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Secondary Interactions Involving Zinc-Bound Ligands: Roles in Structural Stabilization and Macromolecular Interactions

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Abstract

A large number of proteins contain bound zinc ions. These zinc ions are frequently coordinated by a combination of histidine and cysteine residues. In addition to atoms that coordinate directly to the zinc ions, these side chains have groups that can donate or accept hydrogen bonds from other groups. These secondary interactions can help stabilize the zinc-binding sites, can contribute to protein folding and stability, and, on occasion, can participate in interactions with other macromolecules. Five examples of these secondary interactions are discussed: carbonic anhydrase (where secondary interactions involving histidine residues stabilize the zinc-binding site thermodynamically and kinetically), retroviral nucleocapsid proteins and TRAF proteins (where cysteine sulfur to peptide NH hydrogen bonds contribute to the structural relationships between adjacent domains), and zinc finger proteins and TIS11 where secondary interactions participate in protein-nucleic acid interactions.

1. Introduction

Bert Vallee was one of the pioneers of zinc biochemistry. He and his coworkers developed a number of analytical techniques that revealed the presence of zinc in key proteins and enabled the characterization of the structural and reactivity properties of zinc sites that were crucial for function in zinc metalloenzymes. His discovery (with Hans Neurath)[1] that bovine pancreatic carboxypeptidase A is a zinc metalloenzyme increased the number of known zinc metalloenzymes from one (carbonic anhydrase) to two. His group subsequently demonstrated that alcohol dehydrogenases are zinc metalloenzymes and subsequently discovered that this family of metalloenzymes contains two classes of zinc ions, one essential for enzymatic activity and the other for structural integrity and subunit association. These studies presaged decades of work on these and other zinc metalloenzymes as well as the discovery of large classes of proteins that contain only structural zinc ions, essential for proper folding and macromolecular interactions but not directly involved in any catalytic process[2].

The elucidation of the enzymatic reaction mechanisms for enzymes such as carboxypeptidase A[3], carbonic anhydrase[4], and alcohol dehydrogenase[5] revealed that

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exogenous ligands such as water and ethanol bind directly to open coordination sites on zinc where they are polarized and activated. Ligand binding to zinc favors ligand deprotonation resulting in species prepared for subsequent reactions. For proteins containing only structural zinc ions, the influence of the zinc on the bound ligands, which are always derived entirely from the protein, has not been as extensively discussed. These ligands are also polarized by zinc and their protonation and hydrogen-bonding properties modulated by zinc binding. Here, we examine examples from three classes of proteins where this occurs. The first involves carbonic anhydrase II where the free N-H groups on the imidazoles from each of the three zinc-bound histidine residues donates a hydrogen bond to a protein-derived oxygen atom. These secondary interactions help stabilize the zinc-binding site, leading to increased zinc affinity and kinetic stability. The second class is exemplified by two groups of proteins with zinc binding sites defined by three cysteines and one histidine residue. Here, the cysteine residues are deprotonated to their cysteinate state upon zinc binding and participate in key hydrogen-bonding interactions. Specifically, these cysteinate ligands accept hydrogen bonds from peptide backbone N-H groups from residues just on the carboxyl terminal side of the cysteinate residue, helping to orient and rigidify linker regions between adjacent zinc-binding domains. The last class involves examples of proteins that show secondary interactions involving zinc-bound ligands that play important roles in the interactions between these proteins and specific nucleic acid molecules. Together, these examples demonstrate that zinc-bound ligands can play other important structural roles besides acting as binding sites and anchors for the metal ion.

2. Secondary interactions stabilize zinc-binding sites: Carbonic anhydrase

Carbonic anhydrase catalyzes the hydration of carbon dioxide to carbonic acid. The active site of human carbonic anhydrase II comprises a zinc ion bound in a distorted tetrahedral geometry to three histidine residues with an additional coordination site available for binding and activating water. Detailed examination of the carbonic anhydrase crystal structure reveals that the zinc-bound water along with the non-bonding NH groups of each imidazole ring, donate hydrogen bonds to nearby protein ligands [6]. Specifically the NH groups donate their hydrogen bonds to a glutamine carboxamide side chain, a backbone carbonyl oxygen and a carboxylate oxygen of a glutamate residue side chain (Figure 1). Mutation of either the glutamine or glutamate residues involved in these hydrogen-bonding interactions to alanine results in an approximately 10-fold decrease in the affinity for zinc by the protein [7, 8]. The role of these hydrogen-bonding interactions appears to be that they rigidify the zinc-binding histidine residues and orient them for optimal coordination hence lowering the entropic energy for binding and the tendency to reorient to allow zinc release. Furthermore, they stabilize the charge on the zinc center by partially reducing the positive charge on the bound zinc. A key aspect of the significance of these interactions can be seen by the effects of mutating the glutamate residue on the rate of zinc release. Mutation of the glutamate to an alanine or even aspartate increases the k_{off} rate more than 200-fold even though it only decreases the zinc affinity by less than 5-fold. The increase in the zinc dissociation rate is crucial since the half-life of the zinc complex decreases from approximately 5 days to less than 30 minutes at 25 °C. This dramatic difference can probably distinguish between a protein that can stably bind zinc inside (or outside) a cell and a protein that loses its zinc when present in an environment with a low concentration of available zinc. Note that such carboxylate-histidine-metal interactions have been observed in many other proteins[9].

3. Peptide NH to cysteinate sulfur hydrogen bonds help orient adjacent zinc binding domains

Carbonic anhydrase provides an example where secondary interactions involving metal-binding residues could occur even in the absence of the bound metal ion. However, in other cases, binding of the metal substantially perturbs the properties of the amino acid side chains that participate in metal binding. One of the clearest examples of the phenomenon involves metal-binding cysteine residues. The side chain of cysteine includes a thiol group that typically has a pK_a value near 9 and, hence, is protonated at physiological pH. However, in almost all protein metal-binding sites that utilize cysteine, the thiol group binds in its deprotonated form as a thiolate. As was first observed in the iron proteins rubredoxin and ferredoxin, such metal-bound thiolates tend to accept hydrogen bonds from nearby NH groups in the protein backbone[10]. Such peptide NH to cysteinate sulfur hydrogen bonds have subsequently been found to occur in many metalloproteins and appear to play a significant role in stabilizing specific protein structures[11]. Furthermore, these interactions can be directly detected by nuclear magnetic resonance methods with the incorporation of appropriate metal ions[12]. Below are two examples of proteins that contain tandem zinc-binding domains in which these interactions appear to play an important role in stabilizing the relative orientation of adjacent zinc binding units.

The first example is the HIV-1 nucleocapsid protein (NCp7) from the retroviral CCHC-box proteins[13]. This protein recognizes specific structures within the RNA genome of HIV-1 and facilitates packaging the genome into maturing virions[14]. NCp7 contains two CCHC-box sequences of the form Cys-X₂-Cys-X₄-His-X₄-Cys[15, 16] separated by a basic linker of seven amino acids that is essential for interacting with nucleic acids. Each of the zinc-bound cysteinate residues accepts one or more hydrogen bonds from peptide NH groups to stabilize the folded structure through various tight turns, which in turn are stabilized by the NH-S hydrogen bonds [17]. Of particular interest here are the interactions involving the last cysteinate residue of the amino-terminal CCHC-box domain, which in part, determine the relationship between the two domains.

The structure of NCp7 containing both the zinc binding domains has been independently determined[18, 19]. In each structure, the thiolate side chain of the last cysteine on the amino-terminal domain is within hydrogen bonding distance and orientation from the N-H backbone of an alanine residue, which is part of the linker and two residues C-terminal to the cysteine (Figure 2). Similarly, comparable structures have been observed between equivalent residues in other nucleocapsid CCHC proteins[20]. Thus although the two zinc binding domains function as independent folded units, this NH-S hydrogen bond, together with the bend imposed by the conserved proline residue next to the alanine in the linker appear to be important for constraining the relative orientation the two domains suitable for optimal nucleic acid binding. These interactions and the associated structure are present in a complex between the HIV-1 nucleocapsid protein and a high-affinity RNA binding site[13].

The second example involves the Tumor Necrosis Factor Receptor-Associated Factor (TRAF)-like proteins. The archetypes of this family are the TRAF proteins, which play an important role in several signaling pathways. There are seven mammalian TRAF proteins (TRAF1- TRAF7) most of which contain a variable number of adjacent domains that approximate the consensus sequence Cys-X₂₋₅-Cys-X₁₁₋₁₂-His-X₃₋₇-Cys (hereafter referred to as TRAF-like CCHC domains). The TRAF-like domain at the carboxyl terminus of each array has a His residue in place of the last Cys. Importantly, each pair of adjacent domains is joined by a relatively short six amino acid linker. The structures of a number of TRAF-like domains have been determined[21-23]. Each domain folds into an independent structure approximating the beta strand-turn-beta strand-turn-alpha helix fold, observed for the

“classical” Cys₂His₂ TFIIIA-like zinc-binding domains. Nonetheless, examination of the tandem zinc-binding domains in the crystal structure of TRAF6 reveals that the last cysteine of each N-terminal TRAF-like domain is capable of participating in an NH-S hydrogen bonding interaction with the backbone NH of the second amino acid in the linker (Figure 3). The same is observed in the NMR structure of two adjacent zinc-binding domains of TRAF4 and in the crystal structure of SIAH1 (Seven in absentia homolog 1) which is also a member of the TRAF-like protein family. The relatively short linker forces the two adjacent domains to be in contact with one another and the presence of the NH-S hydrogen bond appears to increase the rigidity of this junction. This is manifest by the relatively high degree of conservation of structures of CCHC TRAF-like domain pairs[22].

The close contact between adjacent domains suggests that metal binding by these domains might not be independent. Based on observation, we recently investigated the metal binding by two adjacent domains from TRAF4 and demonstrated that this binding is weakly cooperative[24]. A similar study on the classical Cys₂His₂ TFIIIA-type zinc binding domains with a linker that is one amino acid longer and no substantial interdomain interactions showed no detectable cooperativity[25].

Many TRAF-like proteins have been proposed to function as ubiquitin E3-ligases, which require an elongated stiff platform to enable the transfer of ubiquitin between E2 and the substrate [26, 27]. The CCHC TRAF-like zinc binding domains of these proteins appear to function as a rigid platform, supported, in part, by the secondary interactions involving the terminal metal-bound cysteinate residue.

4. Zinc-bound ligands in protein-nucleic acid interactions

The role of structural zinc-binding proteins in macromolecular interactions such as protein-protein, protein-nucleic acid and protein-lipid interactions has been clearly established. However, the involvement and importance of zinc-bound ligands in intermolecular interactions with these macromolecules has received less attention. Here, we discuss two examples of nucleic acid binding proteins, which use zinc-bound ligands to make intermolecular contacts that are important for nucleic acid binding.

The structure of Zif268 bound to DNA serves as a archetype for understanding how DNA interacts with the classical Cys₂His₂ TFIIIA-like proteins, which are one of the largest class of nucleic acid binding proteins found in eukaryotic transcription factors. These proteins, which are primarily known for their role in transcription regulation, contain a variable number of Cys₂His₂ zinc-binding domains. Zif268 contains three Cys₂His₂ zinc-binding domains with the conserved sequence Cys-X₂₋₄-Cys-X₁₂-His-X₃₋₄-His, separated by a relatively well-conserved seven amino acid linker. Each domain folds into an independent beta strand-turn-beta strand-turn-alpha helix structure stabilized by interactions between conserved hydrophobic residues. The crystal structure of Zif268 bound to DNA revealed that amino acids from the amino-terminal part of the alpha helix make base-specific contacts, facilitated by a network of hydrogen bonding interactions, with base pairs in the major groove of double stranded DNA [28, 29]. Residues in the alpha helix also make contacts with the backbone phosphate groups. Prominent among these is a conserved hydrogen bond formed between the first zinc-bound histidine ligand and the phosphate backbone, in which the non-coordinating N-H group of the imidazole ring donates a hydrogen bond to a phosphate oxygen atom (Figure 4). It has been suggested that this interaction may increase the affinity of the DNA-protein complex by 5-10 fold [8].

The second example involves a family of sequence-specific RNA binding proteins, the TIS11 proteins. These RNA binding proteins are characterized by the presence of zinc-binding domains that approximately the form Cys-X₈-Cys-X₅-Cys-X₃-His and play

important roles in post-transcriptional regulation[30, 31]. They bind to AU-rich elements in the 3'-untranslated region of target mRNAs to promote their deadenylation and degradation. The solution structure of the RNA-binding domain of TIS11d bound to an RNA target has been determined[32]. Each zinc-binding domain interacts with one 5'-UAUU-3' site through a combination of an extensive network of hydrogen bonding interactions between the protein backbone and the bases, and stacking interactions between conserved aromatic residues and RNA bases. The secondary interactions involving the zinc-bound cysteinate and histidine ligands served primarily to stabilize the protein structure. However, the last cysteinate in each domain also forms an S-HN hydrogen bond with the NH group in position 3 of a uracil ring. Interestingly, these are the only sidechain to base hydrogen bonds in TIS11d-RNA complex structure.

5. Conclusions

Zinc-binding domains are typically characterized by zinc ions coordinated by four protein-derived ligands, most frequently histidine and cysteine residues. The primary role of these ligands is to bind the zinc ion. However, in many cases, these ligands also interact through hydrogen bonds with other groups within the protein or a protein complex. These secondary interactions contribute to the ability of bound zinc ions to support protein structures as well as macromolecular interactions. These observations extend those made by Professor Vallee who was not only one of the pioneers of zinc enzymology but also someone who recognized the importance and diversity of zinc binding domains[33].

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Figure 1.

The active site structure of carbonic anhydrase II showing that the three zinc-bound histidine residues participate in hydrogen bonding interactions (dashed lines) with additional groups in the protein.

Figure 2.

A structure of the two zinc-binding domains from the HIV-1 nucleocapsid protein. A key cysteine sulfur to peptide NH hydrogen bond that helps restrict the orientation of the two zinc-binding domains is shown.

Figure 3.

A portion of the structure of human TRAF6 showing two adjacent TRAF-like CCHC domains. The terminal cysteine residue of the amino-terminal domain participates in a sulfur to peptide NH hydrogen bond that contributes to the relatively rigid relationship between the two domains.

Figure 4. The structure of the zinc finger protein Zif268 bound to a DNA target site. Two hydrogen bonds between zinc-bound histidine residues and phosphate groups in the DNA backbone are shown.

Figure 5.
The structure of the RNA-binding domain of TIS11d bound to an RNA oligonucleotide.
Two interactions between zinc-bound cysteine residues and NH groups from key uridine residues are shown.