Bioinorganic Chemistry: A Maturing Frontier

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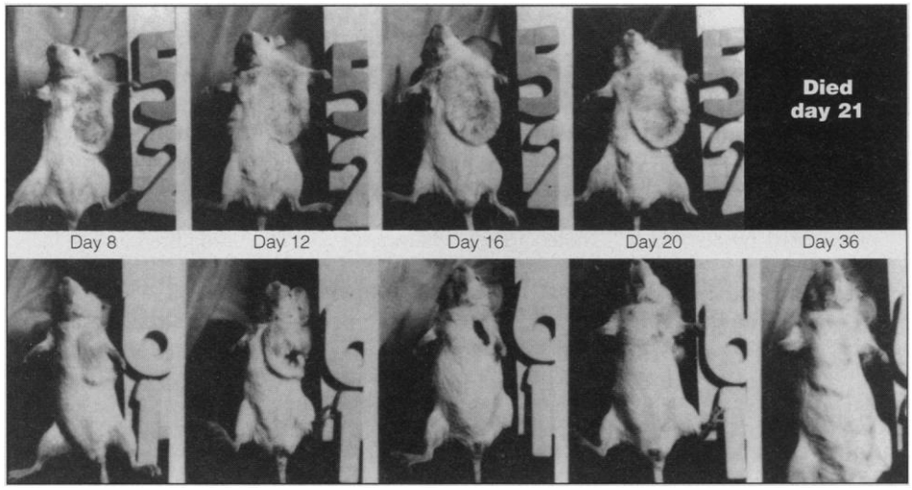
The field of bioinorganic chemistry is burgeoning. Attracting scientists from a variety of disciplines, the problems of this rapidly expanding field are some of the most fascinating ones at the interface of chemistry, biology, agriculture, and medicine. Included are how nature uses metals to convert dinitrogen into ammonia, to turn RNA molecules into enzymes, to regulate which proteins a cell will make, or to block the onset of genetically inherited disorders such as Lou Gehrig's disease. At the same time, bioinorganic chemistry is beginning to show signs of maturity, as can be judged by several recent or soon to be published textbooks (*1*–*4*). Principles are emerging that help guide the thinking of workers in the area and enable connections to be made between seemingly unrelated observations.

Four articles appear in this special issue of *Science* that relate advances at several frontiers of bioinorganic chemistry. On page 701, Karlin surveys metalloenzymes that perform many key chemical transformations that occur in living organisms (*5*), including nitrogen fixation, the conversion of water into dioxygen through photosynthesis, and the utilization of O_2, H_2, CH_4, and NO in metabolism (*6*). But, as Pyle details in her article on page 709 (*7*), RNA molecules can also function as enzymes, and these so-called "ribozymes are always metalloenzymes." Regulating the synthesis of RNA (replication) and protein (translation) molecules is also a function that falls within in the domain of bioinorganic chemistry, as O'Halloran describes on page 715 (*8*). Gene regulation by the so-called zinc finger proteins is so pervasive that this branch of bioinorganic chemistry is now quite familiar to most molecular biologists. And encompassing all of these areas is medicinal bioinorganic chemistry, a topic which Abrams and Murrer cover in their article on page 725 (*9*). Amazingly, heavy metal ions, often thought only to be toxic to life, are being introduced as drugs to treat diseases such as cancer and arthritis and are assuming a role in diagnostic medicine.

Not all metal ions are found in biology. Because biochemical transformations occur roughly on a millisecond time scale, labile metal ions in which the ligands exchange rapidly in and out of the coordination sphere are preferred. Included are Na^+, K^+, Mg^{2+}, Ca^{2+}, most first-row transition metal ions—especially those of V, Mn, Fe, Co, Ni, Cu, and Zn—and Mo ions. Despite their lability, these metal ions usually occupy binding sites in biomolecules that are stabilized by folding of the biopolymer chain. Stability can also be conferred through specialized biological ligands, such as the porphyrin or corrin ring, and by the metal ions to facilitate their removal through chelation therapy. Abrams and Murrer relate how toxic metal ions such as platinum and gold can, under appropriate circumstances, be used as chemotherapeutic agents (*9*). Cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, is among the most successful anticancer drugs (see figure), and auranofin, $[Au(PEt_3)(ttag)]$, where ttag is tetra-*O*-acetylthioglucose, is an oral agent used to treat rheumatoid arthritis. The medicinal applications of such compounds are usually discovered serendipitously, but as bioinorganic chemists unravel the molecular mechanisms, we may soon see the day when rational drug design becomes a reality. Other metal ions not found naturally in biological systems—for example, technetium and gadolinium—have made a major impact in diagnostic medicine where they are used as radiopharmaceuticals or for magnetic resonance imaging (MRI).



Cisplatin cures cancer in mice. Early experiments with mice revealed the remarkable anticancer properties of cisplatin. Two mice with solid sarcoma 180 tumors were observed over a period of time. The top panels show the progression of the tumor in an untreated, negative-control mouse. She died on day 21. The mouse shown in the bottom panels received a single intraperitoneal injection of cisplatin on day 8. Her tumor completely regressed 6 days after treatment, and she lived a normal life-span of 3 years. [Reprinted from (*11*) with permission © Wiley]

assembly of polymetallic units such as iron-sulfur clusters. As discussed by O'Halloran (*8*), assembly and disassembly of one such [4Fe4S] cluster in a protein that binds to "iron-responsive elements" in messenger RNA molecules regulate the translation of several proteins. Such "metalloregulation" may also occur when Zn^{2+} binds to zinc finger proteins that similarly regulate the transcription of genes. The ability of metal ions to bend or fold proteins and nucleic acids thus not only affords structural integrity to these biopolymers but also contributes a key component to the intracellular communications and control network.

Among the processes under tight regulation in cells is the concentration of metal ions. Too little iron, for example, leads to anemia and too much produces toxicity. Medicinal bioinorganic chemists synthesize compounds that bind specifically to toxic

Thus far, I have touched upon factors controlling the choice, uptake, and assembly of metal-containing units, their control and utilization, and their ability to fold biomolecules. Once inside the cell, however, most metal ions bind to specific sites in biopolymers where they perform important functions. What factors control the binding of metal ions to their natural sites in these molecules? Here the thermodynamic and kinetic principles of inorganic chemistry prevail, with selectivity controlled by the charge, size, and hard or soft ligand preferences (*10*) of the metal ion. For example, as indicated by Pyle (*7*), many ribozymes require Mg^{2+} or Mn^{2+} ions for their catalytic activity, and these metals exhibit the same coordination preferences for oxygen over nitrogen or sulfur donors in

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RNA enzymes as they have in metalloproteins. Will the principles governing catalysis in hydrolytic protein enzymes such as carboxypeptidase, a zinc metalloprotein that catalyzes the cleavage of peptide and ester bonds, also apply to ribozymes? In metalloproteins, the positive charge on the metal ion makes it easier to deprotonate coordinated hydroxyl groups supplied by water or alcohol moieties. Moreover, the metal ion facilitates nucleophilic attack on coordinated phosphate or carboxylate esters and can assist in the removal of a leaving group. Rate acceleration also occurs by internal attack within the coordination sphere and by assistance of critically positioned groups near the active site. As research in the young but fascinating field of ribozymes progresses, it will be interesting to see whether these features are mimicked by metal ions involved in RNA catalysis.

In addition to facilitating the binding and activation of substrates by non-redox mechanisms, metal ions perform critical roles in biology as catalysts of electron transfer and atom and group transfer. Although ribozymes are not yet known to function in this manner, metalloproteins do. Karlin describes the unique ability of metal centers in proteins to process the O_2 molecule, a function that is crucial for organisms that live in air (*5*). The transport of dioxygen is performed by metalloproteins containing iron or copper centers, which formally reduce the O_2 molecule upon binding to the deoxy form and then reoxidize it upon its release. This process illustrates the more general bioinorganic principle that substrate binding and oxidation state changes can occur simultaneously in group transfer chemistry. Bioinorganic chemists have successfully modeled the reactions involved in dioxygen transport outside the protein cores. Hemocyanin (Hc), the O_2 transport protein of arthropods and mollusks, contains a pair of copper ions in close proximity. The dicopper(I) center in deoxy Hc reacts with dioxygen to form a peroxo-bridged dicopper(II) adduct in oxy Hc. The structure of the oxy Hc core was revealed through model studies prior to its characterization by protein x-ray crystallography.

One remarkable feature of metal ions in proteins is that the same unit can fulfill a variety of functions. The redox potential, substrate specificity, and reaction rate are all properties that can be tuned by the protein environment. This tuning is achieved either directly, by coordination of different amino acid side chains to the metal, or indirectly, by modulation of hydrophobicity, hydrogen bonding, or the local dielectric constant in the vicinity of the active site. In this sense, some metal cores in proteins may be thought of as bioinorganic chips, recurring modules whose functions in the printed circuit board of the surrounding protein milieu can be modulated according to the requirements of the system. The defining example of such a chip is the metalloporphyrin. In hemoglobin, the iron porphyrin core is bonded to histidine on one side and uses the other site to bind dioxygen for transport. In cytochrome c, the same unit has two axial ligands, a histidine and a methionine, and functions as an efficient electron carrier. And in cytochrome P-450, one axial position is occupied by cysteine and the other by a water molecule, which gets displaced during activation of dioxygen during the catalytic hydroxylation of hydrocarbon substrates.

The principles that govern this tuning of active site reactivity seem also to apply to proteins with related functions but quite different surroundings. For example, metalloproteins involved solely in electron transfer often have core geometries intermediate between those of the oxidized and reduced states of the metal ion in order to minimize structural rearrangements that would occur during the redox reaction. The protein can effect this property by forming a rigid metal-binding cavity.

Despite much recent progress, the frontiers of bioinorganic chemistry are replete with unsolved problems. We know some details about the uptake and storage pathways of iron but very little about the other metal ions. Are there comparable mechanisms, and will general principles emerge? Metal ions flowing through channels in the cell membrane are responsible for neurotransmission and other signaling events in cells. The lithium ion is used to treat manic depressives. Magnetic particles of magnetite have been reported to occur in the human brain. What are the molecular details of these processes and how best can bioinorganic chemists contribute to neurobiology? Recently, a structural role for the potassium ion was suggested to be stabilization of the DNA telomere, a structure at the ends of chromosomes, and a similar unit has been implicated for human immunodeficiency virus RNA. Understanding the structural roles of the alkali and alkaline earth metal ions in biology and the chemically related goal of synthesizing new rare earth ion complexes and improving their application in diagnostic medicine are important challenges for the field. Carbohydrates, the third major biopolymer after proteins and nucleic acids, have been largely neglected by bioinorganic chemists. How, for example, do metal ions catalyze the isomerization of sugars, link proteins to carbohydrates in lectins, or interact with the matrix that comprises the cell wall?

Metalloprotein chemistry, despite the triumphs, still offers numerous challenges. How does the cluster of manganese ions in photosystem II oxidize water to dioxygen; how does methane bind and get selectively oxidized to methanol in methane monooxygenase; are there discrete through-bond pathways for long-range electron transfer to metal centers in proteins; what are the roles of the less well studied metal ions such as vanadium? Finally, I note that the applications of metals in medicine are far fewer than warranted by their potential, perhaps because of the limited numbers of trained inorganic chemists in the pharmaceutical industry. Compositions containing metal ions have long been known to have excellent antiviral activity, heal burns and wounds, stop inflammation, and display analgesic properties. Perhaps the clinical and commercial success of cisplatin will encourage both government funding agencies and private industry to increase support for fundamental, long-range research in medicinal bioinorganic chemistry.

References and Notes

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