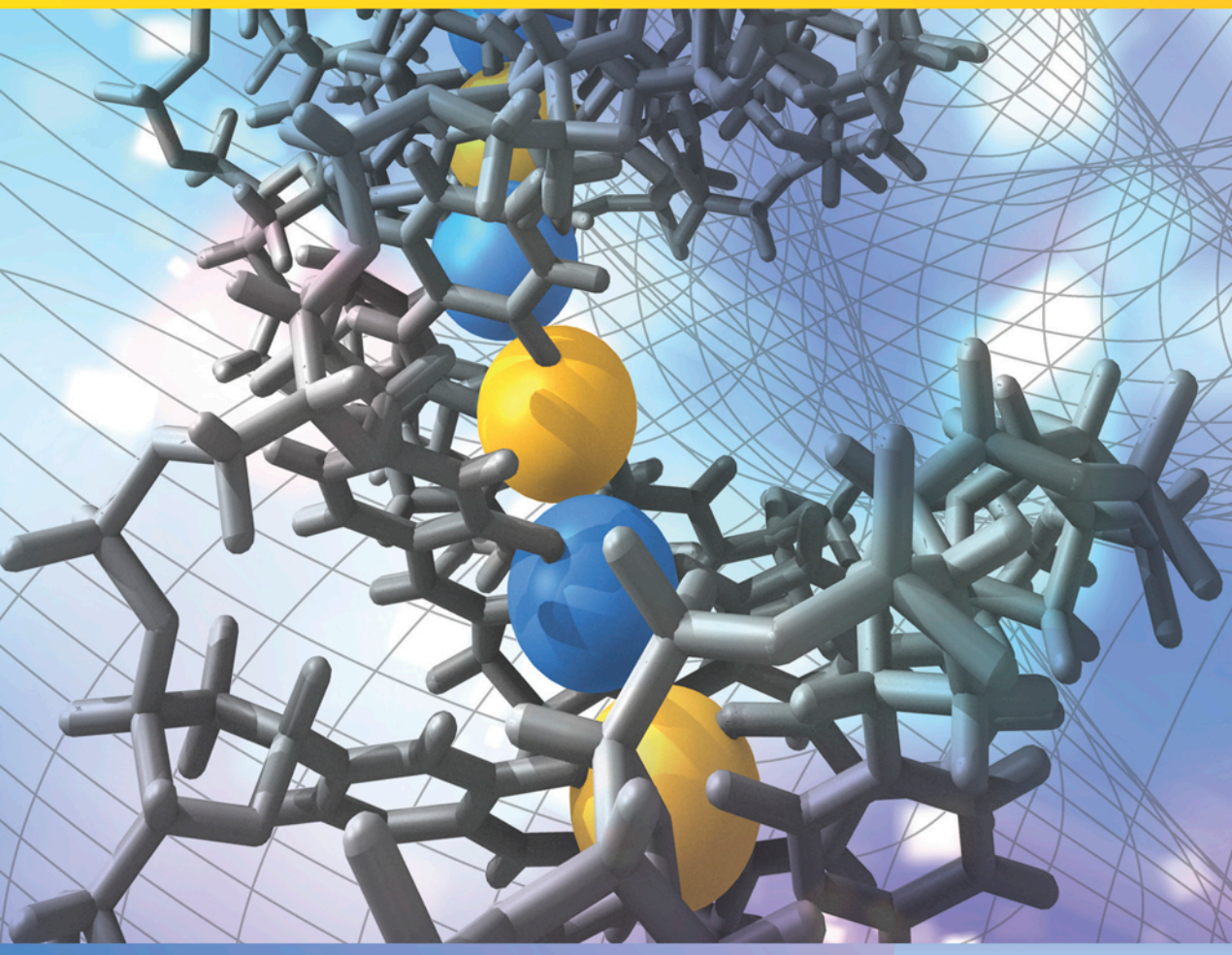
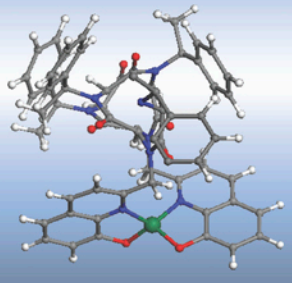
EDITORS | GALIA MAAYAN | MARKUS ALBRECHT

Metallofoldamers

Supramolecular Architectures from Helicates to Biomimetics



WILEY



Metallofoldamers

Metallofoldamers

Supramolecular Architectures from Helicates to Biomimetics

Edited By

GALIA MAAYAN

Schulich Faculty of Chemistry, Technion - Israel Institute of Technology, Israel

and

MARKUS ALBRECHT

Institut für Organische Chemie, RWTH Aachen, Germany

WILEY

A John Wiley & Sons, Ltd., Publication

This edition first published 2013

Registered office
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

Library of Congress Cataloging-in-Publication Data applied for.

A catalogue record for this book is available from the British Library.

ISBN: 9780470973233

Set in 10/12pt, Times by Thomson Digital, Noida, India

Contents

List of Contributors

Catalina Achim, Department of Chemistry, Carnegie Mellon University, USA

Markus Albrecht, Institut für Organische Chemie, RWTH Aachen University, Germany

Martin Berg, Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Germany

Jean-Claude G. Bünzli, Institute of Chemical Sciences and Engineering, École Polytechnique Fédérale de Lausanne, Switzerland

Photovoltaic Materials, Department of Materials Chemistry, Korea University, Sejong Campus

Nicola Castellucci, Dipartimento di Chimica "G. Ciamician", Università di Bologna, Italy

Guido H. Clever, Institute for Inorganic Chemistry, Georg-August University Göttingen, Germany

Arnie De Leon, Department of Chemistry, Carnegie Mellon University, USA

F. Ekkehardt Hahn, Institut für Anorganische und Analytische Chemie, Westfälische Wilhelms-Universität Münster, Germany

Josef Hamacek, Department of Inorganic and Analytical Chemistry, University of Geneva, Switzerland

Jing Kong, Department of Chemistry, Carnegie Mellon University, USA

Dennis Lewing, Institut für Anorganische und Analytische Chemie, Westfälische Wilhelms-Universität Münster, Germany

Arne Lützen, Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Germany

Vasiliki Lykourinou, Department of Chemistry, University of South Florida, USA

Galia Maayan, Schulich Faculty of Chemistry, Technion – Israel Institute of Technology, Israel

Li-June Ming, Department of Chemistry, University of South Florida, USA

Mitsuhiko Shionoya, Department of Chemistry, University of Tokyo, Japan

Claudia Tomasini, Dipartimento di Chimica "G. Ciamician", Università di Bologna, Italy

Yan Zhao, Department of Chemistry, Iowa State University, USA

Raymond Ziessel, Laboratoire de Chimie Organique et Spectroscopie Avancées (LCOSA)

Ecole de Chimie, Polymères, Matériaux Université de Strasbourg France

Foreword

Since their initial presentation 25 years ago, helicates, helical metallosupramolecular architectures, have received wide attention and been subject to numerous investigations. Much of the fascination they exerted and continue to exert on chemists results from their helical structure, and in particular the relationship of double helicates with the emblematic entity, double-stranded DNA. Although this feature was part of our initial motivation, the goal and the impact were much wider. Indeed, as pointed out earlier, the generation of double helicates from designed ligands and suitable metal ions had for us a much broader significance as it marked the start of the implementation of self-organization processes in supramolecular chemistry via metalloarchitectures. It represented an important first step in the formulation of supramolecular self-organization as the outcome of programmed chemical systems, whereby information stored in the structural features of the ligand molecules is processed by the metal cations via their coordination algorithm. In a further extension, the self-sorting processes occurring in mixtures of equilibrating helicates and the generation of anion-dependent circular helicates became the starting points of our studies that were to evolve into constitutional dynamic chemistry.

From double-stranded to multiple stranded, from two centers to multiple centers, from identical metal ions to different ones, the field of helicates developed extensively, exploring structural features, physical properties and coding schemes, and reaching beyond towards a great variety of other metallosupramolecular architectures. Its expansion demonstrated once again that, without claiming to imitate what biology has so powerfully achieved in the DNA molecule, chemistry is exploring a much wider scene, generating entities entirely imagined and fabricated by chemists, exerting the power of chemistry over the expressions of matter.

The present volume displays a selection of the various aspects of the chemistry of helicates and related entities. It takes stock of achievements, but also suggests numerous exciting developments to the imagination of chemists, at the triple interface of organic, inorganic and biological chemistry, as well as, from a more general perspective, towards the precise control of the self-organization of chemical entities through appropriate programming.

The editors have to be congratulated for assembling a remarkable roster of active players in the field, who deserve our warmest thanks for their expert contributions.

Jean-Marie Lehn

Preface

Nature's examples of functional molecular entities are highly versatile. The living world as we know it only exists due to the specific properties and selective interactions of molecules. Hereby, the observed broad functionality of biological systems is mainly based on the structure of biomacromolecules (proteins or DNA), which strongly relies on an intra- or intermolecular connection between polymer strands. This occurs either in a covalent (e.g., –S-S– bridges) or in a noncovalent fashion. In the latter case H-bonding is probably the most prominent bonding motif. In many cases, however, metal ions have a significant role in the structure and function of biopolymers, being able to bind them and consequently to control their overall structural and thus functional features. It is now well established that the identity of the metal-binding ligands and their coordination mode, as well as side chain interactions, have a crucial role in governing metal binding and selectivity in proteins. The coordination of metal ions to proteins and peptides results in conformational changes, which lead to important functions such as catalysis, interactions with specific receptors or target proteins and the inhibition of biochemical or biophysical processes.

The effect of metal ions on conformational changes in proteins and peptides and thus on their function, which is widely discussed in Chapter 1, has inspired the design and synthesis of "metallofoldamers" – synthetic oligomers that fold upon interactions with metal ions to give various stable architectures in solution. Metallofoldamers are one class of "foldamers", which are unnatural oligomers that fold into well defined three-dimensional structures in solution via noncovalent interactions. The design, synthesis, structures and applications of foldamers, as thoroughly described in several reviews and a book, are summarized in Chapter 2. We distinguish between three types of metallofoldamers: helicates (Chapters 3–8), metallo-nucleic acids (Chapters 9–10) and metallo-peptidomimetics (Chapter 11).

The term "helicate" was introduced 25 years ago by J.-M. Lehn and describes a rigid oligomer that spontaneously folds into helical structure upon binding of metal ions to its backbone. The self-assembly principles of helicates are presented in Chapter 3. Due to their simplicity, helicates are the ideal "supramolecular *Drosophila*" to be investigated. Indeed, in the years following their introduction, the chemistry of helicates was intensely studied. This included looking into their structural aspects (Chapter 4) and exploring new ways to construct helicates with various structures and features using unique oligomer backbone chelators (Chapter 5) or nontransition metal ions (Chapter 6). Although the majority of studies were based on the helicate model character, some functional helicates were developed. Thus, liquid-crystalline helicates were designed as supramolecular materials (Chapter 7). In the quest for the creation of more biomimetic helicates, a new generation of helicates is being developed, which includes the use of biopolymers such as peptides and DNA as backbones (Chapters 8–10). This new direction in the field of helicates leads the way to new trends in the field of foldamers – the generation of synthetic

oligomers which are peptide and nucleic acid mimics and can fold upon metal coordination to produce various three-dimensional structures stable in solution (Chapters 9–11). It is important to note that, while in helicates the metal ions template a helical structure via self-assembly, in biomimetic oligomers the metal ions nucleate the formation of a three-dimensional structure in a controlled manner. These biomimetic metallofoldamers are therefore macromolecular coordination compounds in which the overall structure of the macromolecule is influenced by its coordination to metal ions (Chapter 11). This copies nature's example of metallaproteins and opens the door to developing novel functional materials (Chapter 12).

The present book covers the whole field of structure control in oligomeric, polymeric, biomimetic and biological systems spanning the bridge from the simple helicates to polymers and natural or artificial peptides or DNA. This directly leads to prospective applications. We believe that the presented aspects are of interest and we hope that many scientists will be inspired and motivated as we are to do research in this fascinating field of chemistry.

Finally, we would like to deeply acknowledge the authors of the individual chapters for their singular and extraordinary contributions. We are also thankful to our coworkers and colleagues for introducing us to this fascinating field of chemistry, for fruitful discussions and for sharing our interest and passion. We also want to express our gratitude to the Wiley team, especially Paul Deards, who initiated the production of this book, as well as Sarah Tilley and Rebecca Ralf for their professional assistance during the editing and publishing process.

August 2012
Galia Maayan
Markus Albrecht

1

Metalloproteins and Metallopeptides – Natural Metallofoldamers

Vasiliki Lykourinou and Li-June Ming
Department of Chemistry, University of South Florida, USA

1.1 Introduction

By a combination of amino acids with various properties, it is possible to obtain the natural polymers, peptides and proteins, capable of catalyzing key transformations, sustaining energy flow, and maintaining life in a narrow range of allowable conditions and starting materials. The folding, flexibility, and structural changes of peptides and proteins are integral to their functions within living systems. Several factors affect their folding and conformation, including primary sequence, subunit composition, nature and type of subunit interactions, presence of cofactors, and compartment and sequence during folding in a well controlled environment. Emphasis must also be placed on the conformational flexibility of peptides and proteins necessary in the context of their folding and function. In the case of enzymes, such conformational flexibility renders the induced fit process possible during enzymatic catalysis, as found in the large domain movements in hexokinase, HIV protease, and many others upon substrate or inhibitor binding.

Protein conformation, structure, and function are often determined or modulated by metal ions. Therefore, it is instructive to discuss the effect of metal ions or cofactors on the conformational changes of proteins prior to and during their actions. Furthermore, by comparing the folding of metallopeptides and metalloproteins to those whose conformational changes take place in the absence of metal ions can provide further structural and functional information on the metal ions in the former molecules. Such discussion is

Metallofoldamers: Supramolecular Architectures from Helicates to Biomimetics, First Edition.
Edited by Galia Maayan and Markus Albrecht.

important in this context, as many proteins require metal ion(s) for their optimal structure and function.

1.2 Metalloproteins

1.2.1 Metalloproteins are Nature's "Metallofoldamers!"

The term "foldamer" was defined as "polymers with a strong tendency to adopt a specific compact conformation" (Gellman) or "oligomers that fold into a conformationally ordered state in solution, the structures of which are stabilized by a collection of noncovalent interactions between nonadjacent monomer units" (Moore) [1]. In this respect foldamers share common characteristic with proteins and thus the term is adopted to differentiate synthetic oligomers and polymers from "nature's foldamers" such as peptides, proteins, and nucleic acids. The term "metallofoldamers" thus is used to describe foldamers that adopt conformationally ordered states in the presence of metal ions or complexes [2]. Therein, the metal center(s) plays a key structural role in the formation of the specific conformation of the corresponding foldamer.

Metal ions may play a key role in the conformational changes of proteins or peptides crucial in their functions. Given the high occurrence of metal ions as cofactors and their integral role in the function of proteins in carrying out catalytic transformations, it is important to review how they are incorporated in the structure of proteins. The mechanism of metal incorporation ranges from a controlled manner through the use of metallochaperones to the direct incorporation from the cellular pool. In the former case this specific class of proteins binds metal ions and mediates the delivery into target enzymes through protein–protein interactions. In the case of iron transport and heme incorporation, transferrin transports iron into cells and hemopexin delivers apo-heme to the same compartment. For cytochrome-c, the heme must be attached before proper folding occurs [3], whereas the assembly of Fe–S clusters and incorporation into proteins and the folding of the F–S proteins require machinery encoded in the iron–sulfur cluster operon [4].

Nevertheless, metal ion incorporation is not always well controlled in such a manner. There are still many metalloproteins without a known chaperone counterpart for metal delivery and folding. Therefore, metal incorporation has also been suggested to be controlled by the choice of compartment in which the metal incorporation takes place. From studies in cyanobacteria, it becomes apparent that many copper and manganese containing proteins fold in different compartments where metal insertion can be controlled in a way that the Irvin–Williams series of stability can be circumvented [5].

A classic example of the structural role of metal ions that affects the function is the zinc finger domains, wherein the metal ions crosslink α–β domains and thus play a central role in the formation of the defined structures. The metal binding as well as the packing of a hydrophobic core drive the folding process. The zinc coordinates to the Cys_2His_2 motif and drives the folding process, while its removal causes the disruption of the proper folding. These zinc finger domains were first found in the transcription factor TFIIIA, which represents the most common nucleic acid binding motif in transcription factors [6]. Upon binding to DNA, the Zn finger domains undergo further conformational change in order to fit

Figure 1.1 *Crystal structure of zinc finger–DNA complex (of Zif268). The Zn(II) ions (black spheres) in the three "fingers" (cyan) are bound to the protein through two Cys and two His residues and hold the α-helical/β-sheet structural motifs together.*

into the major groove of DNA (Figure 1.1; PDB 1ZAA) [7], representing one typical example of metal- and ligand-mediated conformational change of a natural metallofoldamer.

1.2.2 Metal-Triggered Conformational Change of Proteins

1.2.2.1 Cytochrome c and Heme Binding

The cytochrome c family of electron-transfer proteins has a high α-helix content and a heme cofactor that is postranslationally modified to covalently attach to the protein by two thioether bonds between the vinyl group of heme and two cysteine residues within the motif Cys-Xaa-Xaa-Cys-His [8]. The folding of cytochrome c has been studied by proton to deuterium exchange equilibrium monitored with NMR [9]. The folding sequences involve the interaction of the two terminal helices (N- and C-, respectively) first, followed by joining of the 60s helix. The heme center locks in the designated pocket and this is followed by final coordination of the Met80 in the sixth position. These three helices containing the axial methionine appear to be the minimal structural requirement in cytochrome c folding (Figure 1.2) [10]. The axial heme ligand is not conserved among all the proteins and can be found to be histidine, asparagine, or it can be absent [11]. The cytochrome c family has also been implicated in the apoptotic mechanism of cell death

Figure 1.2 *Comparison of the three-dimensional structures of two different cytochromes c. Left: crystal structure of tuna cytochrome c (PDB ID 3CYT). Right: solution structure of oxidized cytochrome c from* Bacillus pasteurii *determined by NMR. The three helices are in cyan, the extra loop in the tuna enzyme is shown in lavender, the heme is shown in red.*

via a reactive oxygen species mechanism, scavenging of hydrogen peroxide, and in the assembly of cytochrome c oxidase [11].

The absence of the heme from cytochrome c causes complete destabilization of the protein due to a decrease of hydrophobic contacts as the heme resides at the hydrophobic core essential for the folding of the protein, an effect similar to guanidinium chloride denaturation [12]. The denaturant action is related to its competition with water molecules in the protein binding, resulting in an unfolding of the protein structure. Simulations of protein folding have shown that correct folding requires full heme contacts at the folding transition state in addition to the hydrophobic interactions as a critical "folding nucleus" [13]. The computational results are in agreement with H–D exchange NMR results, suggesting the initiation of the folding by the terminal helices followed by the 60s helix for the Met80 loop and β-sheets to build onto. The last helix to form is the 40s loop, concluding the significant folding role of the heme for providing a hydrophobic core to stabilize the protein and coordinating to the His and Met residues which results in further lowering the entropy en route to native folding. The heme center can also complicate the folding process as other residues or small molecules can compete for heme binding, which causes what is termed "chemical frustration" [14]. Folding of cytochrome c can therefore be modulated by choosing solvent conditions that favor one set of heme ligands over others. No crystal structures of the apo protein could be obtained for this reason [10], indicating the significance of the heme cofactor in forming and maintaining the folding this natural metallofoldamer cytochrome c.

1.2.2.2 α-Lactalbumin and its Ca^{2+} Binding and Molten Globule

Calcium binding can cause significant conformational changes, which in turn may mediate a signaling cascade. The "EF-hand" folding is a major Ca-binding motif composed of a helix-loop-helix sequence as found in the multifunctional messenger calmodulin and S100. The assembly of the Ca binding can then be propagated to a protein partner with which the Ca-binding protein is interacting with. In the S100-type of proteins (which regulate cell cycles, cell growth, and differentiation), Ca binding influences protein folding to aid in their dimerization and further interaction with other partner proteins. Likewise, Ca binds α-lactalbumin at a domain containing a helix-loop-helix bend – close to the EF-hand domain – dubbed a Ca-binding "elbow" [8].

α-Lactalbumin is a main protein component of milk, which has been the target for investigation of calcium binding to proteins besides the EF-hand group of proteins and is used as a model for the study of protein stability. It is the regulatory subunit of lactose synthase for the synthesis of lactose from UDP-galactose and glucose in the lactating mammary gland. The protein possesses a single strong Ca^{2+}-binding site, which can also bind Mg^{2+}, Mn^{2+}, Na^{+}, and K^{+}, and a few distinct Zn^{2+}-binding sites. In bovine α-lactalbumin, Ca^{2+} binds to the "elbow" region (Figure 1.3, lavender) via three carboxylates of Asp82, Asp87, and Asp88, two carbonyl groups of Lys79 and Asp84, and two water molecules in a distorted pentagonal bipyramid coordination sphere with the two carbonyl groups at the axial positions. The binding of cations to the Ca^{2+} site increases the stability of α-lactalbumin against heat and various denaturing agents and proteases, while the binding of Zn^{2+} to Ca^{2+}-saturated protein decreases the stability and causes aggregation.

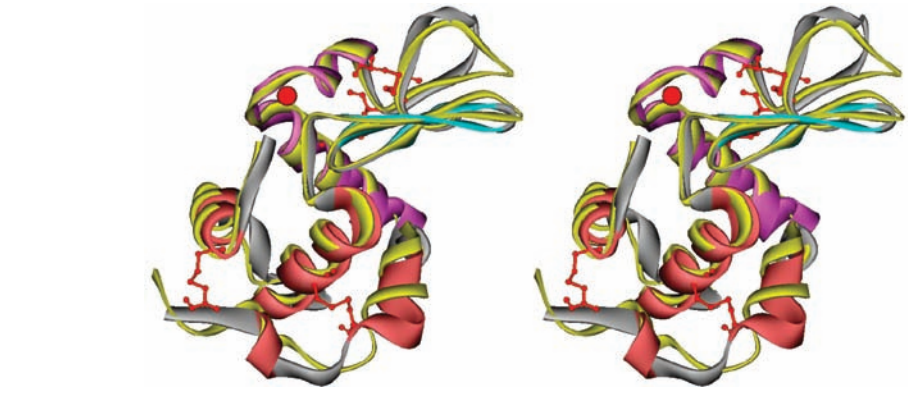


Figure 1.3 *Stereo view of crystal structure (PDB 1F6S) of bovine holo α-lactalbumin, showing the "Ca^{2+} elbow" in lavender. The structure of hen egg white lysozyme (3LZT; yellow) is superimposed onto the α-lactalbumin structure, showing the significant similarity in folding.*

There are four disulfide bonds in α-lactalbumin (Figure 1.3, red), but none in the EF-hand proteins, which dramatically stabilize the protein conformation [8]. The correct folding of the protein, which relies on the correct formation of the proper disulfide bonds, is promoted by the high Ca^{2+}-binding affinity to the protein. Ca^{2+} binding however does not change the secondary structure based on circular dichroism (CD) and fluorescence studies [15,16] which can effectively afford the molten globular state of the protein in the absence of Ca^{2+} possessing a native-like secondary structure but a flexible tertiary structure [16]. The molten globular state of the protein can be obtained by removal of calcium, in the presence of denaturants, or in an acid denaturant state [17].

Ca^{2+} does not have a significant effect on the metal-binding site, but it does affect the cleft at the opposite face (underneath the β-sheets shown in Figure 1.3) of the molecule at the joining of the α-helical (Figure 1.3, light brown) and the β-sheet (Figure 1.3, cyan) lobes by disturbing the H-bonding pattern in this region, which results in a more open conformation in apo α-lactalbumin and demonstrates the significance of Ca^{2+} in the folding of α-lactalbumin [18].

Bovine α-lactalbumin (BLA) shares 38% sequence homology with hen egg white lysozyme (HEWL) with most of the differences at charged residues in BLA. However, their tertiary structures are nearly superimposable with all the four conserved disulfide bonds (Figure 1.3, yellow ribbon). Electrostatic interactions can stabilize partially unfolded conformations, which can aid in the formation of molten globule state in BLA, but not in HEWL. The thermodynamic folding barriers in the two proteins are different with a marginal barrier possible for BLA due to stabilization of partially unfolded conformations in the presence of Ca^{2+}, which stabilizes the fully folded state of the protein [19]. Such electrostatic interactions can be potentially engineered in artificial systems as a means to aid in structure stabilization. These studies again reveal the significance of metal ions in the proper folding and stabilization of proteins in order to afford functional natural metallofoldamers.

1.2.2.3 Metallothionein and Heavy Metal Regulation

The concentration of zinc must be well controlled in the cell, as high concentrations can be toxic and cause mitochondrial dysfunction. Metallothionein plays a central role in zinc homeostasis since it is the major protein important in regulation of the zinc level in the cell and its translocation [20] and it has been shown to induce an effect on brain neurons by binding to neuronal receptors and initiating pathways which cause neurite survival [21,22]. Metallothionein regulates the flow of zinc and copper in the cell and can further prevent poisoning from exposure to toxic cadmium and mercury. Its overall role is suggested to be the control of the distribution of zinc as a function of the cellular energy state and it has been implicated in the following functions [23,24]: (a) intracellular zinc transport, (b) zinc binding and exchange (e.g., with the zinc cluster protein Gal4, zinc finger transcription factors such as TFIIIA, and aconitase) as well as a zinc-specific chaperone, (c) oxidoreductive properties of cysteine bound zinc as cysteine ligands are redox-sensitive regulatory switches [25], (d) controlling cellular zinc distribution as a function of the energy state of the cell as shown by the interaction with ATP, GSH, and ROS and zinc distribution to enzymes in metabolic networks of gene expression and respiration [26], and (e) a possible role in neural activity, storing and distributing zinc for the neuronal network and protecting it against cellular damage as well as neuronal recovery through binding to neuronal receptors initiating signal transduction pathways [22].

One function of metallothionein may resemble that of the iron-storage protein ferritin in terms of its zinc-storage capability. It is a small protein rich in cysteine (20 cysteines in a total of 62 amino acids in human metallothioneine), but without aromatic amino acids such as tyrosine or histidine. The apo protein can bind a total of seven equivalents of divalent metal ions with d^{10} configuration such as Zn^{2+} or Cd^{2+} in two noninteracting domains (Figure 1.4) [27] and up to six Cu^{+} ions in each domain [28]. The protein can bind up to seven Zn^{2+} or Cd^{2+} ions in tetrahedral coordination spheres, or 12 three-coordinate Cu^{+} ions with only cysteine residues. The binding of metal converts the random coil conformation of apo metallothionein into a folded two-domain structure (Figure 1.4). Cd^{2+} binding has been extensively studied with C-13 NMR [29], wherein structural flexibility was observed. The absence of hydrophobic residues for stabilizing a folded form is compensated by the presence of the metal–thiolate core in the folding of this protein.

Apo metallothionein retains the backbone conformation imposed by the formation of the metal–thiolate clusters. REF computational studies indicate a potential H-bonding

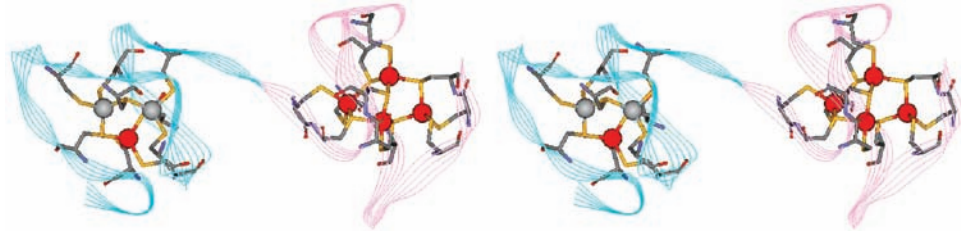


Figure 1.4 *Stereo view of the structure (PDB 4MT2) of Cd_5Zn_2, showing the N- (cyan) and C- (pink) domains with the coordinated Cys residue.*

network present in apo metallothionein which plays a role in the formation of a constant but flexible backbone needed to adjust to the incoming metal to ensure specific metal incorporation [30]. The metal–thiolate clusters then direct the "wrapping" of the protein into its three-dimensional structure. There are two distinct metal binding sites in the C-terminal (a-domain) and N-terminal domains (b-domain). The C-domain contains four Zn^{2+} ions bound to 11 Cys residues with five serving as bridging ligands, while the N-domain binds three Zn^{2+} ions via nine Cys residues and the metal binding follows a non-cooperative model up to four equivalents, as shown by Co^{2+} binding [31,32]. Computational studies also support this metal-binding mechanism. MM3/MD calculations of metallothionein structure after sequential removal of metals show that the last two metals bind to independent tetrathiolate sites from terminal thiolate ligands, which further supports independent Co^{2+} binding to isolated sites prior to metal cluster formation and also confirms that the C-terminal site is the first to bind metal ions [24]. Taken together, metallothionein represents an excellent example for describing the role of metal ions in the proper folding of a natural metallofoldamer.

1.2.3 Conformational Change of Metalloproteins Caused by Ligand Binding

1.2.3.1 Calmodulin and Ca^{2+}/Ligand Binding

The role of metal ions in stabilizing the folded states of small proteins is well established, as illustrated in zinc finger proteins [33] (Figure 1.1). Reversible binding of metal ions, where both the metal-free disordered form and the metal-bound ordered form are functional, is very widely observed among calcium-binding proteins. The coupling of the N- and C-terminal lobes in the EF-hand Ca^{2+}-binding calmodulin is a good example [34]. Since calcium signaling is such an important process in many metabolic systems, it is likely that this kind of reversible order–disorder equilibrium is quite common. The binding of up to four Ca^{2+} ions in the two different globular ends of apo calmodulin [35] causes significant conformational changes to the molecule (Figure 1.5), including straightening of the long interdomain helix (Figure 1.5, red). Calcium binds to the sites with different affinities (i.e., a higher preference for the C-terminal binding site than the N-terminus),

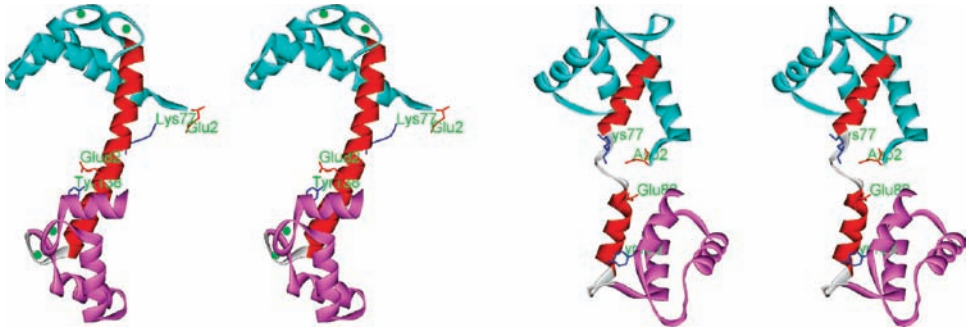


Figure 1.5 *Right: structure of Ca^{2+}-free calmodulin determined with NMR in solution, showing a kink in the middle of the interdomain helix (PDB ID 1CFD). Left: crystal structure of Ca^{2+}-bound calmodulin, showing dramatic conformational change upon Ca^{2+} binding (PDB 1EXR).*

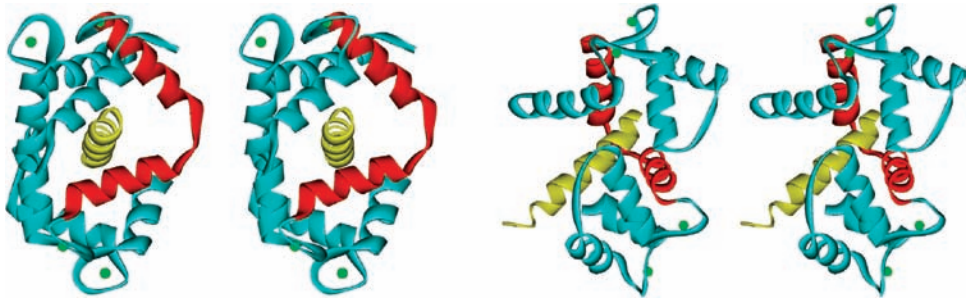


Figure 1.6 *Two different views (rotating by ~90°) of Ca_4-calmodulin-bound "IQ motif" of the Cav1.1 channel (PDB ID 2VAY). The interdomain helix is colored in red, the IQ motif in yellow, and the four Ca^{2+} ions as green spheres.*

resulting in conformational changes and interaction with other proteins and enzymes to perform its regulatory role (Figure 1.5) [36]. Ca^{2+} binding to the C-terminal sites stabilizes the long interdomain helix via a Tyr138–Glu82 interaction, which in turn disrupts two interaction helices by breaking an Asp/Glu2–Lys77 interaction, which is followed by Ca^{2+} binding to the N-terminal sites to form a binding cleft for target proteins [37].

Calmodulin can bind various molecules, including drugs, peptides, and their regulating target proteins. Calmodulin-modulated Ca^{2+} signaling is thus attributed to the different responses of these target molecules to the conformational change of calmodulin upon Ca^{2+} binding. The difference in Ca^{2+} binding and target interactions of the two lobes also enable calmodulin to work out local and global Ca^{2+} sensing and signaling through conformational change [38]. Further, the binding of target molecule to calmodulin can also influence the Ca^{2+} sensitivity of calmodulin [39]. The calmodulin-binding regions in the target proteins are comprised of short helical segments of ~14–26 amino acids with a high occurrence of hydrophobic and basic residues for high affinity and specificity without the need for sequence specificity [40]. Such dramatic conformational change is illustrated in Figure 1.6 for Ca_4-calmodulin binding binding to the "IQ" "motif" in the α_1 subunit of the L-type voltage-dependent Ca^{2+} channel Cav1.1, which undergoes Ca^{2+}- and CaM-dependent channel facilitation and inactivation [41]. The C-terminal conformation of the α_1 subunit is critical for channel function and has been proposed to regulate the gating machinery of the channel [42]. The binding causes a significant conformational change in calmodulin, especially the kink at positions 79–81 of the interdomain helix, which results in wrapping around the peptide (Figure 1.6). Taken together, calmodulin represents one of the best examples showing significant metal- and ligand-induced conformational changes.

1.2.3.2 Carboxypeptidase A Catalytic Mechanism

Another example of dramatic conformational changes in a metalloenzyme is well represented in the action of carboxypeptidase A, a pancreatic proteolytic enzyme. It belongs to a family of exopeptidases responsible for catalyzing the hydrolysis of peptide bonds at the C-terminus of peptides and proteins. It plays a regulatory role or complements the action

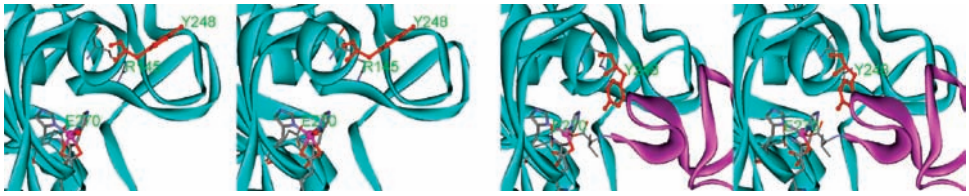


Figure 1.7 *Stereo views of the crystal structure of (left) bovine carboxypeptidase A at 1.25 Å (PDB 1ML) with the nucleophilic water shown in red sphere and (right) the enzyme with a bound potato inhibitor (lavender; PDB 4CPA).*

of other proteolytic enzymes such as trypsin, chymotrypsin, and pepsin to aid in the production of essential amino acids [43]. Carboxypeptidase A is specific to hydrophobic C-terminal amino acid residues (such as phenylalanine, tyrosine, or tryptophan), while the B-type is specific to the charged residues Lys and Arg. Carboxypeptidase A is a monomer of 307 amino acids with a globular shape consisting of both α-helices and β-sheets (Figure 1.7). The active-site zinc ion plays a key role in the catalysis as it participates in the stabilization of the intermediate, the deprotonation of the nucleophilic coordinated water, and the electrostatic interactions critical for recognition of the terminal amino acid in the substrate peptide chain. This enzyme shares a common active-site motif of $H^{69}xxE^{72}$ for zinc binding (with a bidentate carboxylate of E72), along with a second histidine (H196) located far downstream and a water molecule to complete the coordination sphere of the metal (Figure 1.7) [44].

As in the case of hexakinase, this protein also undergoes conformational changes upon substrate binding to the active site "pocket," which closes upon substrate binding. More specifically, the negatively charged residues can interact with Arg145 residues in the active site while the hydrophobic interactions between the substrate and the hydrophobic pocket can help orient the substrate. Upon binding of potato inhibitor via its C-terminal carboxylate (Figure 1.7) [45], the bidentate Glu72 residue becomes monodentate which is accompanied by a >10 Å movement of Tyr248 to form a H-bond with the bound inhibitor along with a ~ 2 Å movement of the backbone of the region around Tyr248 toward the metal. Once again, conformational flexibility in metalloproteins is illustrated herein with carboxypeptidase A during its catalytic action.

1.2.3.3 Aminopeptidase and Alternative Catalysis

Aminopeptidases are widely distributed hydrolytic enzymes catalyzing various processes, such as peptide digestion and hormone production, some requiring metal ion(s) in the active site for full activity. The nuclearity of the active site of metallopeptidases varies from mononuclear in the case of carboxypeptidase, to dinuclear in aminopeptidases, and trinuclear in phospholipase C. Even among the dinuclear aminopeptidases, such as those isolated from bovine lens (bAP) [46], *Escherichia coli* (eAP) [47], *Aeromonas proteolytica* (aAP) [48], and *Streptomyces griseus* (sAP) [49], there is a variation in the structural and mechanistic roles of the metal ions [50]. For example, the dinuclear aAP shows selective metal binding, but mononuclear catalytic activity with the second metal playing a regulatory role [51]. In contrast, the dinuclear sAP (as well as bAP) exhibits dinuclear

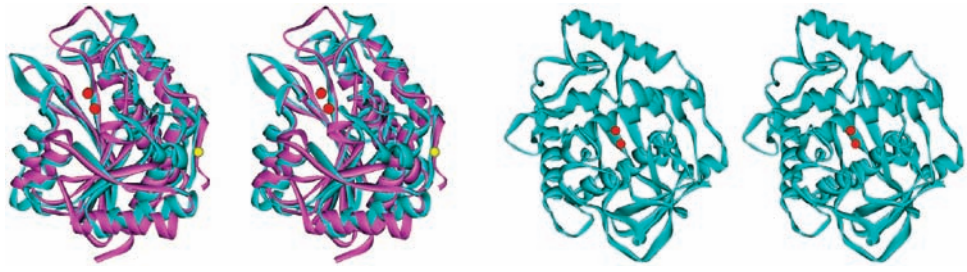


Figure 1.8 *Left: stereo view of superimposed sAP (lavender; PDB ID 1CP7) and aAP (cyan; PDB 1AMP), with the active-site di-Zn in red sphere and Ca (in sAP) in yellow. Right: stereo view of di-Cu catechol oxidase from sweet potato, showing the very different folding from that of sAP. The two Cu ions are shown in red.*

catalysis, despite its very similar folding and active-site coordination (Asp/His on one metal, Glu/His on the other metal, and a bridging Asp) to those of aAP (Figure 1.8), which indicates that the microenvironment such as the proximal amino acid residues around the active-site coordination sphere must be more significant than the folding of the peptide backbone for proper functions and specific activities.

Streptomyces AP is a di-Zn^{2+}-containing 30-kDa enzyme which consists of a central β-sheet core surrounded by helices with the active site found within the β-sheet region (Figure 1.8), wherein the dinuclear site can selectively bind metal ions such as Co^{2+} and Mn^{2+} in the two metal-binding sites of sAP [52]. The various metal derivatives exhibit significant alternative catalysis toward a phosphodiester substrate, despite the fact the latter frequently serves as a transition-state inhibitor, which is not the case for aAP [53]. Moreover, the di-Cu^{2+} derivative of the enzyme shows significant activity toward catechol oxidation [54], despite its protein folding and active-site coordination environment being completely different from those of catechol oxidase with three His residues bound to each Cu (Figure 1.8). The observations presented herein indicate that protein folding in not the only control for showing specific enzyme catalysis (i.e., sAP vs aAP toward alternative catalysis) and the folding and/or active-site coordination sphere does not need to be restricted to a certain pattern to exhibit a specific catalysis (i.e., di-Cu-sAP vs catechol oxidase).

1.2.4 Protein Misfolding: Causes and Implications – Cu, Zn-Superoxide Dismutase

The superoxide dismutase (SOD) family has four distinct groups, that is, Cu-, Zn-, Fe-, Mn-, and Ni-containing, which are responsible for catalyzing the conversion of superoxide anionic radical to O_2 and H_2O_2 to protect the cellular environment from damage by superoxides generated during respiration or through the oxidative activity of immune cells [55]. The Cu,Zn-containing SOD (SOD1) is a dimeric protein, with each monomer consisting of an eight-stranded β-barrel and electrostatic and metal-binding loops (Figure 1.9) [56]. The electrostatic loop features Arg143 for hydrogen bonding to superoxide and Thr137 in conjunction with Arg143 to limit the anions coming into the copper-active site. The catalytic site features a unique bridging His63 residue between the two metal ions at

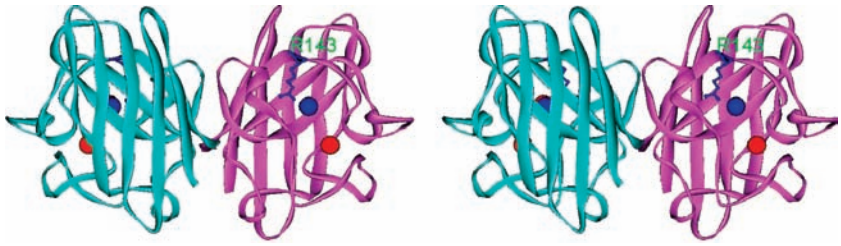


Figure 1.9 *Crystal structure of as-isolated dimeric human SOD1 (PDB ID 2C9U). The Cu ions are shown in blue and Zn ions in red.*

6 Å apart. The coordination site of Cu^{2+} is completed by three more His residues and a water molecule in a square pyramidal geometry, whereas the Zn^{2+} tetrahedral site is comprised of two more His residues and an Asp residue. The catalytic cycle starts with the binding of superoxide to Cu^{2+} by displacing the coordinated water followed by electron transfer to the copper and diffusion of oxygen, which results in a trigonal planar Cu^{+} site. A second electron transferred by another superoxide results in the regeneration of the Cu^{2+} center and the release of peroxide [57].

SOD1 is properly folded through posttranslational modifications which proceed via two distinct pathways, depending on whether or not the copper chaperone CCS is required for the insertion of Cu and the formation of an intramolecular disulfide bond [58]. The formation of disulfide bridges is crucial in the oligomerization of the protein as the reduced metal free protein favors the monomeric state. Proper folding of SOD1 is important since many mutant forms of this protein have been shown to cause amyotrophic lateral sclerosis (ALS), suggested to be due to destabilized or completely unfolded structures and aggregation at room temperature [59]. More specifically, the immature reduced forms of the mutant protein without the formation of disulfide bonds [60] have been implicated in the aggregation process as they can form incorrect intermolecular disulfide crosslinks. The spinal cords of ALS transgenic mice have been found to contain significant amounts of insoluble aggregates composed of such crosslinked multimers, which however are not observed in other tissues such as the brain cortex and liver [60]. Transgenic mice expressing the human mutant G85R SOD1 protein develop paralytic ALS symptoms along with the appearance of SOD1-enriched inclusions in their neural tissues. The crystal structure of this mutant supports that metal-deficient and/or disulfide-reduced SOD1 mutants may contribute to toxicity in SOD1-linked ALS [61].

The unfolding process of SOD1 has been shown to include more than two states, involving other intermediates in the unfolding process. The irreversible inactivation process due to thermal denaturation has distinct features between apo- and holoenzymes, with the rates of inactivation showing a biphasic response as a function of temperature for the apoenzyme but a monophasic function in the case of the apoenzyme [62,66]. The role of the metal ions has been also implicated in the stabilization of the β-barrel structure of the protein fold [63]. Moreover, the unfolded state of the protein is also stabilized by metal ions [64]. The derivative Cu, E-SOD1 without a bound Zn^{2+}, has a lower thermal stability, supporting the primary structural role of Zn^{2+} in this protein. In addition, Co(II)

or Hg(II) can replace Zn(II) in order to maintain the thermal stability of the Cu(II)-free apoenzyme [65]. Overall, the apoenzyme is more sensitive to inactivating processes compared to the holoenzyme. Stability is also affected by the oxidation state of the bound copper. DSC measurements of dithionite reduced native SOD1 containing Cu^{+} and Zn^{2+} reveal one peak at 96 °C while native SOD1 containing Cu^{2+} and Zn^{2+} exhibits two melting transitions at 89 and 96 °C wherein the transition at 89 °C is affected by oxygen in the solution [66]. The effect of metal binding in protein stabilization is not unique to SOD1. For example the Cu-binding in *P. aeruginosa* azurin stabilizes the protein, whereas in beta-2-macroglobulin it causes native-state destabilization [67].

Taken together, the presence and identity of the metal ion bound to SOD1 and the status of the disulfide bonds in SOD1 have significant effects on the folding, stability, and catalytic efficiency of the enzyme. Destabilization and misfolding of this enzyme may result in the formation of aggregations in neural tissues and cause neurodegeneration and ALS. The above sections have briefly described the folding, structure, and function of several natural metallofoldamers–metalloproteins, which serve as a foundation for the further design and investigation of synthetic metallofoldamers.

1.3 Metallopeptides

Analogous to the metalloproteins discussed above which inspired the design and syntheses of metallofoldamers, a number of simple natural products such as oligopeptides and oligoketides and some antibiotics also adopt secondary or specific structures upon binding with metal ion(s) and can serve as templates for functional metallofoldamers. Metal ions play a key role in the actions of synthetic and natural metallopeptides [68,69] and are involved in specific interactions with proteins, membranes, nucleic acids, and other biomolecules. For example, Fe/Co-bleomycin binds DNA, which impairs DNA function and may also result in DNA cleavage; metallobacitracin binds the sugar-carrying undecaisoprenyl pyrophosphate to inhibit cell wall synthesis; and the specific binding of metal ions to ionophores or siderophores results in their transport through the cell membrane either causing disruption of the potential across the membrane or enabling microorganisms to acquire Fe from the environment.

In addition to the α-helical and β-sheet secondary structures, the β-turn is another important secondary structure in peptides and proteins, in which Pro frequently found at the "break point" [70] and the β-turns [71] to afford an anti-parallel β-sheet structure. In addition to Pro, Gly is also a "structure breaker" and frequently associated with Pro to form a turn [72] as observed at the G12–P13 β-turn in Cu,Zn-superoxide dismutase (Figure 1.10). Combining with His, a metal-binding site can form near the turn in metalloproteins, such as the Pro86 turn in the copper site of plastocyanin (Figure 1.10, blue). Peptides are prototypical molecules which can adopt secondary structures to exhibit broad biological activities by interacting with specific receptors or target proteins, including a large number of G protein-coupled receptors, wherein a general "turn motif" is associated with the binding [73]. Peptides are involved in many physiological regulations and bioactivities, such as the opioid peptides dynorphin, endorphin, and enkephalin, galanin (which may regulate nociception), ghrelin (which may stimulate hunger), Ca^{2+}-regulating calcitonin, adrenocorticotrophic hormone, and some neurotoxins. Such peptide-associated activities have triggered the design