai, *Bioinorganic Chemistry*, Allyn and Bacon, Boston, 1977, p. 184.) (b) Clostridial ferredoxin. (Reproduced with permission from E. T. Adman, L. C. Sieker, and L. H. Jensen, *J. Biol. Chem.*, 1973, 248, 3987.) (c) A model for the structure of the Fe_6S_6 active unit. (Reproduced with permission from E.-I. Ochiai, *Bioinorganic Chemistry*, Allyn and Bacon, Boston, 1977, p. 192.)

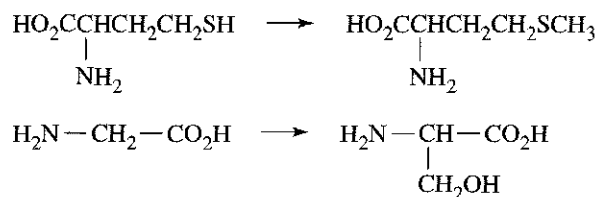
Coenzyme B_{12}

A vitamin known as coenzyme B_{12} is the only known organometallic compound in nature. It incorporates cobalt into a corrin ring structure, which has one less $=\text{CH}-$ bridge between the pyrrole rings than the porphyrins (Figure 16-7). This compound is known to prevent anemia and also has been found to have many catalytic properties. During isolation of this compound from natural sources, the adenosine group is usually replaced by cyanide, and it is in this cyanocobalamin (vitamin B_{12}) form that it is used medicinally. The cobalt can be counted as $\text{Co}(\text{III})$ in these compounds; the four corrin nitrogens contribute electrons and a charge of $2-$, the benzimidazole nitrogen contributes two electrons, and the cyanide or adenosine in the sixth position contributes two electrons and a charge of $1-$. Without the sixth ligand, the molecule is called cobalamin.

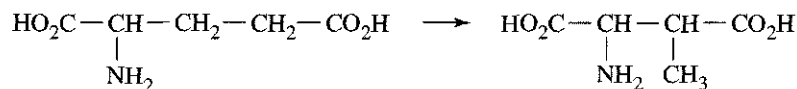
FIGURE 16-7 Coenzyme B₁₂.

Methylcobalamin can methylate many compounds, including metals. The reactions of alkylcobalamins depend on cleavage of the alkyl—cobalt bond, which can result in Co(I) and an alkyl cation, Co(II) and an alkyl radical, or Co(III) and an alkyl anion, with the radical mechanism being the most common. The alkyl products can then react in a number of ways. Some of the reactions include the following:¹²

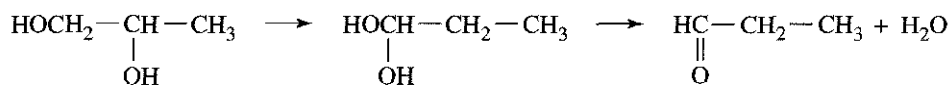
Methylation or Hydroxymethylation



Isomerization



Isomerization and Dehydration



¹²R. H. Abeles, "Current Status of the Mechanism of Action of B₁₂-Coenzyme" in A. W. Addison, W. R. Cullen, D. Dolphin, and B. R. James, eds., *Biological Aspects of Inorganic Chemistry*, Wiley-Interscience, New York, 1977, pp. 245–260.

16-2 Ferritin and transferrin

OTHER IRON COMPOUNDS

Iron is stored in both plant and animal organisms in combination with a protein called apoferritin. The resulting ferritin contains a micelle of ferric hydroxide-oxide-phosphate surrounded by the protein and is present mainly in the spleen, liver, and bone marrow in mammals. Individual subunits of the apoferritin have a molecular weight of about 18,500, and 24 of these subunits combine to form the complex, with a protein molecular weight of about 445,000 and up to 4,300 atoms of Fe in the iron core, stored as ferrihydrite phosphate, $[(\text{Fe}(\text{O})\text{OH})_8(\text{FeOPO}_3\text{H}_2) \cdot x\text{H}_2\text{PO}_4]$. The mechanisms for incorporation of iron into this complex and removal for use in the body are uncertain, but it appears that reduction to Fe(II) and chelation of the Fe(II) are required to remove iron from the core, and the reverse process moves it into the storage core of the complex. It is known from tracer experiments that all the oxygen atoms in the ferrihydrite are derived from water, rather than O_2 . Other iron proteins, called transferrins, serve to transport iron as Fe(III) in the blood and other fluids. One of these has iron bound as Fe(III) by two tyrosine phenoxo groups, an aspartic acid carboxyl group, a histidine imidazole, and either HCO_3^- or CO_3^{2-} , as shown in Figure 16-8.¹³

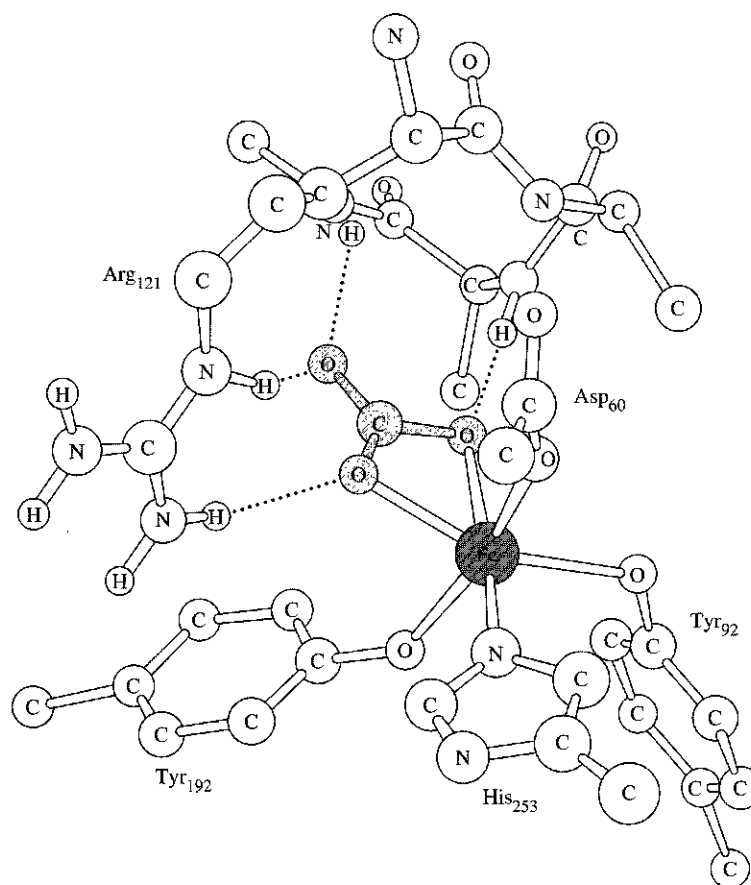


FIGURE 16-8 Lactotransferrin. (Reproduced with permission from S. J. Lippard and J. M. Berg, *Principles of Bioinorganic Chemistry*, University Science Books, Mill Valley, CA, 1994, p. 144.)

¹³R. E. Feeny and S. K. Komatsu, *Struct. Bonding*, 1966, 1, 149–206; E. E. Hazen, cited in B. L. Vallee and W. E. C. Wacker, *Metalloproteins*, Academic Press, New York, 1969, p. 89.

Siderochromes

Bacteria and fungi also synthesize iron transfer compounds, called siderochromes.¹⁴ The common structures are complex hydroxamates (also called ferrichromes or ferrioxamines) or complex catechols (enterobactin), all shown in Figure 16-9. They have peptide backbones and are very strong chelating agents ($K_f \approx 10^{30}$ to 10^{50}), allowing the organism to extract iron from surroundings that contain very little iron or are basic enough that the iron is present as insoluble hydroxides or oxides. Some of these compounds act as growth factors for bacteria and others act as antibiotics. There are also examples in which the iron is bound by a mixture of phenolic hydroxyl, hydroxamate, amine, and alcoholic hydroxyl groups.

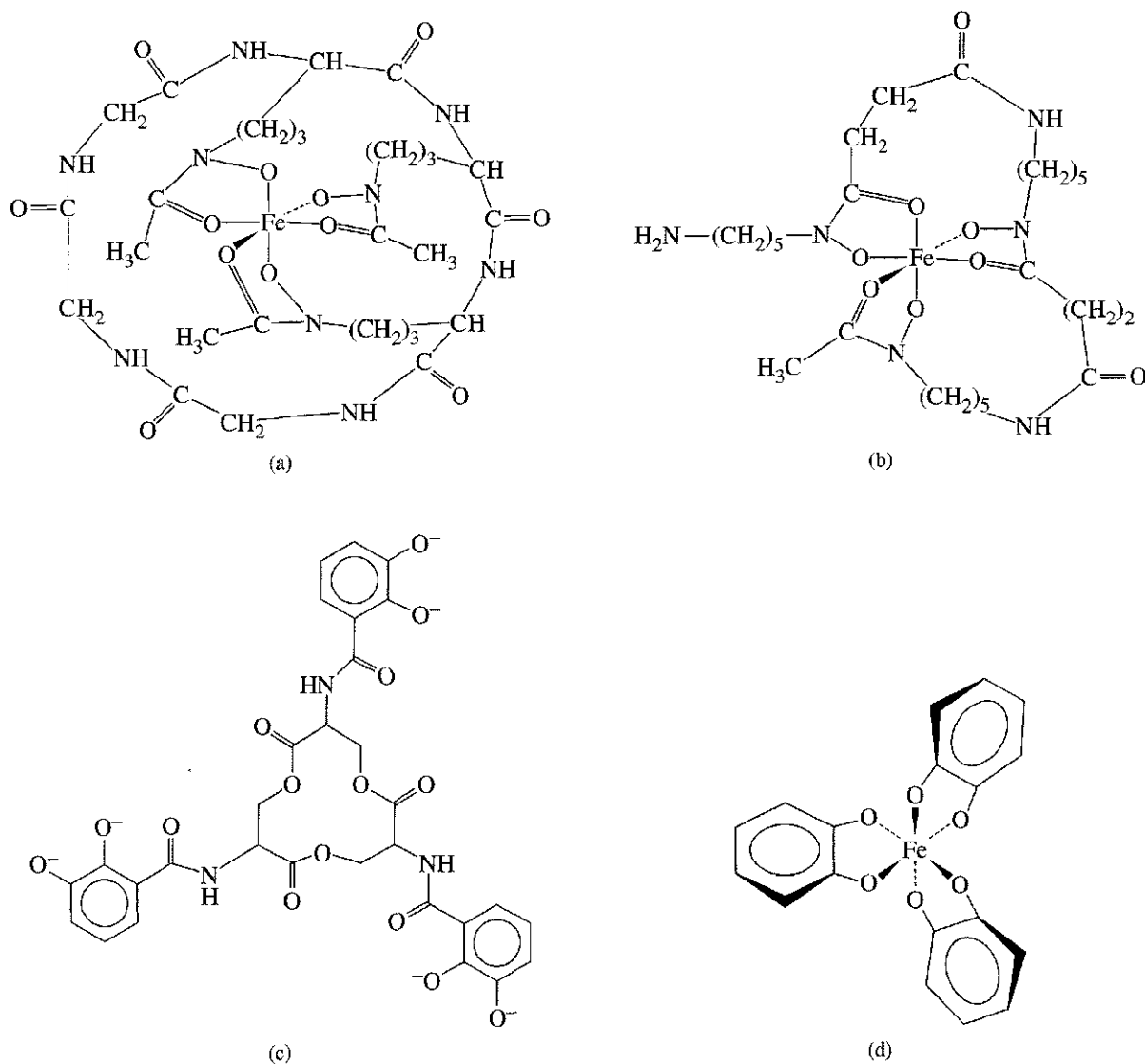


FIGURE 16-9 Ferrichromes, Ferrioxamines, and Catechol Siderochromes. (a) Ferrichrome A. (b) Ferrioxamine B. (c) Enterobactin, a catechol siderochrome. (d) The catechol complex of enterobactin with Fe(III). The trilactone ring (omitted in the drawing) has all *S* conformation in the chiral atoms, which in turn results in a Δ conformation when the six catechol oxygen atoms complex with Fe(III).

¹⁴K. N. Raymond, G. Müller, and B. F. Matzanke, *Top. Curr. Chem.*, 1984, 123, 49.

16-3 ZINC AND COPPER ENZYMES

Zinc is found in more than 80 enzymes. Two of these, carboxypeptidase and carbonic anhydrase, will be discussed here.¹⁵ Copper is also a common metal in enzymes and is present in four different forms. Two of the copper enzymes will also be described.

Carboxypeptidase

Carboxypeptidase is a pancreatic enzyme that catalyzes the hydrolysis of the peptide bond at the carboxyl end of proteins and peptides, with a strong preference for amino acids with an aromatic or branched aliphatic side chain. The zinc ion is bound in a 5-coordinate site by two histidine nitrogens, both oxygens from a glutamic acid carboxyl group, and a water molecule. A pocket in the protein structure accommodates the side chain of the substrate. Evidence indicates that the negative carboxyl group of the substrate hydrogen bonds to an arginine on the enzyme while the zinc bonds to the oxygen of the peptide carbonyl, as shown in Figure 16-10. A Zn—OH or Zn—OH₂ combination

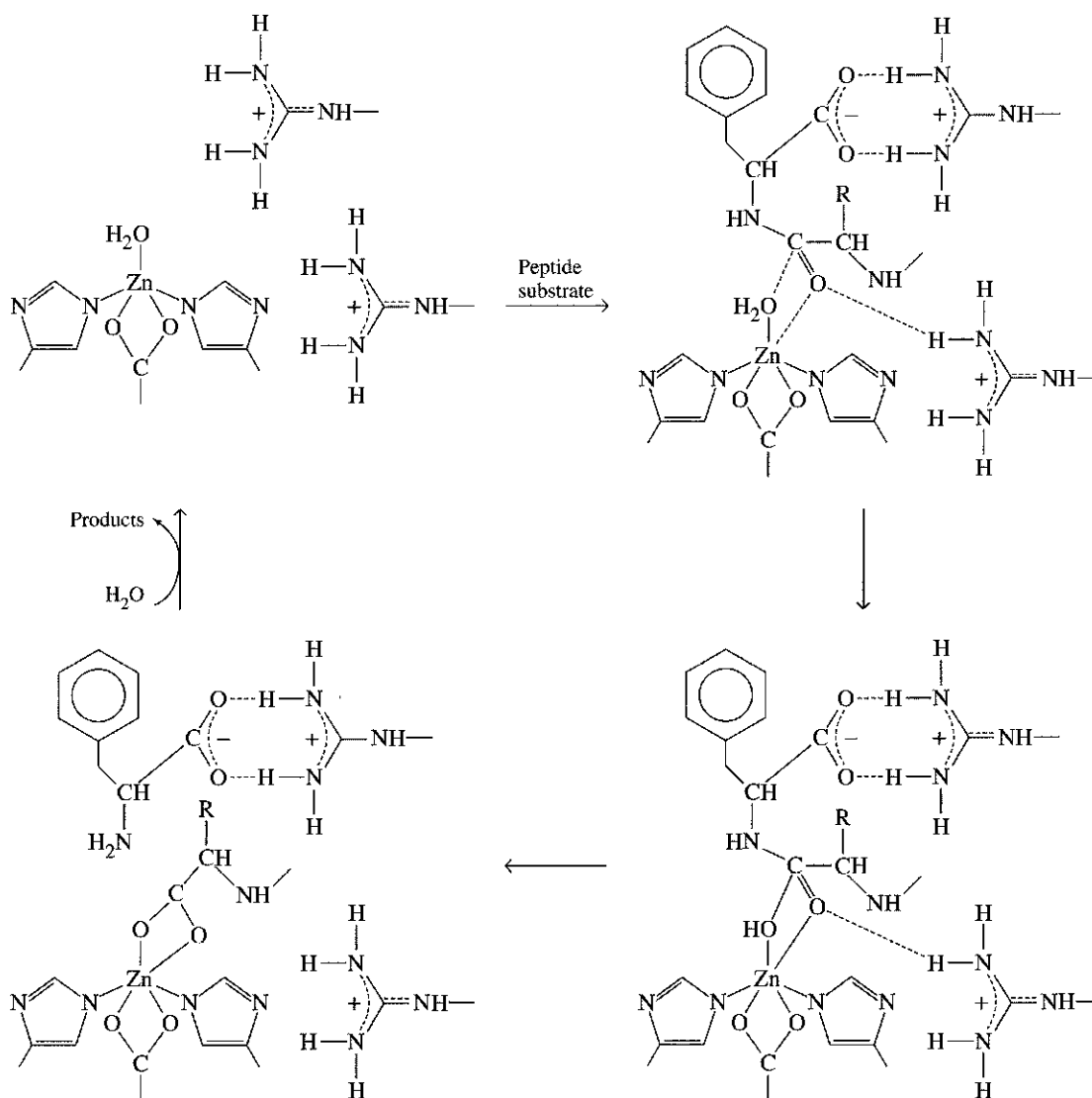


FIGURE 16-10 Proposed Mechanism of Carboxypeptidase Action. Transfer of several hydrogen ions is not shown.

¹⁵I. Bertini, C. Luchinat, and R. Monnanni, *J. Chem. Educ.*, **1985**, 62, 924.

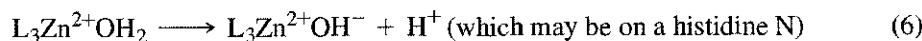
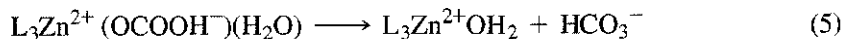
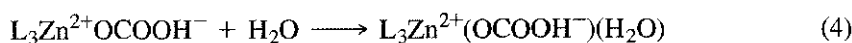
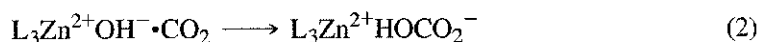
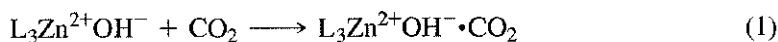
seems to be the group that reacts with the carbonyl carbon, with assistance of a glutamic acid carboxyl group from the enzyme that assists in the transfer of H^+ from the bound water to the amino acid product.¹⁶ An artificial peptidase model compound has been made with a Cu(II) bound by four nitrogens in a chain that ends in a guanidinium ion, all attached to a cross-linked polystyrene.¹⁷ The catalytic activity is high for hydrolysis of amides with carboxyl groups attached, similar to a carboxypeptidase activity. The H^+ on the guanidinium group can hydrogen-bond to the carboxyl group, holding the substrate in position near the Cu, which is the active site.

Carbonic anhydrase

In a few cases, theoretical calculations of transition state and intermediate energies and geometries provide confirmation of experimental studies of the mechanism of enzyme reactions and suggest directions for further study. One of these is the hydration of carbon dioxide catalyzed by carbonic anhydrase, a zinc enzyme. Below pH 7, the uncatalyzed reaction $HCO_3^- + H^+ \rightleftharpoons H_2O + CO_2$ is favored. Above pH 7, the reaction is $HCO_3^- \rightleftharpoons OH^- + CO_2$, catalyzed by carbonic anhydrases I, II, and III. II is particularly active, with a rate enhancement of 10^6 or more, approaching a diffusion-controlled rate.¹⁸

The active site has a zinc(II) ion bonded to three histidine imidazole groups and a fourth site occupied by water or hydroxide ion, $L_3Zn^{2+}OH^-$, where L = imidazole N from histidine. Experimentally, the enzyme loses activity below pH 7, indicating that an ionizable group of pK 7 is part of the active site. In addition, it is known that the product of the hydration of CO_2 is HCO_3^- , as would be expected in neutral or basic solution.

Calculations have shown that water bound to the zinc ion can lose a proton readily, but imidazole bound to the zinc ion cannot. There is still an unsettled question about the first ionization from the zinc-bound water molecule. This reaction seems to be much too fast and is dependent on buffer concentration. The role of the buffer is still unknown, but in some fashion it assists in the reaction. The sequence of reactions usually used to describe the reaction is as follows:



The complex formed in (1) is loosely bound, moving to the more tightly bound product of (2). The transition state for Reaction (3) may be a bidentate hydrogen carbonate,¹⁹ or there may be a proton transfer between the bound oxygen atom and one of the unbound

¹⁶D. W. Christianson and W. N. Lipscomb, *Acc. Chem. Res.*, **1989**, 22, 62.

¹⁷J. Suh and S.-J. Moon, *Inorg. Chem.*, **2001**, 40, 4890.

¹⁸S. Lindskog, *Adv. Bioinorg. Chem.*, **1982**, 4, 116; P. J. Stein, S. P. Merrill, and R. W. Henkens, *J. Am. Chem. Soc.*, **1977**, 99, 3194; *Biochemistry*, **1985**, 24, 2459.; S. Lindskog in T. G. Spiro, ed., *Zinc Enzymes*, Wiley, New York, 1983, p. 77.

¹⁹S. Lindskog, *Ann. N.Y. Acad. Sci.*, **1984**, 429, 61; D. N. Silverman and S. Lindskog, *Acc. Chem. Res.*, **1988**, 21, 30.

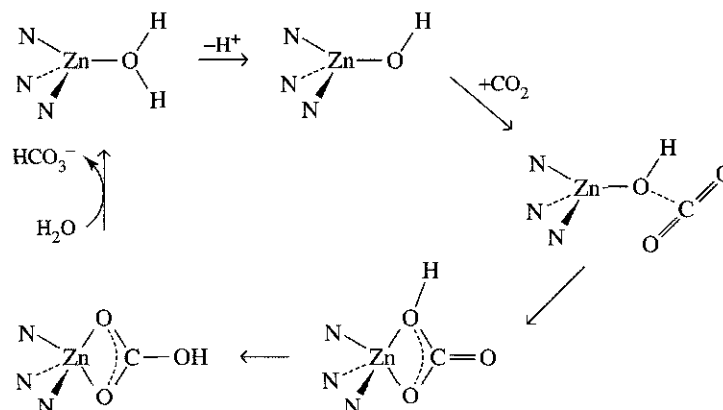


FIGURE 16-11 Proposed Mechanism for Carbonic Anhydrase.

oxygen atoms.²⁰ In either case, the result is probably a bound hydrogen carbonate ion that has the OH group at as great a distance from the Zn as possible. Whether Reaction (5) has a 5-coordinate Zn with addition of the water molecule is uncertain; it may just be part of the transition state. There have been several attempts to determine the mechanism and the transition states by theoretical calculations,²¹ but the details remain uncertain. Some possibilities are shown in Figure 16-11. Future calculations that include more of the protein structure surrounding the active site may reveal the “true” mechanism.

A study of spinach carbonic anhydrase showed very similar kinetic behavior, but also showed that the Zn is bound to a sulfur atom.²² It was concluded that the two enzymes are convergently evolved, with different structures, but have equivalent functions.

Ceruloplasmin and superoxide dismutase

Copper is present in mammals in ceruloplasmin and superoxide dismutase and it is also part of a number of enzymes in plants and other organisms, including laccase, ascorbate oxidase, and plastocyanin. In these compounds, it is present in four different forms, listed in Table 16-3.

TABLE 16-3
Forms of Copper in Proteins

	Absorption Maximum (nm)	Extinction Coefficients ($L\ mol^{-1}\ cm^{-1}$)	Comments
Type 1	600 nm	1000–4000	Responsible for the blue color of blue oxidases and electron-transfer proteins, $L \rightarrow M$ charge transfer spectrum of Cu—S bond
Type 2	Near 600 nm	300	Similar to ordinary tetragonal Cu(II) complexes, but more intensely colored
Type 3	330 nm	3000–5000	Paired Cu(II) ions, diamagnetic, associated with redox reactions of O_2 , where it undergoes a 2-electron change, bypassing superoxide
Cu(I)			Colorless, diamagnetic, no epr spectrum (no unpaired electrons)

²⁰W. N. Lipscomb, *Ann. Rev. Biochem.*, **1983**, 429, 17.

²¹J.-Y. Liang and W. N. Lipscomb, *Biochemistry*, **1987**, 26, 5293; K. M. Merz, Jr., R. Hoffmann, and M. J. S. Dewar, *J. Am. Chem. Soc.*, **1989**, 111, 5636; Y.-J. Zheung and K. M. Merz, Jr., *J. Am. Chem. Soc.*, **1992**, 114, 10498; M. Solà, A. Liedós, M. Duran, and J. Bertrán, *J. Am. Chem. Soc.*, **1992**, 114, 869.

²²R. S. Rowlett, M. R. Chance, M. D. Wirt, D. E. Sidelinger, J. R. Royal, M. Woodroffe, Y.-F. A. Wang, R. P. Saha, and M. G. Lam, *Biochemistry*, **1994**, 33, 13967.

Ceruloplasmin²³ is an intensely blue glycoprotein of the α_2 -globulin fraction of mammalian blood, which acts as a copper transfer protein and probably has a role in iron storage. The structure is known;²⁴ it contains three Type 1 (T1) sites (one of which seems to be inactive) and a Type 2/Type 3 (T2/T3) trinuclear cluster. It is believed to be part of the process of oxidizing Fe(II) to Fe(III) in the transfer of iron from ferritin to transferrin. Reduction of two T1 sites and the T3 pair is fast, but reduction of the T2 Cu site is slow; the pathways of electron transfer between the sites have been investigated, but the complete mechanism is still unknown.²⁵

Bovine superoxide dismutase²⁶ contains one atom of Cu(II) and one atom of Zn(II) in each of two subunits, with a molecular weight of about 16,000. The copper is in a distorted square-pyramidal site, bound to four histidine nitrogens and a water; the zinc is bound to three histidines (including a bridging imidazole ring bound to both metal ions) and an aspartate carboxyl oxygen in a distorted tetrahedral structure, as shown in Figure 16-12. Cu is the more essential metal, which cannot be replaced while retaining activity. On the other hand, the Zn can be replaced by other divalent metals with retention of most of the catalytic activity. The major role of zinc may be to provide structural stability, as evidenced by the stability of the enzyme at high temperatures, but the enzyme with Cu in both sites is still active in the presence of SCN^- , which breaks the histidine bridge between the Cu atoms.²⁷ The superoxide ion, O_2^- , which can be formed by dissociation of oxygen from heme proteins [leaving behind Fe(III)], is relatively unreactive, but one of its products, HO_2 , is very reactive, so the superoxide must be removed quickly. O_2^- is found in several metabolic processes and appears to

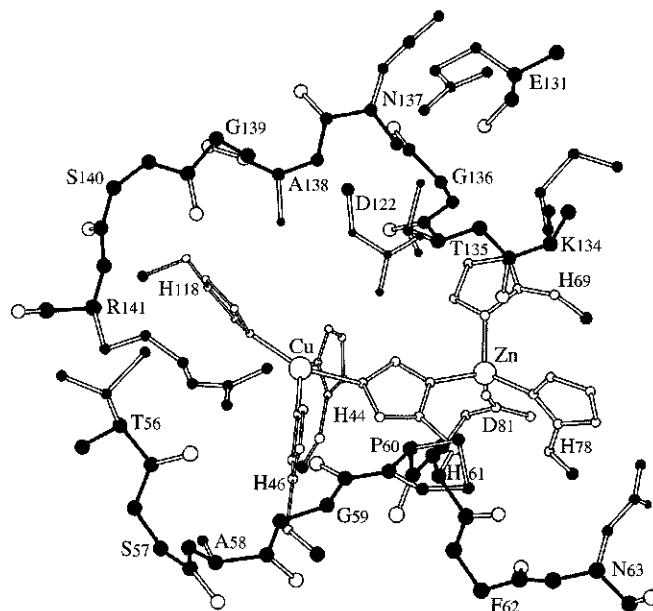


FIGURE 16-12 Active Site of Bovine Superoxide Dismutase. Shown is a drawing of the active site channel as viewed from the solvent. The main chain is shown in black, the ligand side chains as open circles and bonds, and the other side chains as solid atoms and open bonds. (Reproduced with permission from J. A. Tainter, E. D. Getzoff, J. S. Richardson, and D. C. Richardson, *Nature*, **1983**, 306, 284. © 1983 Macmillan Magazines Limited.)

²³S. H. Lawrie and E. S. Mohammed, *Coord. Chem. Rev.*, **1980**, 33, 279.

²⁴I. Zaitseva, V. Zaitsev, G. Card, K. Moshov, B. Bax, A. Ralph, and P. Lindley, *J. Biol. Inorg. Chem.*, **1996**, 1, 15; P. F. Lindley, G. Card, I. Zaitseva, V. Zaitsev, B. Reinhammar, E. Selin-Lindgren, and K. Yoshida, *J. Biol. Inorg. Chem.*, **1997**, 2, 454; V. N. Zaitsev, I. Zaitseva, M. Papiz, and P. F. Lindley, *J. Biol. Inorg. Chem.*, **1999**, 4, 579.

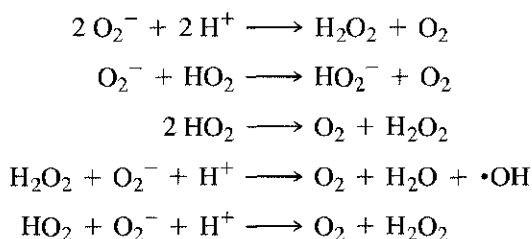
²⁵T. E. Machonkin and E. I. Solomon, *J. Am. Chem. Soc.*, **2000**, 122, 12547.

²⁶I. Fridovich, *Adv. Inorg. Biochem.* **1979**, 1, 67; J. S. Valentine and D. M. de Freitas, *J. Chem. Educ.*, **1985**, 62, 728.

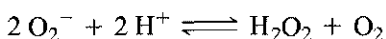
²⁷K. G. Strothcamp and S. J. Lippard, *Biochemistry*, **1981**, 20, 7488.

be essential for a few (e.g., tumor necrosis factor, antibacterial effect of myeloperoxidases), but large amounts form in some pathological conditions and cause serious damage. One pathway of reaction is the formation of OH radicals and singlet oxygen, both of which are toxic.

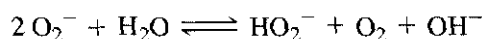
Some reactions of superoxide are the following:



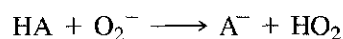
The reactions catalyzed by superoxide dismutases



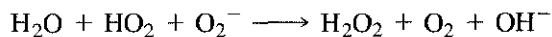
and



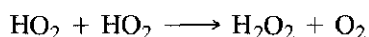
have large equilibrium constants, and can proceed by the reactions



and

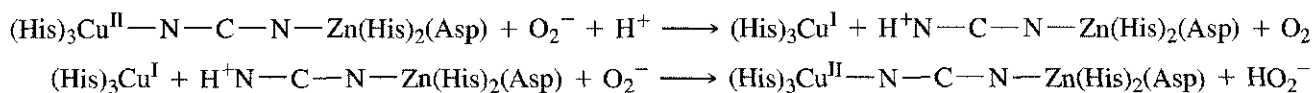


or



Reactions seem to involve a Cu(II)-Cu(I) cycle, with H^+ replacing the Cu(I) on the bridging histidine at one stage. The rate-limiting step is the approach and/or bonding of O_2^- to Cu. In saturated conditions, H^+ transfer may be rate-limiting.

In a simple model of reaction, O_2^- and H^+ react to form Cu(I) and H^+ -histidine-Zn and O_2 , then O_2^- reacts with the enzyme to reform the Cu(II)-histidine-Zn and H_2O_2 :



A more detailed model²⁸ has O_2^- replace H_2O as the fifth ligand on Cu, with hydrogen bonding to an arginine guanidinium group, transfer of H^+ from water to the histidine, and release of O_2 , forming Cu(I) and H^+ -histidine-Zn. These react with O_2^- and H^+ to form the arginine- H^+ - O_2 -Cu species, with hydrogen bonding to the H^+ histidine and water. Release of H_2O_2 reforms the native enzyme. It has also been suggested²⁹

²⁸R. Osman and H. Basch, *J. Am. Chem. Soc.*, **1984**, *106*, 5710.

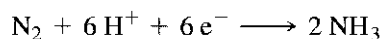
²⁹L. S. Ellerby, D. E. Cabelli, J. A. Graden, and J. S. Valentine, *J. Am. Chem. Soc.*, **1996**, *118*, 6556.

that the Zn-histidine role is to promote the release of HO_2^- from the copper in this final step by forcing the HO_2^- into the axial position, where it would be more weakly bound.

The roles of the copper enzymes in electron transport, oxygen transport, and oxidation reactions have guaranteed continued interest in their study. In addition to studies of the natural compounds, there have been many attempts to design model structures of these enzymes, particularly of the binuclear species. Many of these include both nitrogen and oxygen donors built into macrocyclic ligands, although sulfur has been used as well.³⁰

16-4 NITROGEN FIXATION

A very important sequence of reactions converts nitrogen from the atmosphere into ammonia:



The NH_3 can then be further converted into nitrate or nitrite or directly used in the synthesis of amino acids and other essential compounds. This reaction takes place at 0.8 atm N_2 pressure and ambient temperatures in *Rhizobium* bacteria in nodules on the roots of legumes such as peas and beans, as well as in other independent bacteria. In contrast to these mild conditions, industrial synthesis of ammonia requires high temperatures and pressures with iron oxide catalysts, and even then yields only 15% to 20% conversion of the nitrogen to ammonia. Intensive efforts to determine the bacterial mechanism and to improve the efficiency of the industrial process have so far been only moderately successful; the goal of approaching enzymatic efficiency on an industrial scale is still only a goal.

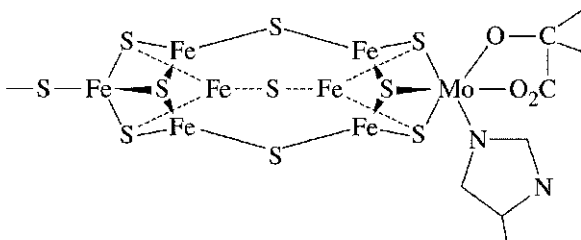
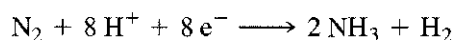


FIGURE 16-13 The FeMo-Cofactor Site of Nitrogenase.

The nitrogenase enzymes responsible for nitrogen fixation contain two proteins. The iron-molybdenum protein contains two metal centers. One, called the FeMo-cofactor, contains molybdenum, iron, and sulfur (Figure 16-13). This may be the site of nitrogen reduction; there are open binding sites on some of the iron atoms in the middle and a pocket large enough for substrate binding. The other site, called the P-cluster, contains eight iron atoms and eight sulfur atoms in two subunits that are nearly cubic.³¹ The P-cluster is believed to assist the reaction by transfer of electrons, but little more is known about the mechanism of the reaction. The second protein contains two identical subunits with a single 4Fe:4S cluster and an adenosine diphosphate (ADP) molecule bound between the two subunits. In some fashion, this protein is reduced and transfers single electrons to the FeMo-cofactor protein, where the reaction with nitrogen takes place. Eight electrons are required for N_2 conversion to 2NH_3 by the enzymes because the reaction also forms H_2 .³² In addition, 16 molecules of MgATP are converted to MgADP and inorganic phosphate.



³⁰K. D. Karlin and Y. Gultneh, *J. Chem. Educ.*, **1985**, 62, 983, K. G. Strothcamp and S. J. Lippard, *Acc. Chem. Res.*, **1982**, 15, 318.

³¹M. M. Georgiadis, H. Komiya, P. Chakrabarti, D. Woo, J. J. Kornac, and D. C. Rees, *Science*, **1992**, 257, pp.1653, 1677.

³²F. B. Simpson and R. H. Burris, *Science*, **1984**, 224, 1095.

The nitrogenase reaction seems to begin with a series of four electron transfers, leading to a reduced form of the enzyme that then can bind four H^+ ions. After these changes, N_2 can be bound as H_2 is released and, finally, the N_2 is reduced to NH_3 and released from the complex.³³ Two alternative sites for the first proton to bind have been suggested, one in the middle of the Fe_6S_3 cluster in the MoFe active site, and the other at an alkoxy oxygen of homocitrate bound to Mo at one end of the cluster.³⁴ Calculations show the possibility of N_2 bonding asymmetrically with one of the N atoms near the center of the four Fe atoms, approximately at the corners of a square on the front face shown in Figure 16-13 and the other sticking out toward the top front.³⁵ This more distant N is positioned to accept H atoms from the nearby S atoms, which could release NH_3 . The remaining N can then accept H atoms in a similar fashion from S atoms to form the second NH_3 .

In addition to the nitrogenases whose cofactor contains the structure $MoFe_7S_9$, others contain clusters with no Mo. Theoretical calculations³⁶ based on an Fe_2S_5 cluster (a portion of the larger cofactor), with one S bridging two Fe(II) atoms, have shown that adding an H atom to the bridging S is required to allow formation of an N_2 bridge between the iron atoms. Once this occurs, addition of H to the N_2 can proceed through exothermic formation of N_2H , N_2H_2 , N_2H_3 , and N_2H_4 . Subsequent steps are less easily predicted, but the suggestion is that the next H atom combines with the H from the bridging sulfur to form H_2 , known to be one of the products of the reaction. The next H atom could add to the N_2H_4 , forming NH_3 and NH_2 , each bound to an iron atom, and a final H creates the second NH_3 , with a large exothermic value. Preliminary calculations based on an $Fe_3S_9^{2-}$ cluster, of the same structure as the cofactor but with more symmetry because Fe replaces Mo, gives similar results, in which addition of H atoms to the bridging sulfur atoms is required to open the structure sufficiently for addition of N_2 .

There have been many attempts to make model compounds for ammonia production, but none have been successful. How the enzyme manages to carry out the reaction at ambient temperature and less than 1 atm pressure of N_2 is still an unanswered question.

Nitrification and denitrification

Oxidation of ammonia to nitrite, NO_2^- , and nitrate, NO_3^- , is called nitrification; the reverse reaction is ammonification. Reduction from nitrite to nitrogen is called denitrification. All these reactions, and more, occur in enzyme systems, many of which include transition metals. A molybdenum enzyme, nitrate reductase, reduces nitrate to nitrite. Further reduction to ammonia seems to proceed by 2-electron steps, through an uncertain intermediate with a +1 oxidation state (possibly hyponitrite, $N_2O_2^{2-}$) and hydroxylamine:



Some nitrite reductases contain iron and copper; other enzymes active in these reactions contain manganese. Reactions catalyzed by copper and iron enzymes with NO, N_2O , and N_2 as products have also been reported.

Nitrite reductase from *Alcaligenes xylosoxidans* is made up of three identical subunits, each with an embedded Cu (Type I) and a Cu (Type II) bound by residues from

³³R. N. F. Thornley and D. Lowe, in T. G. Spiro, ed., *Molybdenum Enzymes*, Wiley-Interscience, New York, 1985.

³⁴T. Lovell, J. Li, D. A. Case, and L. Noodleman, *J. Am. Chem. Soc.*, **2002**, *124*, 4546.

³⁵I. Dance, *Chem. Commun. (Cambridge)*, **1997**, 165.

³⁶P. E. M. Siegbahn, J. Westerberg, M. Svensson, and R. H. Crabtree, *J. Phys. Chem.*, **1998**, *102*, 1615.

two of the subunits. Catalysis³⁷ seems to proceed by binding of NO_2^- to the Type II Cu, with carboxylate from an aspartate residue hydrogen-bonded to one of the NO_2^- oxygens, followed by transfer of an electron from the Type I Cu, transfer of H^+ to the same oxygen from a histidine residue, and release of NO to regenerate the active site hydrogen-bonded to the aspartate through a water molecule, as shown in Figure 16-14. This mechanism is supported by study of mutants in which the aspartate and histidine were modified, which reduced the activity of the enzyme.³⁸

Cytochrome *cd*₁ nitrite reductase from *Paracoceus pantotrophus* has a different mechanism,³⁹ with two identical subunits, each with domains containing a *c*-type cytochrome heme and a *d*₁-type cytochrome heme. Electrons from external donors enter through the *c* heme; the *d*₁ heme is the site of nitrite reduction to NO and oxygen reduction to water. One of the puzzles of the mechanism is how the NO can escape from

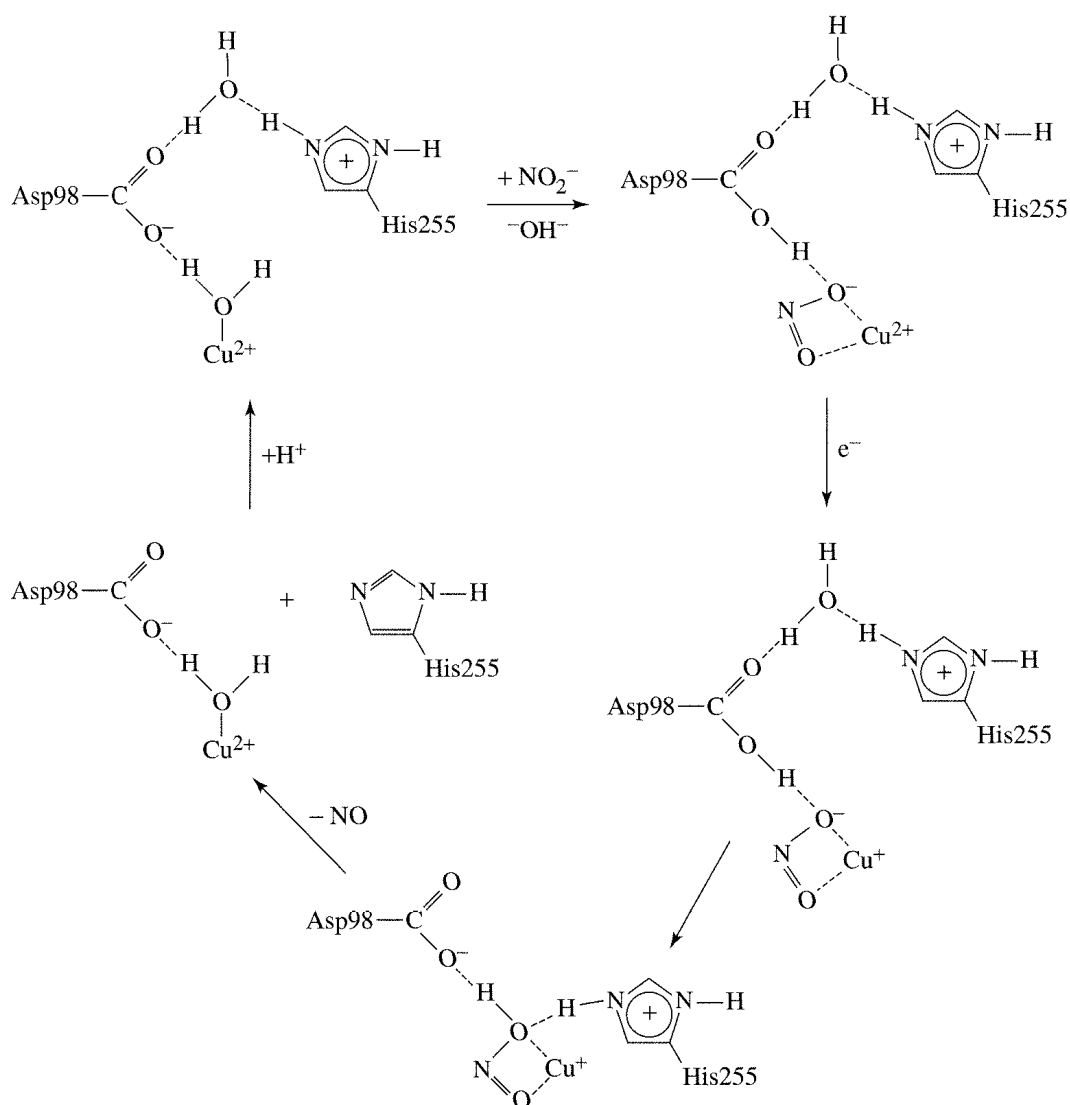


FIGURE 16-14 Proposed Mechanism of Nitrite Reductase. (Redrawn from M. J. Boulanger, M. Kukimoto, M. Nishiyama, S. Horinouchi, and M. E. P. Murphy, *J. Biol. Chem.*, **2000**, 275, 23957.)

³⁷S. Suzuki, K. Kataoka, and K. Yamaguchi, *Acc. Chem. Res.*, **2000**, 33, 728.

³⁸M. J. Boulanger, M. Kukimoto, M. Nishiyama, S. Horinouchi, and M. E. P. Murphy, *J. Biol. Chem.*, **2000**, 275, 23957.

³⁹G. Ranghino, E. Scorza, T. Sjögren, P. A. Williams, M. Ricci, and J. Hajdu, *Biochemistry*, **2000**, 39, 10958.

the heme, for which it has a strong affinity. As in the case of the copper nitrite reductases, protonated nitrogen atoms on histidine residues play an important part. As shown in Figure 16-15, crystallographic evidence points to an oxidized enzyme, with the heme bound by a histidine-Fe bond on the bottom of the heme ring and a tyrosine oxygen on

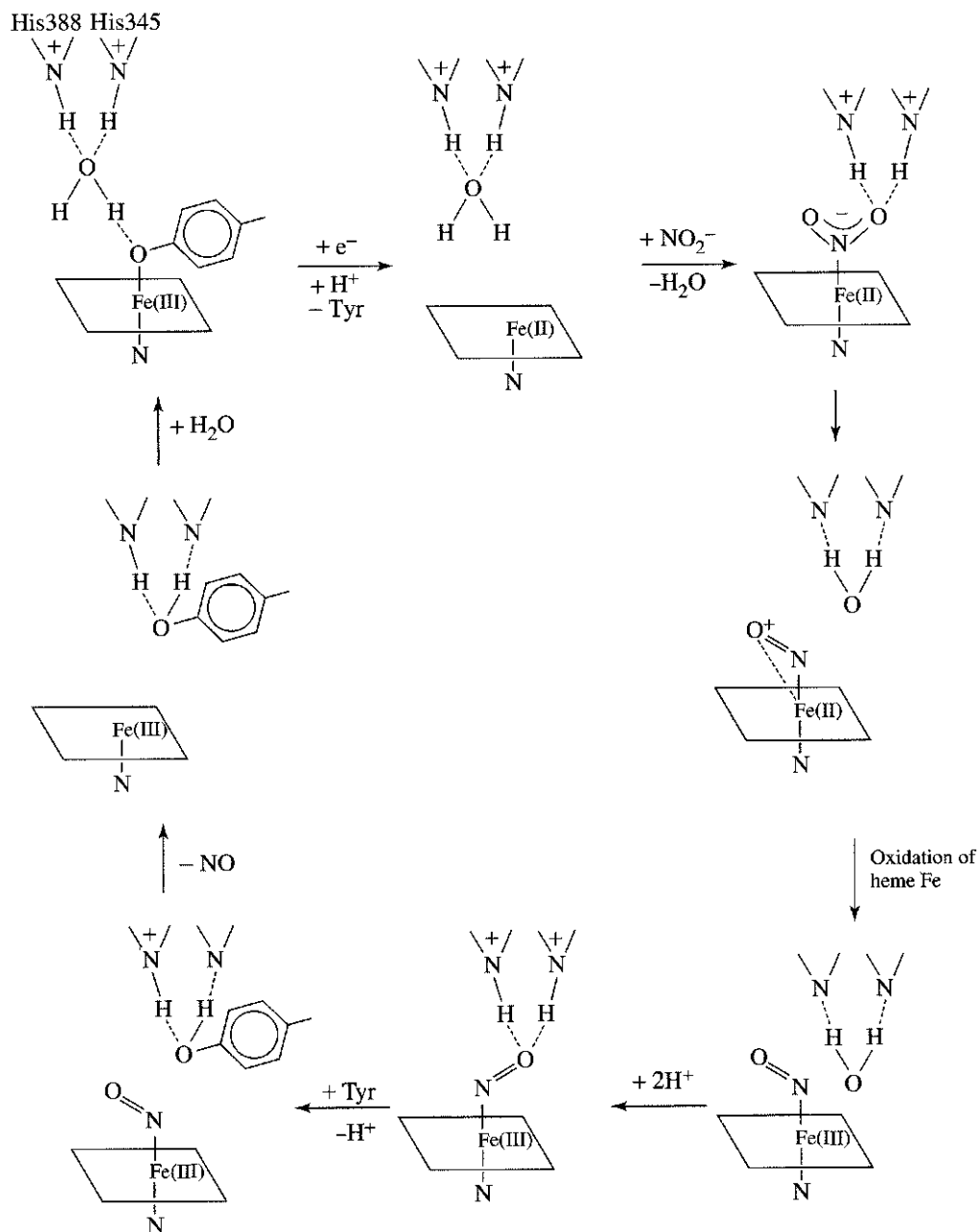


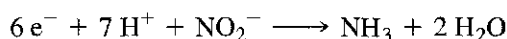
FIGURE 16-15 Possible Routes for Nitrite Reduction by Cytochrome *cd*₁ Nitrite Reductase. (Redrawn from G. Ranghino, E. Scorza, T. Sjögren, P. A. Williams, M. Ricci, and J. Hajdu, *Biochemistry*, 2000, 39, 10958.)

the top. The oxygen is hydrogen-bonded to a water molecule that in turn is hydrogen-bonded to two histidines. On reduction, the tyrosine bond and the water molecule are lost, leaving room for nitrite to enter and bond through the nitrogen atom to the iron, its entry perhaps assisted by the positive charges on the protonated histidines. The two histidines apparently participate in removal of one of the nitrite oxygens (hydrogen bonds to the oxygen become stronger as the N—O bond weakens) and the bent Fe—N—O is thought to change from Fe(II)—NO⁺ to Fe(III)—NO, which remains bent, in contrast to other Fe(III)—NO structures. The two protonated histidine nitrogens may be involved in hydrogen bonding to the NO before it is released and the cycle can begin again. The tyrosine oxygen can replace the NO on the Fe(III), with the release of over 330 kJ/mol of energy.

The reactions proceed by these steps:

- (i) His345 and His388 are protonated in the active site of the unliganded reduced form of cytochrome *cd*₁.
- (ii) Nitrite binds to this doubly protonated enzyme form.
- (iii) Nitrite reduction starts with proton transfer from the histidines to the bound nitrite ion. This process cleaves off a water molecule from the substrate and leaves an NO⁺ cation on the still reduced *d*₁ heme.
- (iv) Electron transfer from the *d*₁ heme to the bound NO⁺ cation creates the more stable [Fe(III)-NO] product complex. The orientation and stability of nitric oxide in this complex depend on possible hydrogen bonding interactions with His345 and His388 in the active site.
- (v) Release of nitric oxide from the *d*₁ heme can happen in more than one way. Delocalization is not continuous in the *d*₁ heme and, as a consequence, the four nitrogen ligands surrounding the iron in the heme plane are not equivalent. Tyr25 or another ligand that can bond temporarily between the histidines may facilitate NO release, influenced by their protonation (or lack thereof).

Still another nitrite reductase, cytochrome *c* NIR, contains five heme groups, only one of which functions as the active site.⁴⁰ A combination of calculations and crystallographic studies has suggested a mechanism in which nitrite replaces a water molecule on one side of the Fe(II) heme (a lysine N is on the opposite side), one of the oxygens of NO₂⁻ is protonated, and the N—O bond is broken with loss of H₂O, leaving a linear Fe(III)—NO species with a low-spin Fe(III). Addition of two electrons and H⁺ leads to Fe^{II}HNO, which is then reduced to Fe^{II}H₂NOH. Yet another electron and another H⁺ allow release of H₂O and formation of an Fe^{III}NH₃ complex. Release of ammonia and a final electron and water addition complete the cycle. Overall, six electrons and seven hydrogen ions react with the nitrite:



With this enzyme, NO is not released, and NO added to the enzyme is only about 1% reduced.

FeS clusters have been known in nitrogenases and other enzymes for some time and have been studied as less complicated species, partly in the hope of elucidating the nitrogenase mechanism and partly because of the large number of possibilities and their interesting nature. The Fe₄S₄ cubane structure is present in high-potential Fe proteins and ferredoxins; nitrogenase contains a Fe-S-Mo region (shown in Figure 16-13) at the

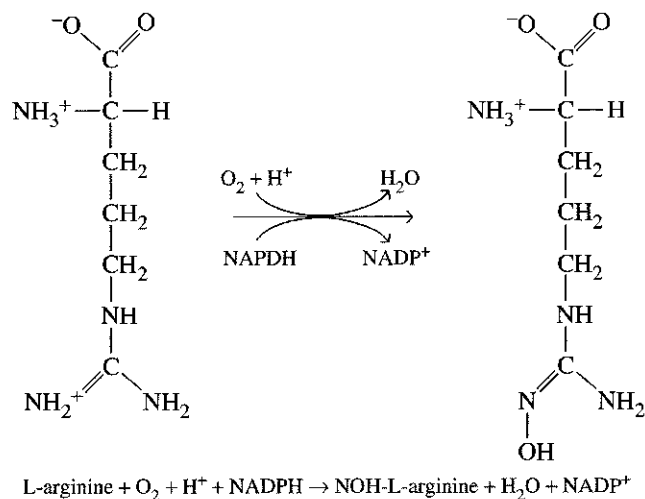
⁴⁰O. Einsle, A. Messerschmidt, R. Huber, P. M. H. Kroneck, and F. Neese, *J. Am. Chem. Soc.*, 2002, 124, 11737.

presumed active site. The clusters show a wide variety of structures and reactions, which makes them difficult to categorize. A review describes a large number of abiological iron-sulfur clusters and their reactions.⁴¹

16-5 NITRIC OXIDE

The importance of NO in biochemistry has only been recognized since the middle of the 1980s, but it was named Molecule of the Year in 1992 by *Science*.⁴² Before that time, it was known primarily as a very reactive gas that is formed during combustion and reacts with oxygen in the air to form NO₂. These two gases, together with tiny amounts of other oxides of nitrogen, are known as NO_x in environmental chemistry, where they are the starting compounds for many reactions. It is now known that another large set of reactions is possible in the body, and the effects of NO are still being discovered.⁴³ For example, overproduction of NO is linked to immune-type diabetes, inflammatory bowel disease, rheumatoid arthritis, carcinogenesis, septic shock, multiple sclerosis, transplant rejection, and stroke. Insufficient NO production is linked to hypertension, impotence, arteriosclerosis, and susceptibility to infection.⁴⁴

NO is synthesized in the body by a number of enzymes, some producing small amounts for nerve transmission and blood flow regulation and some producing large amounts for defense against tumor cells. When large amounts are produced, NO can also have negative effects, such as large blood pressure drops and destruction of tissue, leading to inflammatory disease and degeneration of nerve and brain tissue. The structure of the active site of one of these enzymes, inducible nitric oxide synthase oxygenase, has been determined.⁴⁵ It contains a heme group in a large pocket of the protein, with one side of the iron atom bound to a cysteine sulfur atom and the other side available for substrate binding. It functions by oxidizing arginine in what is believed to be the following two-step reaction:



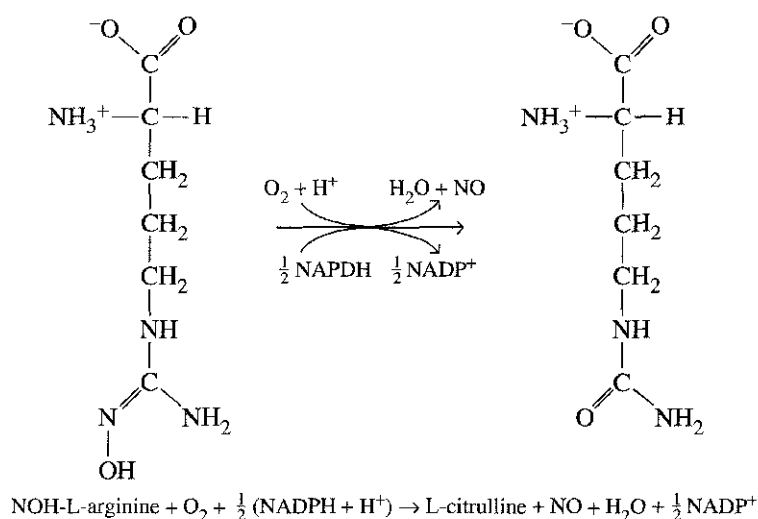
⁴¹H. Ogino, S. Inomata, and H. Tobita, *Chem. Rev.*, **1998**, 98, 2093.

⁴²D. E. Koshland, Jr., *Science*, **1992**, 258, 1861.

⁴³P. J. Feldman, O. W. Griffith, and D. J. Stuehr, *Chem. Eng. News*, Dec. 20, **1993**, p. 26.

⁴⁴S. Moncada and A. Higgs, *N. Engl. J. Med.*, **1993**, 329, 2002; C. Nathan and Q. Xie, *Cell*, **1994**, 78, 915; H. H. Schmidt and U. Walter, *Cell*, **1994**, 78, 919; O. W. Griffith and D. J. Stuehr, *Ann. Rev. Physiol.*, **1995**, 57, 707; O. W. Griffith and C. Szabo, *Biochem. Pharmacol.*, **1996**, 51, 383.

⁴⁵B. R. Crane, A. S. Arvai, R. Gachhui, C. Wu, D. K. Ghosh, E. D. Getzoff, D. J. Stuehr, and J. A. Tainer, *Science*, **1997**, 278, 425.



The energy for these reactions comes from the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP^+ and from the conversion of molecular oxygen to water.

Synthesis of this enzyme is triggered by external stimuli, such as cytokines, released by cancer cells. Once synthesized, the enzyme produces large quantities of NO, which then diffuses into the tumor cells, disrupting DNA synthesis and inhibiting cell growth. The other NO synthases are present at all times, but are activated in a sequence of steps dependent on Ca^{2+} concentration. An activated neuron releases a chemical messenger that opens calcium channels in the next neuron. As Ca^{2+} enters the nerve cell, it binds with calmodulin and the NO synthase to activate it. The reactions described earlier for formation of NO take place, and the NO then activates another enzyme, guanylyl cyclase. From this point on, the effects are uncertain, but may include diffusion back to the first cell and reinforcement of the stimulus. One of the end results seems to be relaxation of smooth muscle, related to the effect seen in blood vessels.

In blood vessels, a similar NO synthase is also activated by Ca^{2+} and calmodulin binding. Increased Ca^{2+} concentration in the endothelial cells of the blood vessels is controlled by calcium channels that can be opened in response to the action of a number of hormones and drugs or by increased pressure in the blood vessel. Again, this activates the enzyme and the NO formed diffuses into the next layer of smooth muscle cells, where it activates guanylyl cyclase to form cyclic guanosine monophosphate (GMP). This compound, in turn, causes a decrease in free Ca^{2+} . Because Ca^{2+} is required for muscle contraction, the net result is muscle relaxation, dilation of the blood vessel, and lowering of the blood pressure. A similar, nonenzymatic effect can be achieved by nitroglycerin, a common heart medicine. It releases NO directly and dilates the blood vessels, thereby increasing blood flow to the heart (and other parts of the body). Maintenance of proper blood pressure appears to require continual synthesis of NO at low levels because the lifetime of NO in the blood or in cells is very short (half-life of a few seconds, depending on the surroundings). NO can also diffuse into the blood, where it decreases clotting ability. In red blood cells, NO is rapidly converted into nitrate by reaction with oxyhemoglobin, in which the Fe(II) is simultaneously converted to the inactive Fe(III) form, or methemoglobin. Other enzyme reactions reduce the Fe(III) back to Fe(II) and restore the activity.

In a different organism, the effect of pH on NO bound to a heme group in the protein nitrophorin 1 helps the bloodsucking insect *Rhodnius prolixus* obtain a meal.⁴⁶

⁴⁶J. M. C. Ribeiro, J. M. Hazzare, R. H. Suxzenzveig, D. E. Champagne, and F. A. Walker, *Science*, 1993, 260, 539.

In the saliva of the insect, the pH is about 5 and the complex is stable. When the complex is injected with the saliva into the blood of a victim, the pH rises to about 7 and the NO is released. The vasodilator and anticoagulant action of the NO make it easier for the insect to draw blood from the victim.

The chemistry of transition metal nitrosyls has been reviewed,⁴⁷ with spectra of many types used to study the electronic structure. Bonding, as described in Chapter 13, can be thought of as a linear complex of NO^+ , isoelectronic with CO and with NO stretching frequencies of 1700 to 2000 cm^{-1} , or a bent complex of NO^- , isoelectronic with O_2 and with NO stretching frequencies of 1500 to 1700 cm^{-1} . The number of electrons on the metal ion and the influence of the other ligands on the metal provide for changes from one to the other during reactions.

NO has a half-life on the order of seconds and is converted to many other products, including NO^+ , NO^- , and ONOO^- , which rapidly decomposes to $\cdot\text{OH} + \text{NO}_2$ or isomerizes to $\text{NO}_3^- + \text{H}^+$ after protonation. Each of these undergoes further reactions, with $\cdot\text{OH}$ and ONOO^- in particular causing many reactions with adverse effects.

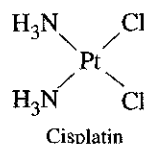
16-6 INORGANIC MEDICINAL COMPOUNDS

Historically, a number of metallic compounds have been used in medicine, including arsenic compounds for the treatment of syphilis and mercury compounds as antiseptics and diuretics.

The general toxicity of these compounds has prompted their replacement, but others have been developed for other diseases. Lithium has activity in the brain and is used to treat hyperactivity, gold compounds are used in arthritis treatment, and antimony compounds are used for the treatment of schistosomiasis. Barium sulfate is used in gastrointestinal X-rays as an imaging agent. Although barium is toxic, the extremely low solubility of the sulfate prevents negative effects. There are other examples in ordinary use, including antacids, fluoride as a tooth decay preventative, and other drugs using copper, zinc, and tin. We will describe only three groups of these compounds, the anti-cancer platinum complexes, gold compounds used in arthritis treatment, and vanadium compounds used in diabetes and cancer treatment.

16-6-1 CISPLATIN AND RELATED COMPLEXES

One compound that is currently being used for the treatment of certain cancers is *cis*-diamminedichloroplatinum(II), or cisplatin. This compound shares the common action of chemotherapeutic agents by preventing cell growth and proliferation. It also shares the common trait of affecting normal cells as well as cancerous cells, but of having a larger effect on the cancerous cells because of their rapid growth rate.



Its effect on cell growth was discovered by B. Rosenberg,⁴⁸ when *E. coli* bacteria placed in an electric field stopped dividing and grew into long filaments, similar to their action when treated with antitumor agents. It was found that the ammonium chloride buffer and the platinum electrode were forming compounds, including cisplatin. Cisplatin acts

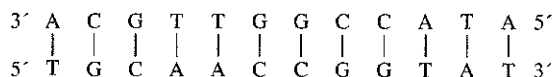
⁴⁷B. L. Westcott and J. H. Enemark, "Transition Metal Nitrosyls," in E. I. Solomon and A. B. P. Lever, eds., *Inorganic Electronic Structure and Spectroscopy*, John Wiley & Sons, New York, 1999, pp. 403–450.

⁴⁸*Chem. Eng. News*, June 21, 1999, p. 9.

on the deoxyribonucleic acid (DNA) of the cells, disturbing the usual helical structure and thus preventing duplication.

Deoxyribonucleic acids are chains of 5-membered deoxyribose sugar rings connected by phosphate links between the 3' and 5' oxygen atoms, with each sugar connected to one of four bases (cytosine, guanine, thymine, and adenine, abbreviated as C, G, T, and A) shown in Figures 16-16 and 16-17.

DNA usually adopts the double-chain twisted ladder structure (the double helix) shown in Figure 16-18 with complementary base sequences allowing hydrogen bonding between the two chains, as in



with the 3' end of one chain opposite the 5' end of the other. In this structure, the planar rings of the bases are stacked in parallel planes on the inside of the helix, with the negative phosphate groups on the outside. Ribonucleic acids (RNAs) have a similar backbone, but the sugars have OH instead of H in the 2' positions and uracil replaces thymine. RNAs have more varied structures and generally do not form the double helix that is common in DNAs.

DNA carries the genetic code that dictates the amino acid sequence during the synthesis of proteins, which in turn dictates the form of life and the details of structure and action. RNA was until recently thought to be primarily a messenger, carrying information

FIGURE 16-16 DNA Backbone Structure. The bases are cytosine, guanine, thymine, or adenine (CGTA).

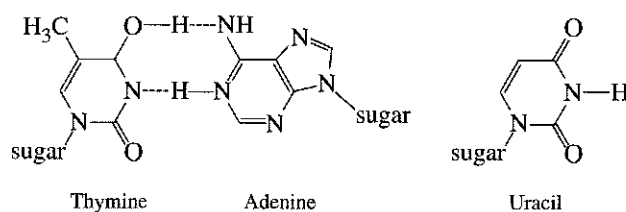
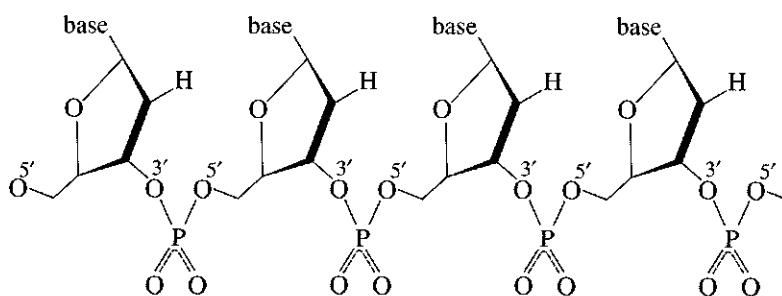
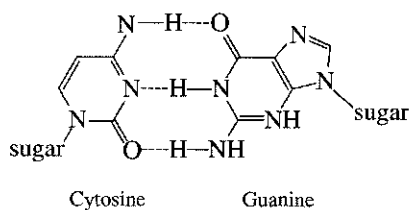


FIGURE 16-17 Purine and Pyrimidine Bases of DNA and RNA. Adenine and guanine are purines, and cytosine, thymine, and uracil are pyrimidines. The hydrogen-bonding combinations of complementary base pairs are shown.



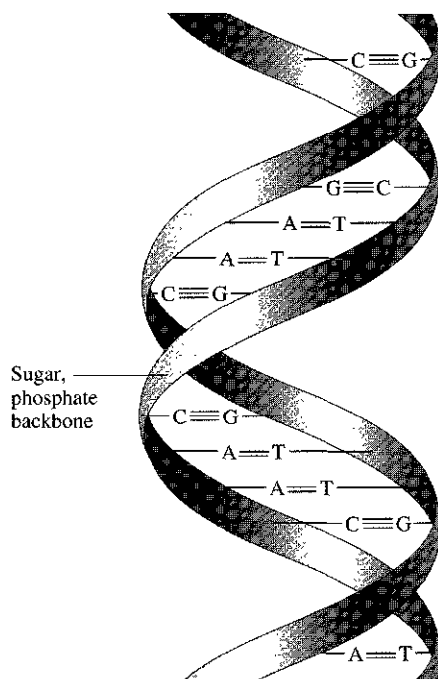


FIGURE 16-18 The DNA Double Helix.

from the DNA to the synthetic site. More recently, however, RNA has been found to have enzymatic activity of its own.

During growth, the DNA molecule “unzips” and new partner molecules are formed on each of the chains, resulting in two molecules where one existed before. Many cancer treatments depend on interrupting this process to prevent the rampant growth characteristic of cancer.

Cisplatin hydrolyzes to the diaqua complex, which then reacts with the nitrogen atoms of guanine in the DNA, forming a crosslink between adjacent guanine bases, usually within the same strand or occasionally between strands. The result is a kink in the DNA helix, with angles up to 34° . This change in shape is enough to interfere with the self-replication of the DNA and slows growth of the cancer. In fact, this treatment actually results in shrinkage of cancers, although the mechanism for this is not yet clear. The structure of cisplatin bound to a short segment of double-stranded DNA is shown in Figure 16-19.

The structure of a protein believed to be involved in anticancer activity when combined with a cisplatin-modified DNA complex⁴⁹ shows a larger kink (61°) in the DNA and intercalation of a phenylalanine ring from the protein into the resulting notch (where the phenylalanine ring is stacked between two base layers). Binding such as this might prevent removal of the cisplatin and other repair reactions of the DNA.

Other compounds have been tested to determine the structural requirements for an effective mutagenic agent. The requirements are as follows:⁵⁰

1. A pair of hard (chloride or oxygen donors) *cis*-anionic ligands subject to substitution by DNA nitrogen bases
2. Water solubility and ability to pass through cell membranes (uncharged complexes)
3. Unreactive ligands on the other sites that are primary or secondary amines

⁴⁹U.-M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo, and S. J. Lippard, *Nature*, **1999**, *399*, 708.

⁵⁰D. B. Brown, A. R. Khokhar, M. P. Hacker, J. J. MacCormack, and R. A. Newman, “Synthesis and Biological Studies of a New Class of Antitumor Platinum Complexes,” in S. J. Lippard, ed., *Platinum, Gold, and Other Metal Chemotherapeutic Agents*, American Chemical Society, Washington, DC, 1983, pp. 265–277.

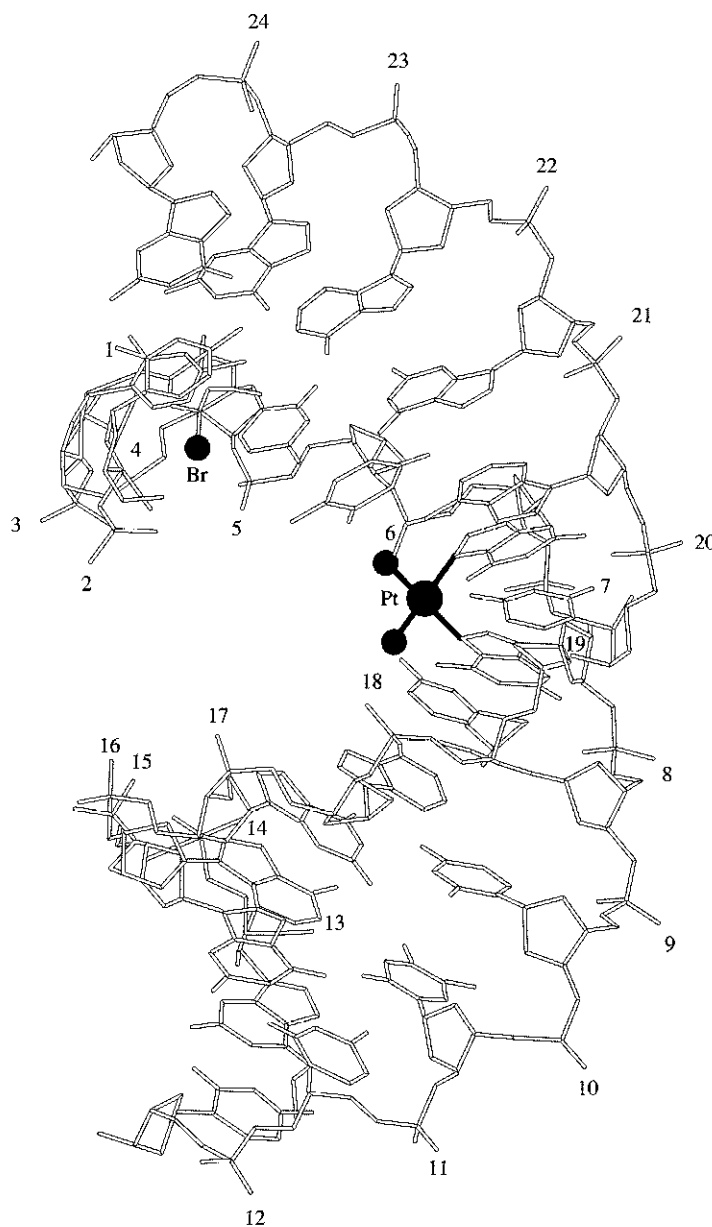


FIGURE 16-19 Structure of a Cisplatin-DNA Complex. Shown is a 26° bend imposed by the GG bonding. (Reproduced with permission from P. M. Takahara, C. A. Frederick, and S. J. Lippard, *J. Am. Chem. Soc.*, **1996**, *118*, 12309.)

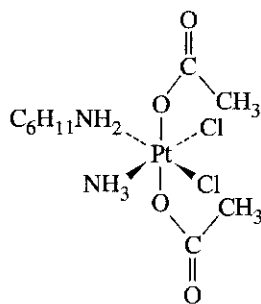


FIGURE 16-20 Bis-(acetato-*O*)-amminedichloro(cyclohexanamine)platinum(IV).

These requirements limit the choices, but a few other compounds related to cisplatin have been used successfully in practice. One goal of research in this area is to find a drug that can be administered orally. (Cisplatin must be given intravenously.) At least one Pt(IV) compound, bis-(acetato-*O*)-amminedichlorobis(cyclohexanamine)platinum(IV), shown in Figure 16-20, has been tested in clinical trials as an orally active antitumor agent.⁵¹ Its action seems to be similar to that of cisplatin, but with the added feature that its ligands protect it from reaction in the digestive system and allow it to be absorbed into the bloodstream.

⁵¹C. M. Giandomenico, M. J. Abrams, B. A. Murrer, J. F. Vollano, M. I. Rheinheimer, S. B. Wyer, G. E. Bossard, and J. D. Higgins III, *Inorg. Chem.*, **1995**, *34*, 1015.

16-6-2 AURANOFIN AND ARTHRITIS TREATMENT

Gold in many forms has been used medicinally for hundreds of years, with relatively few proven benefits and many examples of toxicity. More recently, gold complexes of thiols, Figure 16-21(a) and (b), have been used for treatment of arthritis, but have the major disadvantage that they must be administered by injection into the site of inflammation.

More recently, the compound auranofin, (2,3,4,6-tetra-*O*-acetyl-1-thio- β -glucopyranosato-*S*)-(triethylphosphine)gold(I), Figure 16-21(c), has been developed. It has the advantage that it can be administered orally and still be effective.

The mechanism of action of these compounds is still not known. One possibility is that they act through the formation of gold-sulfur complexes, which can inhibit the formation of disulfide bonds. Because much of the biochemistry of arthritis is still uncertain, the design of drugs for specific action is difficult.

16-6-3 VANADIUM COMPLEXES IN MEDICINE

Several vanadium (IV) compounds have been found to have insulin-like activity. However, their toxicity prevents medical use. (Dipicolinato)oxovanadate(V), $[\text{VO}_2\text{dipic}]^-$, is effective as an oral agent in animals,⁵² and has less toxicity. The acid-base properties of the compound make it likely that it is absorbed in the acidic environment of the stomach or the first part of the small intestine; it protonates at pH ~ 1 .

Several V(IV) compounds, shown in Figure 16-22, also have anticancer activity.⁵³

16-7 STUDY OF DNA USING INORGANIC AGENTS

DNA polymerases work by stitching together two nucleotides, one at the 3' end of a DNA chain and the other a deoxynucleic acid triphosphate. The two are held in position near each other by hydrogen bonding to the template DNA chain and are joined by reaction of the first phosphate of the triphosphate with the OH of the saccharide ring of the other base, releasing diphosphate. Mg^{2+} ions separated by about 390 pm are bound on each side of the phosphorus in the transition state, as in Figure 16-23.

Information on the three-dimensional structure of RNA can be obtained by tethering cleavage agents to known positions and then by studying the fragments produced by the cleavage reactions. This has been used to study *Escherichia coli* ribosomal

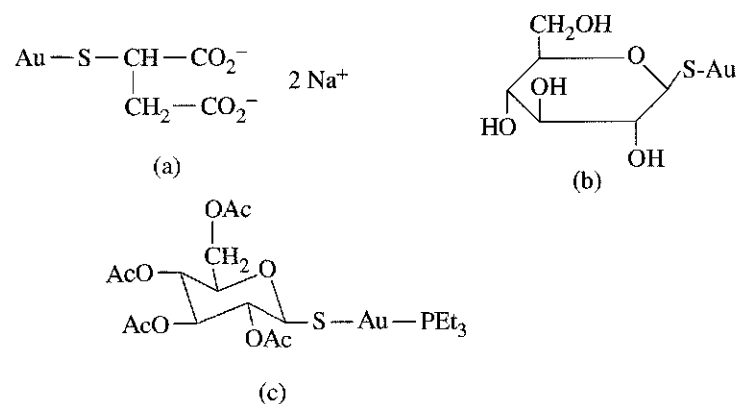


FIGURE 16-21 Gold Antiarthritic Drugs. (a) Sodium aurothiomalate. (b) Aurothioglucose. (c) Auranofin.

⁵²D. C. Crans, L. Yang, T. Jakusch, and T. Kiss, *Inorg. Chem.*, **2000**, *39*, 4409, and references therein.

⁵³F. M. Uckun, Y. Dong, and P. Gosh, U.S. Patent 6,245,808, 2002.

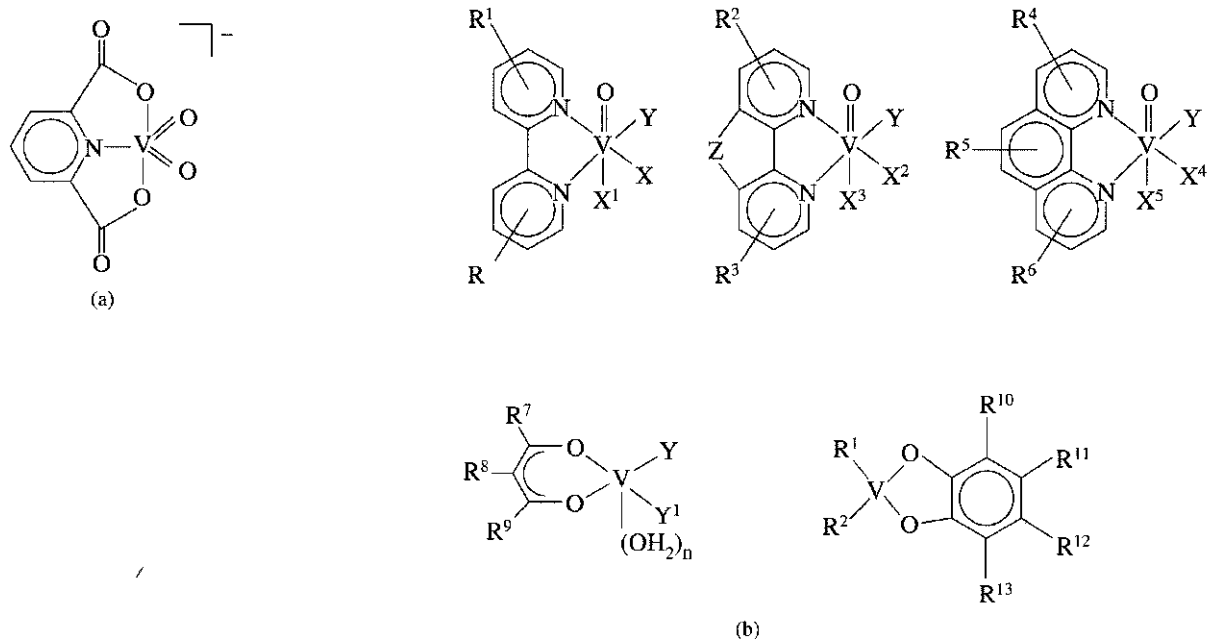


FIGURE 16-22 (Dipicolinato)oxovanadate(V) and Anticancer V(IV) Compounds. R–R⁹ are H, alkyl, alkoxy, halogenated alkyl, alkanoyloxy, or NO₂; R¹⁰–R¹³ are H, halogen, or C₁–C₆ alkyl; and X⁴, X⁵, Y, and Y¹ are mono- or bidentate ligands.

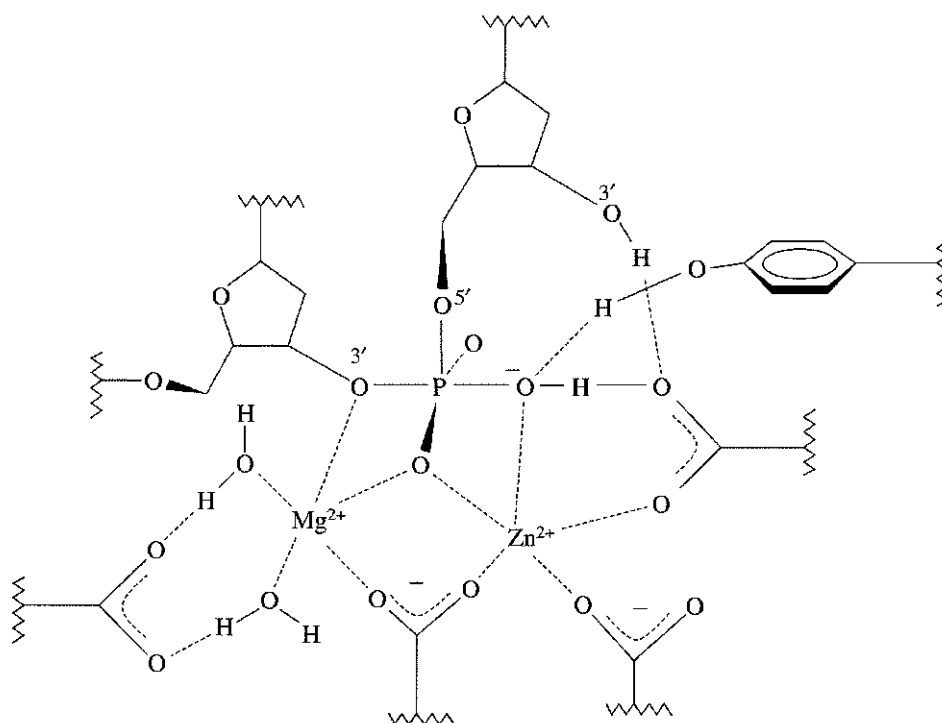
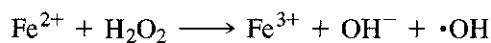


FIGURE 16-23 DNA Polymerase Transition State. (Redrawn from T. A. Steitz and J. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.*, **1993**, *90*, 6498.)

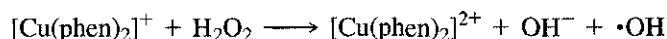
RNA.⁵⁴ Fe(II) as a cleavage agent was attached to the 5' terminus of the 3' fragment and to two other specific sites as 1-(*p*-bromoacetamidobenzyl)-EDTA Fe(II). Addition of H₂O₂ and ascorbic acid generated hydroxyl radicals by the reaction



⁵⁴L. F. Newcomb and H. F. Noller, *Biochemistry*, **1999**, *38*, 945.

These free radicals can diffuse to nearby sites and react with the saccharide ring by abstracting H from the 1' position. Several reactions follow, with the net result that the RNA chain is cleaved, with formation of a small organic molecule as the result of destruction of the saccharide ring. Some of the cleavage points are near the tethering point in the same chain; others are distant in terms of chain position but close in terms of three-dimensional folding. As a result of these experiments, several parts of the chain are now known to be near each other in the folded structure. Similar studies offer the promise of further elucidation of the complete structure. Methidium-EDTA-Fe(II) is another tethered cleavage reagent; it generates superoxide from O₂ and hydroxyl radical from peroxide and is reduced back to Fe(II) by dithiothreitol.⁵⁵

Hydroxyl radicals can also be generated by the reaction



after which thiols or ascorbic acid can reduce Cu(II) back to Cu(I). [Cu(phen)₂]⁺ intercalates (fitting between the parallel rings of the bases) in the minor groove of right-handed double-helix DNA.⁵⁶ Intercalation unwinds the DNA by about 11°, binding to two base pairs. The reaction shows only a slight GC preference. Tethered Cu-phen complexes are also possible; these complexes and their cleavage reactions have been reviewed.⁵⁷

[Ru(en)₂phi]³⁺ (phi = phenanthrenequinone diimine) intercalates with B-DNA. The Δ isomer prefers 5'-GC sites in the major groove and the Λ isomer is site neutral.⁵⁸ On photoactivation, the complex abstracts H3' from the deoxyribose ring and the chain is cleaved, leaving 3' and 5' phosphates, propenoic acid, and 3'-phosphoglycaldehyde consistent with a reaction between O₂ and the 3' carbon, as shown in Figure 16-24.

16-8 ENVIRONMENTAL CHEMISTRY

16-8-1 METALS

Mercury and lead are two of the most prominent metallic environmental contaminants today. Although there have been continued efforts to prevent distribution of these metals and to clean up sources of contamination, they are still serious problems. Other metals and semimetals, such as arsenic, also cause significant health effects. Some of them are described here.

Mercury

Because mercury has a significant vapor pressure, the pure metal can be as serious a problem as its compounds. Although the problem is usually less severe in laboratories today, mercury contamination and poisoning have been a problem in chemistry and physics laboratories for many years. Spills are inevitable when large amounts of the liquid are used in manometers, Toeppler pumps, and mercury diffusion pumps on vacuum lines. Because liquid mercury breaks into tiny drops, cleanup is extremely difficult and contamination remains even after strenuous efforts to remove it. As a result, a low level of mercury vapor is present in many laboratories and can result in toxic reactions. Mercury interferes with nerve action, causing both physical and psychological symptoms. Whether the behavior of the Mad Hatter in Lewis Carroll's *Alice in Wonderland* had an origin in fact is uncertain, but mercury compounds were used in felt making and some hatters were victims of mercury poisoning as a result.

⁵⁵R. P. Hertzberg and P. B. Dervan, *J. Am. Chem. Soc.*, **1982**, *104*, 313.

⁵⁶L. E. Marshall, D. R. Graham, K. A. Reich, and D. S. Sigman, *Biochemistry*, **1981**, *20*, 244; C. Yoon, M. D. Kubawara, A. Spassky, and D. S. Sigman, *Biochemistry*, **1990**, *29*, 2116.

⁵⁷D. S. Sigman, T. C. Bruice, A. Mazunder, C. L. Sutton, *Acc. Chem. Res.*, **1993**, *26*, 98.

⁵⁸T. P. Schields and J. K. Barton, *Biochemistry*, **1995**, *34*, 15037.

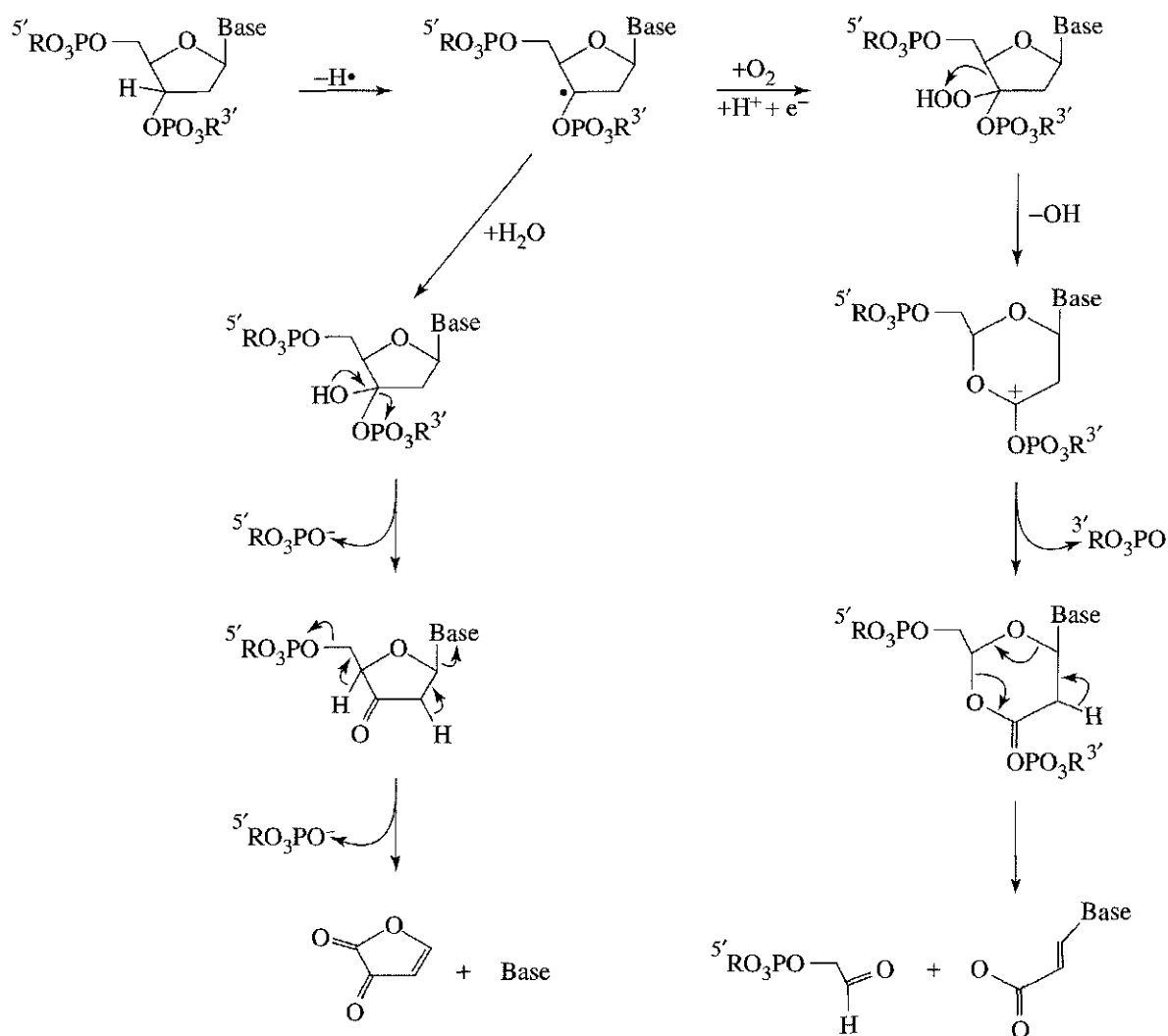
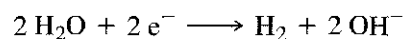
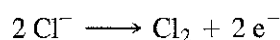


FIGURE 16-24 Proposed Mechanisms of DNA Cleavage, Initiated by $[\text{Ru}(\text{en})_2(\phi)]^{3+}$. (Redrawn from A. Sitlani, E. C. Long, A. M. Pyle, and J. K. Barton, *J. Am. Chem. Soc.*, **1992**, *114*, 2303.)

Several industrial processes use mercury in large amounts, and the resulting potential for spills and loss to the environment is great. One of the largest is the chloralkali industry, in which mercury is used as an electrode for the electrolysis of brine to form chlorine gas and sodium hydroxide:



and



In one tragic incident, an entire community on Minamata Bay in Japan was poisoned, with extremely serious birth defects, very painful reactions, mental disorders, and many deaths. Only after lengthy research was the cause determined to be mercury compounds discarded into a river by a plastics factory. Whether it was inorganic salts or methylmercury compounds seems uncertain, but the contamination was immense and methylmercury compounds were found in the silt and in animals and humans. The methylmercury was readily taken up by the organisms living in the bay and, because the people of the community depended on fish and other seafood from the bay for much of their diet, the entire community was poisoned.

This incident showed the concentrating effect of the food chain and the need for extreme caution in predicting the outcome of dumping any material into the environment. Even though the concentration of methylmercury was low, it was readily taken up by the plants and microorganisms in the water. As these organisms were eaten by larger ones, each organism in the food chain retained the mercury and the concentration of mercury in these predators increased, leading to harmful concentrations in the larger fish and in other organisms eaten by the people.

During the research on mercury reactions in the environment, it was also discovered that insoluble metallic mercury can be converted to soluble methylmercury by bacterial action involving methylcobalamin. Earlier, it had been thought that elemental mercury was unreactive in lakes and rivers; now it is known to be dangerous. As a result, there are now many more toxic metal sources than had once been recognized. Historically, large amounts of metallic mercury were discharged into the Great Lakes and other bodies of water in the belief that it was harmless.⁵⁹ Cleanup of these sites seems impossible, so the problem will remain with us for the foreseeable future.

Although concern about mercury contamination is now more visible, and industries have reduced its release into the environment, the increasing use of mercury in small batteries and other products results in a greater distribution of mercury into the environment as a whole. As a result, the problem is changing from one of a few large sources of contamination to many small ones and techniques for dealing with the problem must change as well. Concerns are being expressed about heavy metal contamination of the atmosphere by incinerators burning municipal garbage and trash, and it is likely that removal of these materials from the trash before burning or scrubbing of the flue gases to remove the volatile products will be needed. Another source of atmospheric mercury (and other elements) is the burning of coal for electric power. In one plant burning 6×10^8 tons of coal per year, 60 tons of mercury, 12,000 tons of lead, 240 tons of cadmium, 3,000 tons of arsenic, 3,000 tons of selenium, 2,400 tons of antimony, 15,000 tons of vanadium, and 120,000 tons of zinc were released as particulates or gases.⁶⁰ It is now believed that the major source of mercury in many lakes is from the atmosphere.

Dimethylmercury has been found in the gases from landfills in Florida.⁶¹ Landfills may be a source of the methylated mercury species that appear in rain. The dimethylmercury quickly breaks down to monomethylmercury, CH_3Hg^+ , a species that is water soluble and is commonly found in fish and other aquatic organisms.

A Canadian study designed to track the cycles of mercury in the atmosphere and lakes is using three stable mercury isotopes, ^{198}Hg for wetlands, ^{200}Hg for uplands, and ^{202}Hg for lakes,⁶² all isotopes that are common in nature (10%, 23%, and 30%, respectively). These isotopes are being added to the area in amounts similar to those received by the most atmospherically polluted lakes in eastern North America over a period of 3 years and their fate tracked by mass spectroscopy to determine the sources of mercury. The isotopes are being applied to the uplands and wetlands by plane during rainstorms and collectors are placed under the forest canopy to determine the amounts reaching the ground. Ground vegetation and litter are also being sampled to determine whether the mercury in them is old mercury that is being recycled or new mercury being added through the atmosphere. Preliminary results have shown different reactions for new mercury initially, but after a few days or weeks the compounds and reactions are the same as for the mercury already in the soil and vegetation.

⁵⁹A. T. Schwartz, D. M. Bance, R. G. Silberman, C. L. Stanisti, W. J. Stratton, and A. P. Zipp, *Chemistry in Context*, 2nd ed., American Chemical Society, Washington, DC, Wm. C. Brown, Dubuque, IA, 1997. Chapter 7, describes the effects on Onondaga Lake in New York.

⁶⁰N. E. Bolton, J. A. Carter, J. F. Emery, C. Feldman, W. Fulkerson, L. D. Hulett, and W. S. Lyon, in S. P. Babu, ed., *Trace Elements in Fuel*, American Chemical Society, Washington, DC, 1975, p. 175.

⁶¹S. E. Lindberg, *Atmos. Environ.*, **2001**, 35, 4011.

⁶²*Chem. Eng. News*, Sept. 24, 2001, pp. 35–38.

Mercury in the Arctic cycles with the seasons between the atmosphere and snow on the ground.⁶³ In the spring, as the sun reappears after the winter darkness, mercury levels in the troposphere decline for about 3 months. At the same time, the level of mercury in the snow increases 100-fold, both as methylmercury and as inorganic compounds of mercury. Later in the year, as the snow melts, the levels in the snow drop and mercury reappears in the troposphere. The elemental mercury in the atmosphere is converted to particulates or reactive species, paralleling a decrease in atmospheric ozone, and is then deposited in the snow. Later in the summer, the mercury levels in the atmosphere increase, probably due to temperature- or sunlight-induced emission of volatile mercury species from the surface.

Lead

Lead is another metal that is widespread in the environment, principally as a result of human activities. Two of the largest sources for environmental lead were paint pigments and leaded gasoline, both now much reduced in importance. White lead [basic lead carbonate, $2\text{PbCO}_3 \cdot \text{Pb}(\text{OH})_2$] was used as a paint pigment for many years and older buildings still have lead-containing paint, frequently under layers of more modern paint. If children living in these buildings eat paint chips, they are likely to ingest significant amounts of lead. In fact, in some cities, lead poisoning of children is a very common problem.⁶⁴ As is the case with mercury, lead can affect nerve action and cause retardation and other mental problems, as well as causing acute illness. Unfortunately, the only cure is complete removal of the paint, a very time-consuming and expensive process.

Although heavy metal glazes are prohibited in commercial manufacture of ceramics in many countries, there are still reports of lead and other toxic heavy metals showing up in dishes imported from countries without similar controls or in ceramic items made by individuals who do not take the appropriate precautions. Because the glaze seems permanent and impervious to water and ordinary foods, it might seem that such materials would not be a hazard, but acidic solutions can extract significant amounts of the heavy metals and result in chronic low-level lead poisoning.

Lead in gasoline is being phased out in industrialized countries, but it is a continuing problem in developing countries. Tetraethyl lead, $\text{Pb}(\text{C}_2\text{H}_5)_4$, has been used as an antiknock compound in gasoline for many years. When this compound is present, a low grade of gasoline burns as efficiently in automobile engines as a higher grade without the lead. Unfortunately, the lead from the gasoline has been distributed throughout the environment. Some studies have found increased lead levels in roadside plants and soil, and the population in general has been exposed to higher levels of lead as a result of this use. Laws requiring the use of unleaded gasoline in newer cars have required other changes in the engines and in the refining of gasoline to compensate.

Catalytic converters

The use of catalytic converters to reduce the amount of unburned hydrocarbons in exhaust gases is an additional example of the use of metals. Reactions of these unburned hydrocarbons in the atmosphere are described later, in the section on photochemical smog. The catalyst currently used is a cordierite or alumina support treated with an Al_2O_3 wash coat containing rare earth oxides and 0.10% to 0.15% Pt, Pd, and/or Rh, which catalyzes the combustion of hydrocarbons in the exhaust gases to carbon dioxide and water. Platinum,

⁶³W. H. Schroeder, K. G. Anlauf, L. A. Barrie, J. Y. Lu, A. Steffen, D. R. Schneeberger, and T. Berg, *Nature*, **1998**, 394, 331.

⁶⁴M. W. Oberle, *Science*, **1969**, 165, 991; P. Mushak and A. F. Crocetti, *Environ. Res.*, **1989**, 50, 210.

palladium, and nickel are among the most reactive (and widely used) catalytic materials. They are used in many different specific compounds and physical forms for reactions of surprising specificity in the petroleum and chemical industries.

In another of the many interactions between problems and their solutions, catalysts in catalytic converters are poisoned by lead. For this reason, cars with catalytic converters are required to use only unleaded gasoline. One negative side effect of the use of catalytic converters is an increase in N_2O emission. The converters reduce NO and NO_2 to N_2O , which has less immediate effects but has a greenhouse effect (described later in this chapter).

Still another recently discovered side effect is the deposition of platinum, palladium, and rhodium along roadsides as a result of catalyst breakdown.⁶⁵ The amounts are small (maximum was 70 ng/g of Pt), but it has been suggested that the amounts approach those that would make recovery economically feasible because the material could be easily collected in comparison with usual mining operations.

Arsenic

Efforts to remove toxic materials from industrial sites, homes, and farms have unearthed other problems. For example, during the 1930s, farmers fought grasshopper infestations with bran poisoned with arsenic compounds. Fifty or more years later, burlap bags of arsenic-laced bran were found in barns and storage sheds, where they were potentially serious hazards. Several states have begun programs to locate and remove these poisons for safe disposal but, because there is no way to detoxify a heavy metal, the material will remain toxic forever. The only possible way to alleviate the problem is to seal the material in a toxic waste dump and take every possible means to prevent leaching or other ways of spreading the material or to find some other use for the heavy metal compounds that is profitable enough to make reprocessing feasible. So far, such uses have been very rare.

Arsenic is also present in groundwater whenever it percolates through minerals containing arsenic compounds. One area where this is particularly common is Bangladesh. In an attempt to reduce waterborne illness, international agencies have been helping drill wells to provide water uncontaminated by bacteria and other surface contaminants. However, increased incidence of birth defects and other health problems began developing at the same time. It was finally discovered that the problem was the high levels of arsenic in the water, 50 ppm or higher. Drinking water standards in the United States require levels less than 50 ppb; a new standard will require reduction in public water supplies to 10 ppb by 2005.⁶⁶ There is now evidence that As(III) disrupts endocrine function even at very low concentrations and interferes with DNA repair capacity.⁶⁷ As further research uncovers the detailed mechanisms of these actions, even more stringent limits may be indicated, in spite of their high costs.

Other heavy metals are also toxic, but fortunately are less widespread and are present in smaller amounts. Mine tailings (waste rock remaining after the valuable minerals have been removed) and waste material from processing plants are major sources of such metals. Many major rivers and lakes have sources of metal contamination from industries whose processes were developed and facilities were built before control of waste was recognized as a major problem.

⁶⁵J. C. Ely, C. R. Neal, C. F. Kulpa, M. A. Schneegurt, J. A. Seidler, and J. C. Jain, *Environ. Sci. Technol.*, **2002**, *35*, 3816.

⁶⁶Details can be found at www.epa.gov/safewater/ars/arsenic.html.

⁶⁷A. S. Andrew, M. R. Karagas, and J. W. Hamilton, *Int. J. Cancer*, **2003**, *104*, 263; R. C. Kaltreider, A. M. Davis, J. P. Lariviere, and J. W. Hamilton, *Env. Health Persp.*, **2001**, *109*, 225.

Radioactive waste

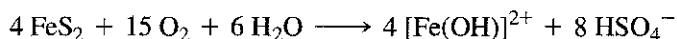
Disposal of radioactive waste is a continuing controversial topic. Some argue that the technical problems have been solved and that only politics remain in the way of efficient permanent storage of such wastes, primarily in the Yucca Mountain site now being constructed in Nevada. Others maintain that the technical problems are far from being solved and, in addition, that the long half-lives of some of the isotopes will require protection of the disposal sites for hundreds or even thousands of years. At this time, it is impossible to predict the outcome, beyond noting that no location is perfect, either geologically or politically. Reports of contamination of water and land around processing sites have led to even more suspicion of any reported solution, and have made the choices even more difficult. An additional concern is the need to transport the wastes to whatever storage site is selected. Although some argue that the containers and transportation modes (either by truck or rail) have been developed and adequately tested, others argue that public safety demands even more than has been done. One factor that has been neglected in much of the discussion is that new fuel rods have been delivered to nuclear reactors for years with few incidents. Again, the question is what degree of safety is required and how it can be guaranteed.

As in the case of the heavy metals described earlier, the problem is the permanent nature of the atoms. Even though they are undergoing radioactive decay, the process is one that will leave some radioactive materials for thousands of years, and the radiation will be dangerous for that length of time. A related problem is the wide variety of elements in much of the radioactive waste. Spent fuel rods from nuclear reactors contain ^{238}U in large amounts, ^{235}U in small amounts (largely depleted by the chain reaction), ^{239}Pu , fission and other decay products of a bewildering variety, and the metal cladding material that has become radioactive because of the intense neutron flux of the reactor. Structural materials from decommissioned reactors and byproducts from ore processing and isotope enrichment plants are other examples of relatively high-level wastes. Low-level wastes from laboratories and hospitals present different technical difficulties because of their relatively large volume but low radioactive level. For some purposes, concentration of such wastes would be desirable, but loss to the environment during processing is an additional problem. As a relatively small but very important part of the overall problem of waste disposal, disposal of radioactive waste will long be the subject of many fiercely fought battles.

16-8-2 NONMETALS

Sulfur

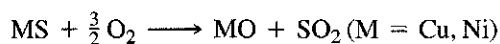
Mine tailings are a source of both metal and nonmetal contamination. A common material in coal mines is iron pyrite, FeS_2 . As a contaminant of coal, this compound and similar compounds contribute to the production of sulfur oxides in flue gases when coal is burned. As a material in mine tailings, it contributes both iron and sulfur to water pollution when the sulfide is oxidized in a series of reactions to sulfate and the Fe(II) oxidized to Fe(III):



Because Fe(III) is a strongly acidic cation, the net result is a dilute solution of sulfuric acid containing Fe(II), Fe(III), and other heavy metal ions dissolved in the acidic solution (pH values of 2 to 3.5 have been measured). In areas with played-out mines, such solutions are common in the streams and rivers, effectively killing most plant and animal life in the water.

When coal containing sulfur compounds is burned, the resulting sulfur dioxide and sulfur trioxide can result in atmospheric contamination. There is much worldwide controversy regarding such contamination, because it travels across political and natural boundaries, and those who generate the contamination are rarely those who suffer its direct consequences. The sulfur oxides and nitrogen oxides from high-temperature combustion are readily dissolved in water droplets in the atmosphere and returned to the earth as acid rain. Although the evidence is still being debated, there seems little doubt that such acid rain has damaged forests and lakes globally, as well as attacking building materials and artistic works. Studies of the damage to limestone statues and building materials show an accelerating rate of destruction, with many carvings and sculptures becoming completely unrecognizable over a relatively short time.

Although the amount of sulfur released by smelting is only about 10% of the total released into the atmosphere, the dramatic effects of sulfur oxides can be seen locally around smelting industries, where nickel or copper are mined and purified. The major ores of these metals are sulfides, and the method of extracting the metal begins with roasting the ore in air to convert it to the oxide:



When compared with the United States, a larger fraction of the sulfur dioxide generated in Canada is caused by smelting operations, because more of Canada's power generation is hydroelectric and the total amount of power generated is smaller. Two sites that have been studied thoroughly are in Trail, British Columbia, and Sudbury, Ontario. When the area around Trail was studied from 1929 through 1936, after 30 to 40 years of smelter operation, no conifers were found within 12 miles and damage to vegetation could be seen as far as 39 miles from the source.⁶⁸ Similar effects could also be seen around Sudbury, with evidence of acidified lakes up to 40 miles away. Efforts to control the emission of SO₂ and SO₃ have reduced the contamination, but recovery of the environment is a very slow process.

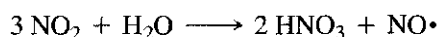
One advantage of the recovery of sulfur oxides from smelting is that the amounts are large enough to be economically useful; in most cases, the concentration of sulfur dioxide and sulfur trioxide found in power plant flue gases is so small that it is simply an added expense to remove them. Two techniques are used, removal of the sulfur compounds from the coal before burning and scrubbing of the stack gases to remove the oxides. Because FeS₂ is much more dense than coal, much of it can be removed by reducing the coal to a powder and separating the two by gravitational techniques. Leaching with sodium hydroxide also removes much of the sulfide contaminant, but scrubbing of the stack gases with a substance such as an aqueous slurry of CaCO₃ is still required for complete removal. The resulting CaSO₃ and CaSO₄ must also be disposed of or used in some way. Other techniques require gasification of the coal (partial combustion in steam to CO and H₂) and scrubbing of the gas to remove the resulting H₂S, combustion of a fluidized bed of finely pulverized coal and limestone, or complete conversion of SO₂ to SO₃ on a V₂O₅ catalyst and removal of SO₃ as H₂SO₄.

Nitrogen oxides and photochemical smog

Nitrogen oxides are also major contaminants, primarily from automobiles. The combustion process in automotive engines takes place at a high enough temperature that NO and NO₂ are formed. In the air, NO is rapidly converted to NO₂, and both can react with the hydrocarbons that are also released by cars. The resulting compounds are among the primary causes of smog seen in urban areas, particularly those where geography prevents easy mixing of the atmosphere and removal of contaminants. Although

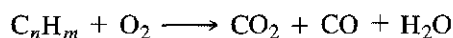
⁶⁸C. G. Down and J. Stocks, *Environmental Impact of Mining*, Wiley, New York, 1977, p. 63.

improvements have been made, there are still serious problems. The nitrogen oxides can also form nitric acid, which can contribute to acid rain:

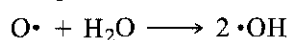


Photochemical smog can form whenever air heavily laden with exhaust gases is trapped by atmospheric and topographic conditions and exposed to sunlight. Ozone and formaldehyde formed in the atmosphere from nitrogen oxides and hydrocarbons are also major contributors to the smog. Some major reactions in this sequence are shown here.⁶⁹

Reactions during combustion of gasoline include the following:



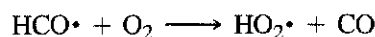
Traces of ozone can be photolyzed, with hydroxyl radical the most important product:



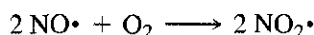
Another important species, the hydroperoxyl radical, is formed by the photolysis of formaldehyde:



(M is an unreactive molecule that removes kinetic energy from the products after this exothermic reaction.)



Oxidation of $\text{NO}\cdot$ at high concentration yields $\text{NO}_2\cdot$



and oxidation of NO by $\text{HO}_2\cdot$ at low NO concentrations, which is more common, also yields NO_2 :

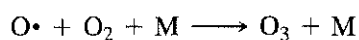


Photolysis of NO_2 forms oxygen atoms:

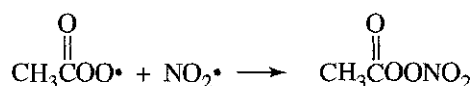
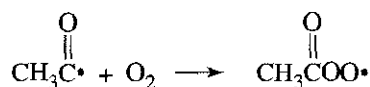
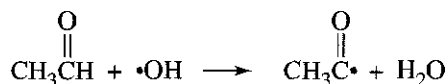


(This requires light with $\lambda < 395 \text{ nm}$, at the ultraviolet edge of the visible region.)

Finally, production of ozone occurs:



Oxygen atoms and ozone react with NO and NO_2 to form NO_2 , NO_3 , and N_2O_5 . These products then react with water to form HNO_2 and HNO_3 . They also react with hydrocarbons to form aldehydes, oxygen-containing free radical species, and finally alkyl nitrites and nitrates, all of which are very reactive and contribute to eye and lung irritation and the damaging effects on vegetation, rubber, and plastics. One of the most reactive is peroxyacetyl nitrate, formed by the reaction of aldehydes with hydroxyl radical and NO_2 :

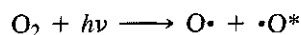


⁶⁹B. J. Finlayson-Pitts and J. N. Pitts, Jr., *Atmospheric Chemistry: Fundamentals and Experimental Techniques*, Wiley, New York, 1986, pp. 29–37.

Photochemical reactions of the aldehydes and alkyl nitrites generate more radicals and continue the chain of reactions.

The ozone layer

Although it is an injurious pollutant in the lower atmosphere, ozone is an essential protective agent in the stratosphere. It is formed by photochemical dissociation of oxygen,



(This requires light of $\lambda < 242$ nm, in the far UV.)

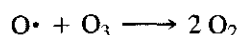
The activated oxygen atoms, O^* , react with molecular oxygen to form ozone:



The ozone formed in this way absorbs ultraviolet radiation with $\lambda < 340$ nm, regenerating molecular oxygen:

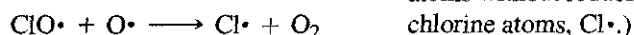
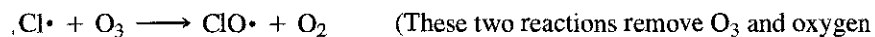
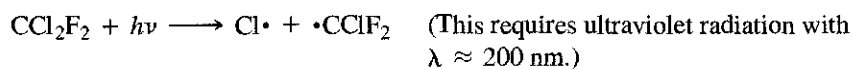


followed by

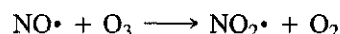
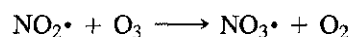


This mechanism filters out much of the sun's ultraviolet radiation, protecting plant and animal life on the surface of the Earth from other damaging photochemical reactions. This natural equilibrium is affected by compounds added to the atmosphere by humans. The most well known of these compounds are the chlorofluorocarbons, especially CF_2Cl_2 and CCl_3F , known as CFC 12 and 11, respectively. The names can be deciphered by adding 90 to the numbers. The resulting sequence of numbers gives the number of carbon, hydrogen, and fluorine atoms; the number of chlorine atoms can be deduced from this information. These compounds were once widely used as refrigerants, blowing agents for the manufacture of plastic foams, and propellants in aerosol cans. Because their damaging effects have been demonstrated conclusively, substitutes for chlorofluorocarbons have been found and nonessential uses are now restricted.

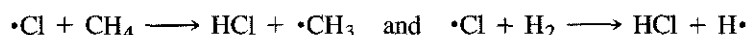
The destruction of ozone by these compounds is caused, paradoxically, by their extreme stability and lack of reaction under ordinary conditions. Because they are so stable, they remain in the atmosphere indefinitely and finally diffuse to the stratosphere. The intense high-energy ultraviolet radiation in the stratosphere causes dissociation and forms chlorine atoms, which then undergo a series of reactions that destroy ozone:⁷⁰



Other compounds, such as NO and NO_2 , also contribute to the chain of events:

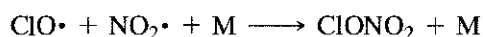


The chains are terminated by reactions such as

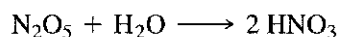


⁷⁰M. J. Molina and F. S. Rowland, *Nature*, 1974, 249, 810; F. S. Rowland, *Am. Sci.*, 1989, 77, 36.

that are followed by combinations of the new radicals to form stable molecules such as CH_4 , H_2 , and C_2H_6 , and by a reaction that ties up the chlorine:



Although Rowland and Molina had predicted depletion of ozone concentrations by these reactions, there were many who doubted their conclusions. The phenomenon that finally brought the problem to the attention of the world was the discovery of the ozone "hole" over the Antarctic in 1985.⁷¹ During the winter, a combination of air flow pattern and low temperature create stratospheric clouds of ice particles. The surface of these particles is an ideal location for reaction of NO_2 , OCl , and O_3 . These clouds contain nitric acid hydrate, formed by



and



These reactions, plus



remove chlorine from the air and generate Cl_2 on the surface of the ice crystals. In the spring, increased sunlight splits these molecules into chlorine atoms and the decomposition of ozone proceeds at a much higher rate. The reaction with NO_2 that would remove ClO from the air is prevented because the NO_2 is mostly tied up as HNO_3 in the ice. The polar vortex prevents mixing with air containing a higher concentration of ozone, and the result is a reduced concentration of ozone over the Antarctic. As the air warms in the summer, the circulation changes, the clouds dissipate, and the level of ozone returns to a more nearly normal level. Within 2 years of the discovery of the ozone hole, the international community had accepted this as evidence of a global problem, and the Montreal Protocol on Substances that Deplete the Ozone Layer was signed. It set a schedule for decreasing use and production of CFCs and eventually for their complete ban. Unfortunately, the limitations put on CFC production and use have led to a black market and illegal international trade.

The size and duration of the Antarctic ozone hole have declined,⁷² but this appears to be the result of unusual stratospheric weather patterns rather than a reduction in CFCs. It is uncertain how long it will take for ozone levels to return to their previous amounts; estimates of 50 years or more are common, depending on assumptions about new sources.

Whether reduction in use of these chlorofluorocarbons will be sufficient to prevent serious worldwide results caused by destruction of the ozone layer remains to be seen. Predictions based on the materials already in the atmosphere indicate that the damage will be significant, even if production could be stopped immediately, but such predictions are based on untested computer models and are subject to considerable error. Production has stopped or declined drastically in most countries, but the compounds proposed as substitutes are primarily those containing C, H, Cl, and F with lower stability. Whether they really reduce the effects is still uncertain, and complete replacement

⁷¹J. C. Farman, B. G. Gardiner, and J. D. Shanklin, *Nature*, **1985**, 315, 207.

⁷²*Chem. Eng. News*, Oct. 7, 2002, p. 26.

will require years.⁷³ Methods for recycling CFCs from air conditioners and refrigeration units have been developed, but there are still large amounts of CFCs in use that will eventually make their way into the atmosphere.

More recent observations have detected a similar ozone hole in the Arctic, but it is smaller and much more variable, largely because the temperatures vary more there.⁷⁴ Volcanic activity that injects sulfur dioxide into the atmosphere also has an effect that depends on temperature and on the height of the SO₂ injection. The SO₂ reacts with air to form SO₃, which then reacts with water to form sulfuric acid aerosols. These volcanic aerosols, particularly at cold polar temperatures, reduce the nitrogen oxide concentration of the air and activate chlorine species that destroy ozone, as do the polar stratospheric clouds described earlier. Because these aerosols are stable at warmer temperatures (~200 K) than the natural stratospheric clouds, and because they can exist at lower altitudes, they can have significant effects. Until the level of chlorine is reduced to preindustrial levels, low temperatures and volcanic activity are likely to create Arctic ozone holes each spring as a result of reactions during the winter.

The greenhouse effect

Another atmospheric problem is the greenhouse effect. The major cause of the problem in this case is carbon dioxide, released by combustion and decomposition of organic matter. Other gases, including methane and CFCs, also contribute to the problem. In this effect, visible and ultraviolet radiation from the sun that is not absorbed in the stratosphere and upper atmosphere reaches the surface of the Earth and is absorbed and converted to heat. This heat, in the form of infrared radiation, is transmitted out from the Earth through the atmosphere. Molecules such as CO₂ and CH₄, which have low-energy vibrational energy levels, absorb this radiation and reradiate the energy, much of it toward the Earth. As a result, the energy cannot escape from the Earth, and its surface and the atmosphere are warmed. A new greenhouse gas, SF₅CF₃, has recently been found.⁷⁵ Its origin is unknown, and its concentration in the atmosphere is only about 0.1 parts per trillion, but it is a very long-lived gas (several hundred years) and has the largest greenhouse effect found. It may come from high-voltage breakdown of SF₆, which is used extensively as an insulator in high-voltage equipment.

Although there are still objections and the details are a source of controversy (largely because of inadequate computer models and lack of sufficient data for good projections), there is general agreement that the greenhouse effect is occurring and that only the timing and amount of warming are uncertain. An international conference in Kyoto, Japan, in 1997, reached preliminary agreement on reduction in greenhouse gases, but implementation of the agreements will be difficult and lengthy, with the United States officially objecting to the reductions in CO₂ production proposed.

The arguments about global warming are complicated by seemingly contradictory evidence. For example, Antarctica has been growing colder overall in recent years,⁷⁶ but the average temperature of the Antarctic Peninsula, which extends northward

⁷³L. E. Manauer, *Science*, **1990**, *249*, 31.

⁷⁴A. Tabazadeh, K. Drdla, M. R. Schoeberl, P. Hajjil, and O. B. Toon, *Proc. Natl. Acad. Sci. U.S.A.*, **2002**, *99*, 2609.

⁷⁵W. T. Sturges, *Science*, **2000**, *289*, 611.

⁷⁶P. T. Doran, J. C. Prisco, W. B. Lyons, J. E. Walsh, A. G. Fountain, D. M. McKnight, D. L. Moorhead, R. A. Virginia, D. H. Wall, G. D. Clow, C. H. Fritson, C. P. McKay, and A. N. Parsons, *Nature*, **2002**, *415*, 517.

toward South America, has been rising. (A giant ice shelf broke off in early 2002.)⁷⁷ Another recent analysis⁷⁸ shows that the fluctuations are the result of cooling in the stratosphere, caused by the loss of ozone that would otherwise absorb solar energy. The result is a stronger flow of air around the South Pole, leading to both effects.

If there is a significant warming, even as much as a 3° to 4°C increase in the average temperature over large portions of the Earth, the consequences are expected to be extreme. Rainfall patterns will change drastically, the oceans will rise with significant melting of the polar ice caps and thermal expansion of the water (two small towns in Alaska suffering from erosion and thawing of the permafrost are already planning to move),⁷⁹ and every part of the Earth will be affected. Efforts are being made to reduce the production of CO₂ and the release of hydrocarbons into the atmosphere, but the sources are so diffuse that it is difficult to have much effect. Major sources of methane in the atmosphere are rice paddies, swamps, and animals. Methane is produced as a result of decay of underwater vegetation in paddies and swamps and as a result of the digestive processes in ruminants. Increasing population, coupled with increasing agriculture and more grazing animals, increases the amount of methane released.

GENERAL REFERENCES

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