9.2: Toxic Effects of Metals

Two Classes of Toxic Metal Compounds

As intimated in the previous section, the presence of excess quantities of an essential metal can be as deleterious as insufficient amounts. This situation can arise from accidental ingestion of the element or from metabolic disorders leading to the incapacitation of normal biochemical mechanisms that control uptake and distribution phenomena. These possibilities constitute one major class of metal toxicity. The other broad class results from entry of nonessential metals into the cell through food, skin absorption, or respiration. The toxicities associated with this latter class have received much recent attention because of the public health risks of chemical and radioisotopic environmental pollutants.

In this section, we survey examples of both categories, and discuss ways in which bioinorganic chemistry can contribute to the removal of toxic metals and restoration of normal function. One way involves chelation therapy, in which metal-specific chelating agents are administered as drugs to complex and facilitate excretion of the unwanted excess element. The use of desferrioxamine to treat iron poisoning is one example of this approach. A second role of bioinorganic chemistry is to identify fundamental biological mechanisms that regulate metal detoxification, and to apply the principles that emerge to help control the toxic effects of metal ions in the environment. Recent studies of mercury resistance and detoxification in bacteria provide an elegant example of the way in which biochemistry and molecular biology can be used to elucidate events at the molecular level. This work, which has uncovered the existence of metalloregulatory proteins, is described in some detail in Section III.F below. It represents a benchmark by which other investigations into the mechanisms of metal-detoxification phenomena may be evaluated.

Copper Overload and Wilson's Disease[8]

Wilson's disease results from a genetically inherited metabolic defect in which copper can no longer be tolerated at normal levels. The clinical manifestations are liver disease, neurological damage, and brown or green (Kayser-Fleischer) rings in the cornea of the eyes. Patients suffering from Wilson's disease have low levels of the copper-storage protein ceruloplasmin; the gene and gene products responsible for the altered metabolism have not yet been identified. Chelation therapy, using $K_2Ca(EDTA)$, the Ca^{2+} ion being added to replenish body calcium stores depleted by EDTA coordination, 2,3-dimercaptopropan-1-ol (BAL, British Anti-Lewisite), or d-penicillamine to remove excess copper, causes the symptoms to disappear. The sulfhydryl groups of the latter two compounds presumably effect removal of copper as Cu(I) thiolate complexes. Wilson's disease offers an excellent opportunity for modem methodologies to isolate and clone the gene responsible for this altered Cu metabolism, ultimately providing a rational basis for treatment.

Iron Toxicity[9]

Chelation therapy is also used to treat iron overload. Acute iron poisoning, such as that resulting from accidental ingestion of $FeSO_4$ tablets, results in corrosion of the gastrointestinal tract. Chronic iron poisoning, or hemochromatosis, arises from digestion of excess iron usually supplied by vessels used for cooking. A classic case of the latter is siderosis induced in members of the Bantu tribe in South Africa, who consume large quantities of beer brewed in iron pots and who suffer from deposits of iron in liver, kidney, and heart, causing failure of these organs. The chelating agent of choice for iron toxicity is the siderophore desferrioxamine, a polypeptide having a very high affinity for Fe(III) but not for other metals. Ferrioxamine chelates occur naturally in bacteria as iron-transport agents. Attempts to mimic and improve upon the natural systems to provide better ligands for chelation therapy constitutes an active area of bioinorganic research (see Chapter 1).

Toxic Effects of Other Essential Metals[10,11]

When present in concentrations above their normal cellular levels, most of the other metals listed in Table 9.1 are toxic. Calcium levels in the body are controlled by vitamin D and parathyroid hormones. Failure to regulate Ca^{2+} leads to calcification of tissue, the formation of stones and cataracts, a complex process about which little is understood (see Chapter 3). Chronic manganese poisoning, which can occur following ingestion of metal-oxide dust, e.g., among miners in Chile, produces neurological symptoms similar to Parkinson's disease. Neuron damage has been demonstrated. Although Zn toxicity is rare, it can lead to deficiencies in other essential metals, notably calcium, iron, and copper. Cobalt poisoning leads to gastrointestinal distress and heart failure. Metal poisoning by those elements has been treated by chelating agents, most frequently CaNaiEDTA), but the selectivity offered by the ferrioxamine class of ligands available for iron has not even been approached. Fortunately, there are few cases involving these metals.

Plutonium: A Consequence of the Nuclear Age[12]

Some of the chelating agents developed to treat iron toxicity have found application as therapeutics for plutonium poisoning. Diethylenetriaminepentaacetic acid (DTPA) salts and siderophores are especially effective. Some improvement over the naturally occurring chelates has been made by tailoring the ligand to encapsulate completely the eight-coordinate Pu(IV) center. Although few individuals have been affected, ingestion of ^{239}Pu, for example, as small particles of PuO_2, at nuclear-reactor sites can have dire consequences. ^{239}Pu emits high energy ex particles, leading to malignancies of bone, liver, lung and lymph nodes, to which tissues it is transported by transferrin. With a maximum tolerated dose of only 1.5 μg, plutonium is among the most toxic metals known. We turn now to other, more classic examples of such industrial pollutants.

Mercuy Toxicity[13] and Bacterial Resistance[14-17]

Mercury is released into the environment as Hg(II) ions through weathering of its most common ore, HgS, red cinnabar. Organomercurials of general formula RHgX used in agriculture have also entered the environment as toxic waste. Both RHgX and HgX_2 compounds bind avidly to sulfhydryl groups in proteins, which can lead to neurological disease and kidney failure. Metallothionein is a favored protein target, which may help to limit mercury toxicity. A highly publicized case occurred in 1953 at Minimata, Japan, where 52 people died after eating mercury-contaminated fish and crustaceans near a factory waste outlet. The volatile, elemental form of mercury, Hg(0), is reportedly nontoxic, but its conversion to alkylmercury compounds by anaerobic microorganisms utilizing a vitamin B-12 biosynthetic pathway constitutes a serious health hazard.

Because of the high affinity of mercury for sulfur-donor ligands, mercury poisoning is treated by BAL; N-acetylpenicillamine has also been proposed. Recently, a very interesting natural detoxification system has been discovered in bacteria resistant to mercury; this system, when fully elucidated, might provide important strategies for treating heavy-metal poisoning in humans.

Presumably under environmental pressure, bacteria have developed mechanisms of resistance to HgX_2 and RHgX compounds in which mercury is recycled back to Hg(0). At least five gene products are involved in the bacterial mercury-resistance mechanism. MerT and MerP mediate the specific uptake of mercury compounds. MerB, organomercury lyase, and MerA, mercuric reductase, catalyze two of the reactions, given in Equations (9.1) and (9.2). Plasmids encoding the genes for these two proteins have been isolated. A typical arrangement of genes in the *mer* operon

$$RHgX + H^+ + X^- \xrightarrow[lyase]{organomercury} HgX_2 + RH \tag{9.1}$$

$$Hg(SR)_2 + NADPH + H^+ \xrightarrow[reductase]{mercuric} Hg(0) + NADP^+ + 2RSH \tag{9.2}$$

region of these plasmids is shown in Figure 9.1. The most thoroughly studied gene product is MerR, a metalloregulatory protein that controls transcription of the *mer* genes. In the absence of Hg(II) the MerR protein binds to DNA as a repressor, preventing transcription of the *merT, P, A,* and *B* genes (Figure 9.1) and negatively autoregulating its own synthesis. When Hg(II) is present, transcription of these genes is turned on. Interestingly, the MerR protein remains bound to the same site on DNA whether acting as an activator in the presence of Hg(II) or as a repressor in its absence. Random and site-specific mutagenesis studies implicate several cysteine residues in the carboxyl terminal region of the protein as candidates for the mercury-binding site.

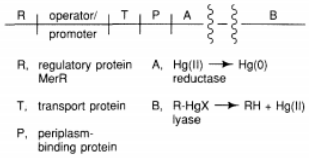
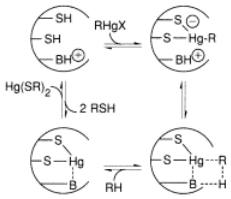


Figure 1, Reference 14).

Organomercury lyase, encoded by the *merB* gene, achieves the remarkable enzymatic step of breaking Hg-C bonds (Equation 9.1). It is a 22-kDa protein with no metals or cofactors. Two cysteine-sulfhydryl groups on the protein have been postulated to effect this chemistry, as depicted in Equation (9.3). Stereochemical studies of the Hg-C bond cleavage revealed retention of configuration, indicating that cleavage of the Hg-C bond probably does not proceed by a radical pathway. A novel concerted S_E2 mechanism has been suggested. The enzyme turnover numbers, ranging from 1 min^{-1} for CH_3HgCl to 240 min^{-1} for butenylmercuric chloride, although slow, are ~10^5-10^8-fold faster than the nonenzymatic rate.



(9.3)

Mercuric ion reductase, the FAD-containing *merA* gene product, has several pairs of conserved cysteines. From site-specific mutagenesis studies, cysteine residues in the sequence 134-Thr-Cys-Val-Asn-Val-Gly-Cys-140 are known to comprise a redox-active disulfide group; in addition, a redox-inactive pair of cysteines near the carboxyl terminus is also required for the selective reduction of Hg(II). Exactly how the enzyme achieves the chemistry shown in Equation (9.2) is currently uncertain, but the redox activities of the flavin and disulfide/ thiol centers are undoubtedly involved. This enzyme serves both to detoxify mercury supplied directly from the environment as Hg(II) salts and to complete clearance of Hg^{2+} generated by the MerB protein from RHgX compounds. Clearly, Nature has invented a remarkable system to detoxify mercury in this fascinating class of Hg-resistant bacteria.

Cadmium and Lead Toxicity[18]

Gastrointestinal, neurological, and kidney toxicity are among the symptoms experienced by acute or chronic exposure to these heavy metals. The use of unleaded gasoline and the removal of lead-containing pigments from paint have substantially diminished the quantity of this element released to the environment each year. Cadmium sources include alkaline batteries, pigments, and plating. Lead poisoning can be treated by chelation therapy using $CaNa_2$(EDTA) (acute) or penicillamine (chronic). Although both Cd(II) and Pb(II) bind to sulfhydryl groups in thionein, we have little information at the molecular level on the mechanisms by which these elements induce toxicity.

Metals as Carcinogens[19,20]

Although most metal ions have been reported to be carcinogenic, the three most effective cancer-causing metals are Ni, Cr, and, to a lesser extent, Cd. Nickel subsulfide, Ni_2S_3, found in many nickel-containing ores, has been extensively studied and shown to be carcinogenic in humans and other animals. In short-term bioassays including mutagenesis, enhanced infidelity of gene replication *in vitro* and altered bacterial DNA repair were observed. Chromium is most carcinogenic as chromate ion (CrO_4^{2-}), which enters cells by the sulfate uptake pathway and is ultimately reduced to Cr(III) via a Cr(V)-glutathione intermediate species. The latter complex binds to DNA to produce a kinetically inert and potentially damaging lesion. Despite the fact that much information is available about metal-DNA interactions, molecular mechanisms of metal-induced carcinogenesis have not been elucidated. Two aspects of the problem are tumor initiation and tumor development, which are likely to involve different pathways. As new methods become available for studying the molecular events responsible for cancer (oncogenesis), it should be possible for bioinorganic chemists to unravel details of how metals act as carcinogens and as mutagens. Since cancer has genetic origins, metal/nucleic-acid chemistry is likely to be prominent in such mechanisms. As discussed later, metal-DNA interactions are an important aspect of the antitumor drug mechanism of *cis*-$[Pt(NH_3)_2Cl_2]$.

Summary

Toxicity can arise from excessive quantities of either an essential metal, possibly the result of a metabolic deficiency, or a nonessential metal. Both acute and chronic exposure can be treated by chelation therapy, in which hard-soft acid-base relationships are useful in the choice of chelating agent. Since chelates can also remove essential metals not present in toxic amounts, ligands with high specificity are greatly desired. The design and synthesis of such ligands for chelation therapy remains an important objective for the medicinal bioinorganic chemist. Until recently, studies of the toxic effects of metals and their removal, sometimes categorized under "environmental chemistry," have been empirical, with little insight at the molecular level. Application of the new tools of molecular biology to these problems has the potential to change this situation, as illustrated by rapid progress made in cloning the genes and studying the gene products of the mercury-resistance phenotype in bacteria. The discovery of such resistance phenomena in mammalian cells, and even the remote prospect of transferring Hg-resistant genes from bacteria to humans, are exciting possibilities for the future.