Molding metallocenters for biology

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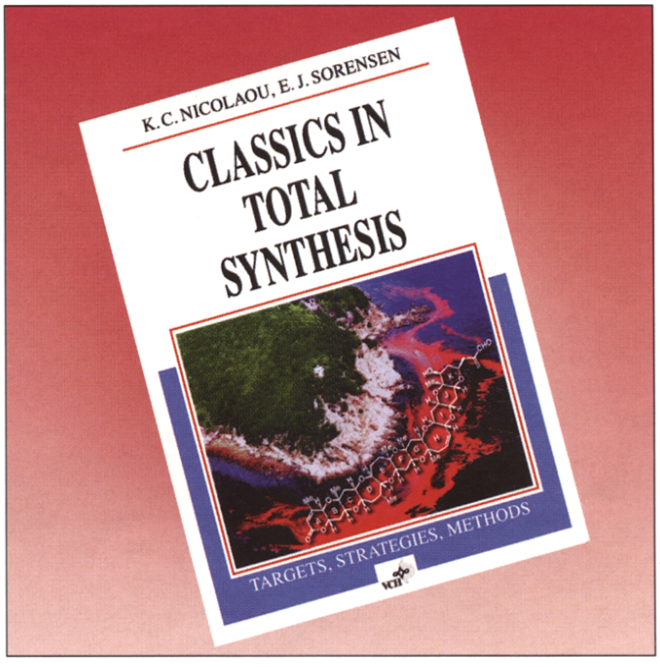
Mechanisms of Metallocenter Assembly edited by RP Hausinger, GL Eichhorn and LG Marzilli, VCH Publishers, Inc., 1996, 268 pp. $115.00 hardcover (ISBN 1-56081-920-0).

The field of bioinorganic chemistry and the large subfield of metallobiochemistry are burgeoning. It is only 25 years since the first volume dedicated to this field was published (*Bioinorganic Chemistry*, R. Dessy, J. Dillard, L. Taylor, editors, Advances in Chemistry Series #100, American Chemical Society, 1971) and this conference proceedings was a broad survey of the relatively few metalloproteins then known, coupled with a few attempts to make analogies between these and chemical systems. The latter were in the realm of inorganic chemistry, while the biochemical systems were traditionally in the province of biochemistry, biophysics, microbiology, and physiology. This border has all but been erased. Now we have journals and several new textbooks devoted to the interdisciplinary area of bioinorganic chemistry, and the great breadth of this field is becoming apparent, at least in outline form.

In earlier days, the focus was almost exclusively on proteins and their metallocenters. Inorganic analogs were pursued to approach the structural and functional properties of the biological site. While this endeavor is still a central thrust of research in bioinorganic chemistry, it has been joined by other crucial components, many of which involve the interaction of metals with nucleic acids (either directly, or indirectly through proteins acting as molecular switches) and/or the manner in which metals are secured from the environment and (eventually) incorporated into the active form of the metallosite. This biological metal-ion processing is a multifaceted activity that involves: modification of the environment by the organism to mobilize the metal (e.g., by changing the pH or effective redox potential of the surroundings); excretion of low molecular weight chelators to bind and solubilize metal ions (e.g., the myriad siderophores used to chelate iron); recognition of the metal or its complex at the cell surface; movement of the metal (or complex) into the periplasm or into the cell (involving specialized active transport systems); storage of the metal inside the cell (in small or large aggregates); and finally, assembly of the active site and its proper placement in the protein host. It is this latter area,



the assembly of the active metallocenter, that is the subject of this excellent volume edited by Hausinger, Eichhorn and Marzilli. This collection of reviews appears to be Volume 11 in the series 'Advances in Inorganic Biochemistry,' with a new publisher, VCH. If the quality of this volume is an indication of the general standard of the volumes in this series, the publisher and editors are strongly encouraged to continue their publication.

What helps make this book both accessible and useful is the first chapter, which is a clear overview of the field, by Hausinger, the senior editor of the volume. Here the various ways in which metalloprotein sites are assembled are systematically analyzed. The analysis leads naturally to classification of possible assembly modes in general terms. Most importantly, the classification is buttressed by specific reference to chapters in the volume and to additional material in the literature that illustrate particular assembly strategies.

The degree of complexity in metal processing varies remarkably. In some cases, the metal center is formed within the protein without any (other) chemical modification of the protein and without any special (nonprotein) ligand for the metal. An example of such assembly is found in the dinuclear iron clusters of hemerythrin and rubrerythrin described in Chapter 2 by Kurtz. In other proteins, post-translational modification of specific residues is critical. Chapter 3 by Drakenberg, Sunnerhagen and Stenflo details the γ-carboxylation of glutamic

acid, used to equip calcium-binding proteins with closely lying carboxylate groups to accommodate the Ca^{2+} ion and its high coordination number.

In some cases, an incredibly complex ligand is provided for the metal. For heme iron (porphyrin) and for B12 (corrin), the ligand has an elaborate organic biosynthetic pathway, which has been established in great detail. These pathways, however, only give a tetrapyrrole with a hole in the middle, and it is the respective job of ferrochelatase, described in Chapter 4 by Dailey, and the cobalt insertion system described in Chapter 5 by Blanche *et al.*, to insert the divalent iron or cobalt ion into the respective ligand to give the metal tetrapyrrole. This complex (cofactor) then binds as a unit to numerous proteins that have evolved to exploit the additional chemical reactivity imparted to the resultant enzyme by this special site. The protein component plays the complementary role of providing specificity toward particular substrates and reactions.

In many electron-transfer proteins and redox enzymes, the assembly of a complex sulfur-bridged metal cluster is required. Our knowledge of these largely inorganic syntheses, performed in a controlled fashion within the cell, is primitive in comparison to the exquisite detail now established for the synthetic organic sequences in porphyrin or corrin biosynthesis. Nevertheless, powerful combinations of molecular genetics, biochemistry, and biophysics are now being marshalled to untangle these complex cluster biosynthetic systems. While key components of these systems have generally been recognized, the full structural and temporal elucidation of their activity has not yet been achieved. Chapter 6 by Muchmore, Jack and Dean on the biosynthesis of the iron-molybdenum cofactor of nitrogenase (FeMoco) and Chapter 9 by Maier and Bock on nickel incorporation into hydrogenases aptly reveal the state of our knowledge (and ignorance) of metallocluster assembly. Chapter 7 by Volini offers a hypothesis concerning the sulfurization or desulfurization of iron-sulfide clusters as a respiratory control mechanism. These chapters highlight the importance of sulfur processing with organic (e.g., cysteine, cystethionine, protein persulfide) or inorganic (e.g., thiosulfate) sulfur serving as sources or intermediates in the sulfur donation process.

Chapter 8 by Moncrief and Hausinger on 'urease metallocenter assembly' describes what is known about assembly of the dinuclear carbamate-bridged nickel active site of this historically and metabolically interesting enzyme. The lack of overlap with the hydrogenase nickel assembly system is notable and indicates that a given metal may be processed in quite distinct ways for the different proteins in which it is found. The two copper proteins discussed in this volume amplify this point. The biosynthesis of the dicopper site in N_2O reductase discussed in Chapter 10 by Zumft and Kroneck and the tyrosinase Cu center described in Chapter 11 by Lee illustrate the genetic and biochemical uniqueness of the pathways for these two Cu proteins. Interestingly, in the synthesis of the Cu center in tyrosinase, a chaperone function is proposed for one of the gene products.

The final chapter, by Brouwer, on metallocluster assembly in zinc and copper metallothioneins reveals the structural definition and surprising lability of these proteins. Metallothioneins appear to be involved both in the delivery of essential metals and in the detoxification of unwanted metals (such as cadmium).

The twelve chapters in this book, admirably complete though most of them are, do not comprehensively cover the field of metallosite assembly. There are obvious gaps. For example, there is no chapter on magnesium insertion into chlorophyll or on molybdopterin biosynthesis (for the molybdenum or tungsten cofactors.) Clearly, complete coverage would be impossible, and the necessary focusing is compensated for by the introductory chapter, which gives leading references to the most obvious omissions.

This collection amply illustrates the tremendous progress in metallocenter assembly that has been made in the last 10 years (with a very high percentage of references from 1985 to 1994). Of equal importance for a review volume, the collection also plumbs the depths of our ignorance. The gaps in our knowledge are enormous. But, as this volume shows, at least we are becoming aware of the existence of the gaps. For example, in the assembly of FeMoco we know of four definite, five probable, and three possible gene products that are directly involved in the biosynthesis. The functions for many of these proteins have been proposed and are being tested using elegant combinations of genetics, biophysics, and biochemistry. Hypotheses about scaffold proteins, molecular props, transferases, and chaperones pervade the volume and promise further experimental progress and perhaps some additional generalizations on the processes and mechanisms of metallocenter assembly.

In summary, this excellent book should help stimulate the field. The collection provides a useful reference to the state of the art in this highly active and important field of study. The authors and editors are to be congratulated for their efforts.