Bioinorganic chemistry and drug design: here comes zinc again

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The structures and reactions of metal ions in proteins are of tremendous interest in bioinorganic chemistry, as is the potential for metals in creating novel medicines. New results combine these aspects in describing an unexpected mode for metal-mediated drug efficacy that relies on well-established principles of metalloprotein structure.

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The discipline of bioinorganic chemistry aims to elucidate the mechanisms by which metal ions (usually transition metals) aid biological processes [1,2]. One third of all enzymes are metalloenzymes in which metal ions play catalytic or structural roles. The mechanisms by which metalloenzymes activate small molecules such as N_2, CH_4, O_2, NO and H_2O are under intense study [3], and the roles of metal ions in hydrolytic catalysis by proteins and by ribozymes are captivating [4,5]. Of equal importance is also the means by which the metal ions get into cells and subsequently into these metalloenzymes in the first place, and the field of metalloregulation seeks to describe how genes are turned on and off in response to changes in metal-ion concentration [6,7].

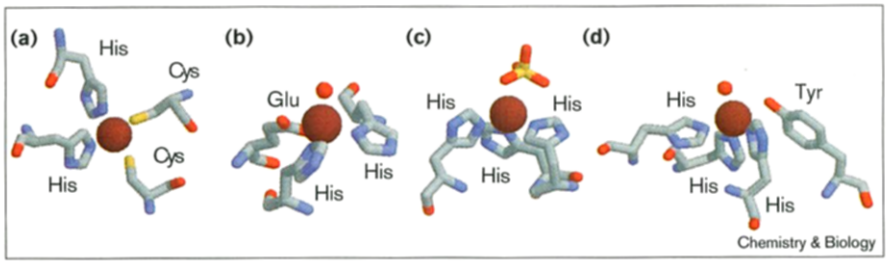
A number of pharmaceutical products and approaches utilize metal ions [8]. The most well known example of these is the cancer chemotherapy agent cisplatin (*cis*-$Pt(NH_3)_2Cl_2$) that is widely used in treatment of advanced cancer and provides an 80–90% success rate for testicular cancer patients [2]. Other metal drugs include gold compounds for treatment of arthritis, sodium nitroprusside for hypertension, technetium and rhenium radionuclides for diagnosis and therapy, and paramagnetic metal complexes for magnetic resonance contrast agents [9]. Recently, photodynamic therapy using metalloporphyrins and their derivatives has attracted significant attention [10]. Yet, despite these success stories, it has been noted that the "applications of metals in medicine are far fewer than warranted by their potential" [2]. In a recent paper, Katz *et al.* [11] report a new mode for metals in medicine discovered by traditional pharmaceutical techniques that center on protein crystallography and the conventional screening of organic small molecules. The authors report that zinc ions can mediate the binding of a small organic molecule to the active site of a serine protease that does not ordinarily contain a metal ion. Involvement of the zinc ion greatly increases the affinity and selectivity of the inhibitor.

Zinc biochemistry

Zinc is a star player in bioinorganic chemistry [1]. Many hydrolytic enzymes, such as carboxypeptidase and carbonic anhydrase, utilize zinc in their active sites [12]. More recently, DNA-binding proteins that use zinc to organize structural elements, called zinc fingers, have been elucidated [13]. Sufferers of the common cold turn to treatment with zinc lozenges that appear to shorten the duration and severity of cold symptoms under the right circumstances [14], possibly because of the involvement of zinc ions in the primary immune system [15]. Zinc is well suited to these roles because it is present at relatively abundant concentrations and binds to a number of donor atoms present in proteins. Bonding of transition metals to organic ligands is often described by the 'hard–soft' principle where more polarizable lone pairs such as those in sulfur are considered 'soft' and less polarizable lone pairs such as those in oxygen are considered 'hard'. In a hard–soft sense, zinc is a 'borderline' Lewis acid that binds to imidazole (histidine), carboxylate (aspartate and glutamate), thiolate (cysteine), and alkoxide (serine, threonine and tyrosine) donors (Figure 1) [12]. The d^{10} dication is redox-inert, so hydrolytic enzymes can utilize zinc without heading down inadvertent redox pathways that would destroy the protein or substrate. Most bioavailable metal ions that bind to such a wide set of donors have some degree of redox activity [3].

Establishing the coordination environments that metal ions adopt in proteins is a major goal in bioinorganic chemistry [16]. Because of the affinity of zinc for a wide range of donors, there are many different ligand sets in zinc proteins. Interested readers are referred to a recent comprehensive review [12], but some prominent examples of zinc proteins are shown in Figure 1. The zinc finger proteins exhibit a very soft environment consisting of two cysteine residues and two histidine residues in a tetrahedral array. This coordination environment has a high specificity for zinc because the soft thiolate ligands do not bind hard metal ions well. The zinc ion in carboxypeptidase A is coordinated to two histidine residues and a glutamate, leaving an open site in the tetrahedral array for a catalytically important water molecule. Similarly, the active site of the enzyme carbonic anhydrase comprises a zinc ion situated on top of three histidine residues with a water ligand available for catalysis. The enzyme astacin contains a five-coordinate zinc ion with three histidine residues, tyrosine, and a coordinated water

Figure 1



Naturally occurring coordination environments for zinc. Four representative proteins with coordinates taken from the Brookhaven Data Bank. Structures of the zinc centers in **(a)** the zinc finger protein Zif268 from accession code 1AAY [25], **(b)** carboxypeptidase A with a coordinated water molecule from 2CTB [26], **(c)** the sulfate complex of carbonic anhydrase from 1CAI [27] and **(d)** astacin from 1AST [28].

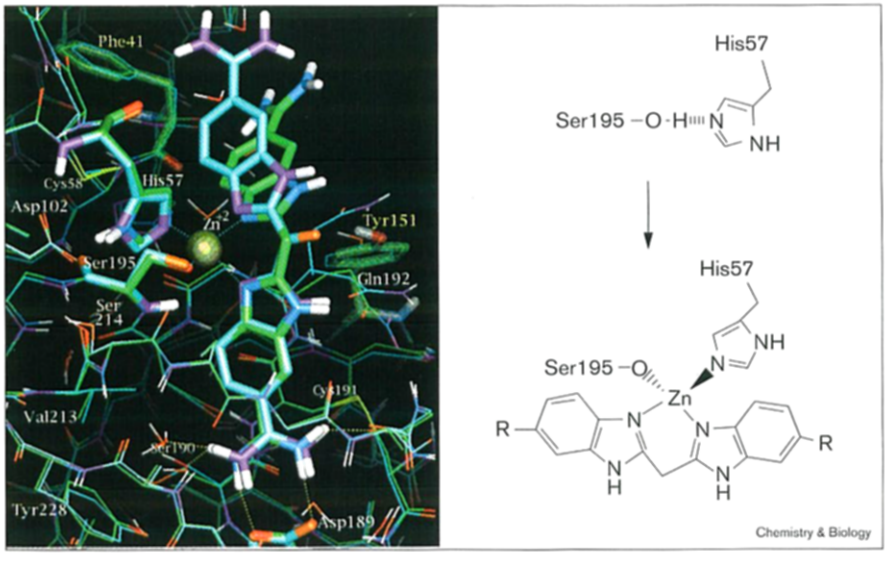
molecule. In just the four examples shown, zinc is found with thiolate, carboxylate, imidazole and alkoxide ligation, so zinc provides the unique combination of versatile coordination with a failure to undergo redox chemistry.

A new role

Now, Katz *et al.* [11] describe a new role for zinc in mediating the binding of small molecule inhibitors to enzyme active sites. The authors have targeted serine proteases, a therapeutically interesting family of targets, with small molecule inhibitors based on bis(benzimidazoles), such as BABIM. These small molecule inhibitors bind to serine proteases with high affinity, giving values of K_d as low as 6 nM [11]. Upon careful examination of the X-ray crystal structures of the enzyme–inhibitor complexes, a novel mode of enzyme inhibition is found in which the enzyme and inhibitor have recruited an exogenous zinc ion to bridge the inhibitor and active-site residues. The enzyme activity of serine proteases is based on a 'catalytic triad' of active-site residues that includes a serine residue (Ser195) and a histidine residue (His57), that must be hydrogen bonded to each other for the enzyme to function [17]. Binding of a zinc ion breaks the Ser–His hydrogen bond, and the resulting lone pairs coordinate the zinc ion (Figure 2). The other two coordination sites on zinc are filled by the two imidazole nitrogen atoms of the inhibitor, creating an environment that approximates coordination by three histidine residues and a (presumably deprotonated) serine residue. This environment is very similar to that of carbonic anhydrase in which the zinc ion is coordinated by three imidazoles and a hydroxide ion [4,12].

Experiments with and without an exogenous EDTA chelator show that the binding of the zinc ion enhances the affinity of the drug molecules by as much as 17,000-fold [11]. Even more exciting, however, is the observation that the selectivity of the drug is enhanced. For example, one of the drugs showed a preference for thrombin over tryptase and trypsin that was enhanced by a factor of 4,800 when zinc was present, even though all three of these enzymes contain the catalytic Ser195 and His57 residues needed to coordinate the zinc ion. The selectivity must be a result of the geometry of the active-site pocket in the distorted protein, which must change conformation to bind

Figure 2



Scheme showing the capture of the catalytic residues of chymotrypsin by zinc ion and BABIM. Structure reproduced with permission from [11].

the metal ion. The ternary complex must then be able to accommodate the tetrahedrally coordinated zinc ion and the accompanying drug molecule. This interaction requires much more specificity than binding the drug in the absence of zinc, because the organic ligand alone is flexible at the methylene carbon that joins the two imidazole rings. Katz *et al.* [11] have identified a loop in thrombin that exhibits potential contacts with the metal-bound drug, providing a conceptual framework for engineering selectivity for or against thrombin.

Prospects

As Katz *et al.* [11] note, these results suggest a large number of new metal–protein–drug ternary complexes that are physiologically significant. There are many other enzymes that have nucleophilic groups in the active site that can coordinate metal ions, so extension of these concepts to other protein–drug pairs and other metal ions is straightforward. Combinatorial methods of drug screening in combination with regulation of metal-ion concentrations will no doubt lead to many new 'metalloproteins' that consist of a metal ion bridging an active-site residues and a chelating drug. Of relevance here are recent studies showing that cobalt complexes target active-site residues and inhibit certain enzymes selectively [18].

Furthermore, although zinc is attractive because of its abundance, redox-inertness, and wide repertoire of coordinated ligands, sequestration of other metal ions by special protein–drug complexes is a further possibility. There have been significant efforts aimed at engineering metal sites into proteins using amino acid ligands resulting in new metal-ion-binding sites created by mutagenesis in proteins that do not bind metal ions [19], in synthetic proteins that bind metal ions [20] and in changes in metal-ion specificity in proteins that ordinarily bind a metal ion but now exhibit different preferences [21]. The concept of Katz *et al.* [11] in using the metal ion to promote selectivity between the protein and drug might be reversed so that the protein–drug combination is specific for a certain metal ion. Such specificity might be desirable under conditions where binding a given metal ion is important, for example in sensing or in following metal-ion homeostasis [22].

All these new applications will rely on continued advances in bioinorganic methods. Characterization of the structures of metal-ion-binding sites by X-ray crystallography and spectroscopic methods will expedite the discovery of new physiologically relevant metal–protein complexes. Computational methods and algorithms for predicting the structures of metal ions in protein and chelate environments [23,24] will now be of increased importance both for solving the structures of experimentally derived metalloproteins and for designing new metal-binding drugs that have high specificities and potencies. Bioinorganic chemistry has been called 'a maturing frontier' [2] — probably mature enough to shepherd the forthcoming barrage of new metal–drug–protein complexes towards realizing their full potential.

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