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Zinc and Sulfur: A Critical Biological Partnership

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In the world of chemistry and mineralogy, the mutual affinity of zinc and sulfur is well-recognized. The earliest detected modes of zinc coordination in biology were different, however. The ligands associated with catalytic zinc ions in hundreds of enzymes proved to be nitrogen and oxygen, i.e., histidine and glutamate/aspartate, while the sulfur of cysteine served as a ligand less frequently (*1*, *2*). The discovery of the so-called zinc fingers in the early 1980s changed this balance. Their coordination of zinc involves two cysteine and two histidine ligands (ZnS_2N_2 coordination) (*3*, *4*). Now "zinc finger" is a generic term that includes motifs with one histidine and three cysteine ligands (ZnS_3N) and with cysteine ligands only (ZnS_4). Since 3% of the ~32000 genes in the human genome encode proteins with zinc fingers (*5*), the zinc−sulfur (Zn−S) interaction is the predominant mode of binding in proteins. Thus, the animate and inanimate worlds after all do not seem to differ in the frequency at which Zn−S bonds occur. Zn−S bonds serve as structural braces in protein domains that participate in protein−protein, protein−nucleic acid, and protein−lipid interactions (*6*, *7*). Yet biology has utilized the functional potential of the Zn−S interaction in a most efficacious manner. The Zn−S interaction offers specific mechanisms for enzyme catalysis, establishes reactivities of zinc sites that hitherto were believed to have only a structural role, allows zinc to be tightly bound and yet to be available, and, importantly, generates redox-active coordination environments for the redox-inert zinc ion. These activities are critically involved in the regulation of protein structure and function, and in mobility, transfer, redistribution, and sensing of cellular zinc, as is becoming evident from the continuing exploration of the protein metallothionein and other proteins involved in cellular zinc homeostasis.

Zn−S Bonds in Mononuclear Sites

At least two aspects of the Zn−S(Cys) interaction are important for enzymatic catalysis. For one, the polarizability of a sulfur ligand and the relative ease with which it can dissociate provide ways of modulating the Lewis acidity of catalytic zinc ions. In cytidine deaminase, the Zn−S distance of one of the two cysteines of the catalytic zinc increases as the transition state is approached (*8*). The concept of the Zn−S bond serving as a "valence buffer" was developed for this dynamic event, because sulfur can accommodate changes of charge at the catalytic zinc (*8*).

The second aspect is also related to the properties of the zinc ion as a Lewis acid. However, it is better described as an effect of the metal on the ligand rather than an effect of the latter on the metal. Thus, much as zinc lowers the pK_a of a bound water molecule to provide a hydroxide as a nucleophile at physiological pH, it can lower the pK_a of a coordinated thiol for the same purpose. Enzymes involved in methylations and alkylations employ such an activation of the ligand (*9*, *10*). In the *Escherichia coli* Ada protein, a DNA repair protein, one of the thiolates in a ZnS_4 site is a zinc-activated nucleophile that reacts with the methyl group of a phosphotriester (*11*). This discovery has significantly influenced our thinking about biological Zn−S coordination as it demonstrated that a site with characteristics of structural zinc sites, in fact, can be reactive. The realization of this intriguing reactivity has inspired work with the aim of answering the following questions: How does zinc coordina-

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tion affect thiolate reactivity? What is the order of reactivity in the series of ZnS_2N_2, ZnS_3N, and ZnS_4 complexes? How is it possible that some ZnS_4 complexes are reactive while others are apparently inert? How can one of four of the same ligands be activated specifically?

Kinetic studies with model complexes for the reaction of the Ada protein demonstrated that a zinc tetrathiolate complex reacts much faster in methyl transfer than either a zinc dithiolate complex or a free thiolate. The results are in agreement with the dissociated ligand being the nucleophile (*12*). In studies with other zinc thiolates, the zinc-bound thiolate is the nucleophile (*13*). In addition to the composition of the ligand set, hydrogen bonding and protonation are important factors in modulating the reactivity of zinc thiolate complexes. An intramolecular amide NH to sulfur hydrogen bond can decrease the rate of alkylation more than 30-fold (*13*, *14*). Thus, hydrogen bonding can deactivate certain thiolates in complexes containing multiple thiolates. Protonation of a thiolate also drastically decreases its nucleophilicity. Zinc coordination counteracts this effect as it lowers the pK_a of a thiol−thiolate equilibrium by approximately 2 orders of magnitude (*10*). Thus, metalation, i.e., zinc binding, activates the ligand, while protonation deactivates it. In most cases, the resolution of X-ray structures of proteins is not high enough to distinguish a coordinated thiol from a thiolate. However, a structure of rubredoxin with zinc substituted into the tetrathiolate site, which in the native protein is occupied by iron, was determined at a sufficiently high resolution to determine that the ligands are all thiolates (*15*). A high-dielectric medium or additional polarizable ligands favor deprotonation of a thiol, making four deprotonations in the formation of a tetrathiolate complex favorable in aqueous solution, in particular at solvent-exposed surfaces in proteins with lysines or arginines as second-shell ligands (*16*). Yet experimental data and theoretical considerations suggest that thiols can serve as ligands in zinc sites (*17*, *18*). Examination of the effects of electrostatic screening and protein packing in zinc fingers demonstrated a continuum from purely structural to reactive sites and identified labile zinc fingers as those with poorly screened scores (*19*).

Redox-Active Zinc Proteins

Perhaps the most important aspect of why biology utilizes thiolate ligands in zinc sites is the redox activity that the sulfur ligand confers on the complex (*20*). Generation of an oxidoreductive environment where the *ligands*, not the metal, are oxidized and reduced with concomitant release and binding of zinc is an extraordinary property of zinc proteins. It allows redox control of zinc and couples cellular zinc availability with redox metabolism (Figure 1). Thus, differences in the coordination environment of zinc in proteins determine whether the metal is metabolically inert or reactive. Aside from metallothionein, redox-active sulfur ligands are now recognized as being important for several protein functions. The heat-shock protein Hsp-33 of *E. coli* operates by a redox zinc switch that induces chaperone activity when a large conformational change occurs upon oxidation of the thiolate ligands of its zinc site (*21*, *22*). In the anti-sigma factor rsrA from *Streptomyces coelicolor*, the zinc thiolate site is a sensor for disulfide stress (*23*). Stress-induced disulfide formation between the cysteine ligands of zinc expels the metal and stabilizes a protein conformation that

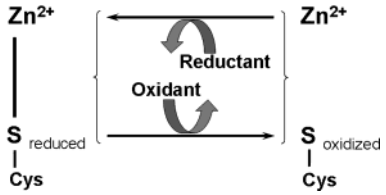

FIGURE 1: Ligand-centered redox chemistry of the Zn−S interaction. The oxidation and reduction of the sulfur ligand are coupled with the binding and release of zinc, thus generating redox zinc switches in proteins. Reversible redox reactions occur between the thiol and disulfide states of sulfur. While under biological conditions the oxidation of cysteine to cysteinesulfenic acid is also reversible, oxidations of cysteine to cysteinesulfinic acid and cysteinesulfonic acid are irreversible.

does not bind the sigma factor. The redox-active zinc site of replication protein A is essential for its single-stranded DNA binding activity and is involved in the recognition of damaged DNA (*24*). Likewise, two of the three cysteine ligands of the catalytic zinc in betaine-homocysteine methyltransferase can be oxidized to a disulfide with concomitant expulsion of zinc (*25*). Finally, it has been suggested that the zinc finger motif in the monomer of cellulose synthase is involved in oxidative dimerization of the enzyme (*26*). These redox zinc switches are a basic principle of controlling protein function. They affect not only tertiary structure but also quaternary structure, where zinc serves a direct role in protein−protein interactions and the assembly of macromolecular complexes (*27*). Some of these interactions employ pairs of thiolate ligands from each protein. In nitric oxide synthase, for example, the zinc site bridges the two protomers. Oxidation of the thiolate ligands releases zinc and uncouples nitric oxide synthesis (*28*). It remains to be determined whether similar interactions in heterologous protein−protein interactions are also modulated by redox reactions and/or control of the availability of zinc. The release of zinc from protein kinase C by specific lipid second messengers or reactive oxygen species (*29*) certainly underscores the fact that redox signaling at Zn−S sites is a more common, and perhaps central, event in redox biology (*30*).

The oxidative activation of matrix metalloproteinases (*31*) is yet another example of the redox activity of Zn−S sites. In the zymogen form of these enzymes, a thiolate group contributed by a cysteine side chain blocks the catalytic site by completing the ZnN_3S coordination (*32*). Reaction of this Zn−S bond with glutathione disulfide (*33*) or other oxidants such as nitric oxide (*34*) activates the proteinase. Finally, the redox activity of Zn−S sites has been exploited for therapeutic purposes. Drugs containing a disulfide bond react with the cysteines in the ZnS_3N site of the HIV nucleocapsid protein NCp7, or the cysteine ligands in the human papillomavirus E6 protein (*35*), forming a mixed disulfide intermediate, and thereby releasing zinc (*36*, *37*). Zinc thiolate−disulfide interchange is also a mechanism of potent inhibition of β-lactamases with zinc thiolate coordination (*38*).

In contrast to simple thiol−disulfide equilibria, redox zinc switches at Zn−S sites are linked to the binding and release of zinc. Hence, the availability of zinc is a controlling factor. It turns out that Zn−S bonds are also critically involved in controlling the availability of zinc. Therefore, in the remainder of the article, much broader implications of Zn−S interactions will be discussed, namely, their functions in

binding zinc tightly and yet allowing it to be distributed dynamically in the cell.

A General Role of Zn−S Bonds in the Cellular Distribution of Zinc

Zinc participates in enzymatic catalysis and protein structure stabilization much more widely than any other transition metal (*39*). The numerous roles of zinc in proteins amplify the question of how the cell ensures distribution of zinc to all the zinc proteins in a timely and coordinated manner. Recent discoveries provide compelling evidence that a rather complex homeostatic system exerts tight control over cellular zinc and warrants that zinc does not interfere with the roles of other metal ions, that the concentration of its free ion is kept very low to avoid unspecific interactions with deleterious effects, and that the demands of all zinc-dependent cellular functions are met. In addition to metallothionein and all the factors that control its gene expression and protein functions, the cellular homeostatic system comprises sensor proteins and families of membrane proteins involved in cellular zinc influx, efflux, and vesicular sequestration (*40*). The following discussion will address the role of the Zn−S bonds in cellular zinc distribution in multicellular eukaryotes. This choice is based on the observations that from bacteria to yeast, to fungi, and to plants and animals Zn−S sites in proteins are used with increasing frequency (*41*), that the homeostatic systems in prokaryotes and eukaryotes are fundamentally different (*40*, *42*), and that the multiplicity of isoforms of metallothioneins and their extensive gene regulation pertains mainly to zinc metabolism in vertebrates.

Conceivably, cellular zinc distribution could be based on thermodynamic gradients provided by differential stabilities of zinc complexes. Without changes in the amounts of the binding species, however, only unidirectional zinc transfer to sites of greater stability would be possible. If an abundant zinc protein binds zinc as tightly (or even tighter) as a less abundant one, in essence the hierarchy of distribution would be established by the amount of protein available. On the other hand, if an abundant zinc protein binds zinc less tightly, zinc would dissociate and metabolic energy would be wasted in assembling a protein that is not fully functional, because it relies on zinc as the limiting factor for its activity. Clearly, such a distribution system based on thermodynamic gradients would be unsatisfactory and would moreover pose a serious challenge to the cell when discriminating among competing metal ions. Available data indicate that despite extensive variability in their coordination environments *intracellular* eukaryotic zinc proteins bind zinc tightly with stabilities (pK_D) of 10−12 (Table 1). Thus, among these proteins, a major thermodynamic driving force for zinc transfer does not exist. The tight binding of zinc to proteins has an important consequence in that it limits the amount of "free" zinc ions in the cell to a very low range, several orders of magnitude lower than the overall cellular zinc concentration, which is estimated to be in the range of 200 μM (*52*). Concentrations of 1×10^{-10} (*53*), 2.4×10^{-11} (*54*), and $<5 \times 10^{-10}$ M (*55*) of free zinc have been determined. These compare relatively well with a free zinc concentration of $1-2 \times 10^{-10}$ M in bovine and equine plasma (*56*). Therefore, the same considerations apply to *extracellular* proteins, which generally also bind zinc very tightly (Table 1). In cardiomyocytes, a basal level of $<1 \times 10^{-10}$ M zinc increases by 1 order of magnitude to reach nanomolar concentrations upon electrical stimulation (*57*), which incidentally makes this the only study, thus far, to report the magnitude of fluctuations in cellular free zinc concentrations under physiological conditions. These measurements place free zinc concentrations in eukaryotes in the range of picomolar to low nanomolar, in accord with the prediction that "under tight feedback control over equilibrium concentrations of free ions...to a first approximation the free ion concentration of each metal is the inverse of its binding constant" (*58*). The necessity for a tight control of zinc ions in this range of concentrations becomes more obvious when one assesses the consequences of an excess of cellular available zinc ions. Thus, zinc strongly inhibits many biological processes. Even nanomolar concentrations can inhibit enzymes *in vitro*, e.g., glycolytic enzymes, but also enzymes involved in signaling such as caspases and protein tyrosine phosphatases (*59*), and mitochondrial function (*60*), suggesting a physiological regulatory mechanism, in which zinc affects specific targets in energy metabolism and signaling (*59*, *61*, *62*).

Table 1: Stability Constants of Eukaryotic Zinc Proteins

zinc complex	stability constant pK_D ($-\log K_D$)	ref
carbonic anhydrase II, human	11.4	*43*
carboxypeptidase A, bovine	10.5	*44*
metallothionein, human or rabbit liver	12.9 (pH 7.0) 13.5 (pH 7.4)	*45*,*46*
glyoxalase I, human erythrocytes	10.6	*47*
angiotensin-converting enzyme, rabbit lung	8.2	*48*
sonic hedgehog, human	<10	*49*
stromelysin, human	10.7	*50*
dipeptidyl peptidase III, rat liver	12.3	*51*

If thermodynamic gradients do not constitute a major driving force for zinc distribution, there must be other mechanisms for making zinc available. It has been suggested that such mechanisms include "zinc trafficking factors that control the kinetics of metal exchange between proteins" (*63*). Metallothionein (MT) will serve as an example here by exhibiting in detail chemical, biochemical, and kinetic mechanisms that are unique for zinc when it is bound to the sulfhydryl group of cysteine. Among all the proteins in Table 1, zinc binds most tightly to MT. Effectively, MT is a thermodynamic sink for zinc, but it can also be a source of zinc, e.g., through coupling with cellular redox metabolism.

Zinc Thiolate Cluster Structures and Zinc Coordination Dynamics

Metallothionein (MT) was the first protein where zinc was found to be associated with sulfur (*64*). It has the most complex Zn−S coordination of all zinc proteins known so far. In vertebrate class I MTs, 20 of the 60−68 amino acids are strictly conserved cysteine residues. They are the only ligands that bind seven zinc ions in multiple zinc-containing motifs, so-called zinc thiolate clusters. The characteristic features of these clusters are bridging sulfur ligands that form networks of electronically coupled bonds in multinuclear complexes. A three-zinc cluster (Zn_3S_9 coordination, three sulfur bridges) and a four-zinc cluster (Zn_4S_{11}, five sulfur bridges) are located in the two domains of MT, respectively (*65*, *66*) (Figure 2). In contrast, class II MTs exhibit great

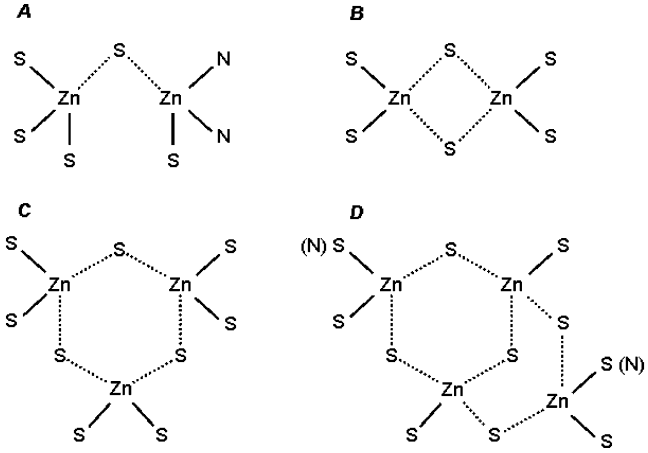

FIGURE 2: Zinc thiolate clusters. (A) Cluster in the dimerization domain of V(D)J recombination-activating protein Rag1. (B) Cluster in the Gal4 type of fungal transcription factors. (C) The three-metal cluster in the N-terminal domain of MT-1 and -2 has six terminal and three bridging ligands (total of nine ligands), providing a coordination that is formally identical for each zinc in terms of bridges and end-on coordination. Such clusters are present in the pre-SET domain of histone lysine methyltransferases. (D) The four-metal cluster in the C-terminal domain of MT-1 and -2 also has six terminal ligands, but five bridging ligands, providing two sets of coordination environments, two with two end-on ligands and two with one end-on ligand. A similar cluster is present in a cyanobacterial MT (SmtA). However, in SmtA, one of the two cysteine ligands is replaced with a histidine in each of two zinc ions that have two terminal ligands. Dotted lines connect the zinc ions and the bridging sulfurs.

structural diversity. The three-dimensional structure of only one class II zinc MT, a cyanobacterial MT, has been determined (*67*). Its zinc thiolate cluster ($Zn_4S_9N_2$, five sulfur bridges) bears a close resemblance to the four-zinc cluster in vertebrate MT, with the notable difference being that two of the zinc ions have one histidine and one cysteine ligand. The other two types of zinc thiolate clusters in biology are present in the Rag1 protein ($Zn_2S_5N_2$, one sulfur bridge) (*68*) and in the Gal4 type of clusters in fungi (Zn_2S_6, two sulfur bridges) (*69*) (Figure 2). In all of these clusters, the zinc ions are in tetrahedral environments with four ligands.

A three-zinc cluster such as the one in the N-terminal domain of MT occurs in one domain of a family of lysine methyltransferases (*70*). While the cluster structure is conserved in these proteins, the amino acid sequences between the ligands differ from those in MT. This lack of conservation makes it difficult to search databases for analogous cluster motifs and to predict how many of them occur in the zinc proteome, i.e., the ensemble of zinc proteins in a given genome (*71*). Zinc thiolate clusters add yet another dimension to the intricacies and dynamic functions of biological Zn−S coordination. They are so remarkable that further discussion of their properties is warranted.

Thermodynamically, MT is one of the most stable zinc sites in eukaryotes with a dissociation constant of 1.4×10^{-13} M ($pK_D = 12.9$) (Table 1). Yet zinc in MT exchanges with zinc in solution with relative ease (*72*). For comparison, carbonic anhydrase, which also binds zinc tightly with a K_D of 4×10^{-12} M ($pK_D = 11.4$) (Table 1), exchanges zinc extremely slowly, with a half-life on the order of 1 year (*73*). These properties are telling evidence of a dynamic function of the zinc ions in MT and illustrate that differences in coordination environments, i.e., only nitrogen ligands in carbonic anhydrase and solely sulfur ligands in MT, can control the kinetics of zinc binding in sites of high thermodynamic stability.

Intermolecular exchange of zinc between MT isoforms is fast, and the two observed kinetic phases have been attributed to the clusters in each domain, with the exchange from the three-metal cluster being faster (*74*). NMR spectroscopy also revealed the *intramolecular* dynamics of the metal ions in MT. Cadmium exchanges rapidly within the three-metal cluster ($0.2-2.7\ s^{-1}$) and slowly within the four-metal cluster ($1 \times 10^{-3}\ s^{-1}$) or between the three-metal and four-metal clusters (*75*). Since all zinc sites are tetracoordinate without space to bind another ligand, these reactions are thought to occur through ligand exchange in which transient intermolecular metal−thiolate bonds are formed (*75*). Indeed, when MTs are separated by a dialysis membrane that is impermeable to the protein but permeable to zinc, zinc does not exchange (*45*). Thus, the thiolate coordination environments of the clusters endow MT with special features. They are thermodynamically stable to retain zinc but have sufficient kinetic lability for the protein to both accept and donate zinc.

Metallothionein and Zinc Release

The protein completely envelops the zinc atoms, prompting the question of how the buried zinc is then released from MT. In contrast to the zinc atoms, some of the sulfur ligands are accessible at the surface of MT (*65*). A host of reactive compounds oxidize the thiolate ligands and release zinc from MT *in vitro* (*20*). Remarkably, the redox potential of MT with 20 reduced cysteines is so low that common biological oxidants such as glutathione disulfide (GSSG) release zinc from MT (*76*, *77*). Since reactions with GSSG and other disulfides are rather slow, additional factors might enhance zinc release. Thus, selenium compounds catalyze the oxidation of MT by disulfides or oxidize MT directly and release zinc (*78*, *79*). Such selenium redox catalysis efficiently couples thiol−disulfide pairs and MT and would allow zinc release by an oxidative reaction in an overall reducing environment such as the cytosol (*79*). Indeed, selenite and membrane-permeable disulfides release zinc in cardiomyocytes and in cultured neurons, respectively (*80*, *81*). When cells are treated with hydrogen peroxide, MT is the source of the released zinc (*82*). Further, nitric oxide (NO) or its adduct with thiols releases zinc from MT (*83*) and from intracellular stores in various cell types (*84*, *85*). Specificity in zinc release reactions is embodied in consensus sequence motifs for catalytic *S*-nitrosylation in both domains of MT-III. Only this isoform reacts rapidly with *S*-nitrosothiols by transnitrosation, i.e., a process of direct NO transfer without the intermediate formation of free NO (*86*). A relation between nitric oxide signaling and cellular zinc distribution is increasingly receiving attention (*87*, *88*). Regardless of whether an enzymatic reaction, a short-lived reactive species, or multiple pathways release zinc, the important point is that the molecular properties of the Zn−S bond couple zinc and redox metabolism in a manner that allows oxidants to release zinc and reductants to promote zinc binding. Thus, the cellular redox state profoundly affects the coordination of

the redox-inert zinc ion, its cellular availability, and its functions.

Thionein and Zinc Binding

All high-resolution structural work has been performed on MT preparations containing a full complement of seven metal atoms, and almost all experiments were interpreted on the basis of the presumption that the holoprotein is the only functional species. Hence, whether significant amounts of the apoprotein thionein (T) exist in cells is critical for the understanding of functions of this protein. While evidence for the existence of T in some neoplastic cell lines has been adduced (*89*), a differential fluorescence labeling assay of tissue homogenates has demonstrated that in the liver and kidney of the rat there is almost as much T as MT under normal physiological conditions (*90*). Different tissues have different MT/T ratios, suggesting that changes in this ratio affect the availability of zinc in the cell (*90*). The very existence of T in some tissues demonstrates that zinc is not readily available to saturate the binding sites of T. In fact, the sulfhydryl groups of T represent a significant chelating capacity that keeps the free zinc concentration in many cell types very low, at least in the absence of other mechanisms controlling its availability. Binding of zinc to T is highly cooperative in the formation of both clusters in MT-1 and -2 (*91*). This cooperativity is critical as it allows binding in an all-or-none fashion and thus the coexistence of MT and T.

Control of Zinc Transfer Reactions and Availability of Cellular Zinc

Compared to the rates of reactions between apoenzymes and free zinc ions, MT does not accelerate the incorporation of zinc into proteins as would be expected if MT were to have "insertase" activity (*92*−*94*). Instead, MT is a reservoir in which zinc is tightly bound and from which zinc can be made available by specific kinetic control mechanisms (*20*, *74*, *95*). It has been estimated, for example, that it would take hours to reconstitute zinc-requiring enzymes with picomolar concentrations of free zinc ions (*96*). Therefore, kinetic control of the availability of cellular zinc is necessary to provide zinc for the catalytic, structural, and regulatory functions of zinc in proteins on a biological time scale.

In the absence of any redox agents, MT does not release all of its seven zinc ions to an apoenzyme. It contains at least one that is more prone to be released than the others (*92*, *97*). Zinc transfer experiments with peptides comprising the individual domains of MT have established that this zinc ion originates from the three-zinc cluster in the N-terminal β-domain (*98*). Transfer of zinc from MT to the apo forms of zinc-requiring enzymes is modulated further by interactions between MT and ATP (*95*, *99*) or glutathione (GSH) (*100*). GSH inhibits the release of zinc in the absence of GSSG but enhances it in its presence in a manner that depends on the GSH/GSSG redox ratio (*92*). Binary complexes of MT and either GSH or GSSG have been detected by mass spectrometry (*101*). Collisionally induced dissociation of these complexes results in the removal of one zinc ion from MT by GSSG, but none is removed by GSH. These experiments demonstrate how changes in the cellular GSH/GSSG redox potential could be employed for metal transfer from MT to the apo forms of zinc proteins, whose zinc stability constants are inherently lower (Table 1).

On the other hand, the reduced thiols of its 20 cysteines make T a very efficient endogenous chelating agent and zinc acceptor that can rapidly reactivate enzymes inhibited by zinc, even when zinc ions are bound at the inhibitory sites with nanomolar binding constants (*59*, *61*, *62*). Further, T suppresses the binding of transcription factor IIIA to 5S RNA and the 5S RNA gene and the binding of Sp1 to DNA, suggesting that it also modulates the actions of zinc finger proteins (*102*, *103*). Since the chelating capacity of T depends on how many of its 20 cysteines are reduced, changes in the cellular redox potential will affect its capacity to inhibit or activate enzymes or other zinc-dependent cellular processes. Once MT is oxidized completely, zinc transfer is essentially irreversible. Reduction of oxidized T to reduced T, which can be brought about by GSH in the presence of a selenium catalyst (*79*), restores the full capacity of the protein to bind zinc and reverses the direction of zinc transfer. Thus, zinc transfer can occur in either direction, from proteins to T and from MT to proteins, depending on the redox state and the MT/T ratio, and additional agents provide kinetic pathways for zinc transfer (*104*).

Ever since the discovery of MT (*64*), its function has been shrouded in uncertainty (*105*). The chemical properties of MT and T as the zinc donor and acceptor, respectively, and their redox properties provide a general framework for their physiological function(s). The translocation of MT/T to the intermembrane space of liver mitochondria, where it releases or binds zinc and modulates respiration (*106*), export of the protein into the extracellular space (*107*, *108*), and its translocation into the nucleus (*109*, *110*) all suggest a general role of MT/T in directing the flow of zinc across some cellular membranes and controlling the availability of zinc in various compartments. However, the observation that mice with genetic knockouts at either MT-1 and -2 loci or the MT-3 locus are viable has not yet contributed to defining its physiological function (*111*, *112*). The knockout mice exhibit many phenotypic changes when tests with regard to zinc or redox metabolism are performed. Such changes are in agreement with a role of MT in retaining zinc under zinc deficiency and in buffering excess zinc (*113*, *114*). Clearly, the phenotype has to undergo more rigorous testing, including additional phenotypes where all of the MT genes have been interrupted simultaneously.

A discussion of MT would be incomplete without a short reference to the MT-III isoform. MT-III has been isolated from brain tissue on the basis of an assay that determines the growth and survival promoting effects of Alzheimer's disease extracts on rat embryonic cortical neurons. Because of this biological activity, the protein was named growth inhibitory factor (*115*). The activity was found to be specific for this isoform and has been mapped to an N-terminal β-domain C-P-C-P motif, which is characteristic for this isoform (*116*). The molecular basis for the function of MT-III as a growth inhibitory factor is unknown.

Zn−*S Bonds in Proteins that Sense the Availability of Cellular Zinc*

Zinc-sensing transcription factors participate in the induction of T. MTF-1 (metal response element-binding transcription factor-1) has six zinc fingers (*117*, *118*) and is essential

for basal as well as metal-induced expression of MT and other proteins (*119*). Not only is the Zn−S interaction critical for the function of its zinc fingers, but another zinc tetrathiolate site is required for zinc-induced transcription in most vertebrates (*120*). A transcription factor that is active in zinc-limited yeast cells utilizes at least two of its seven zinc fingers for sensing zinc (*121*). A homologous protein that senses low levels of zinc in vertebrates has not yet been identified. Thus, zinc sensing is yet another manifestation of a specifically biological function that has evolved on the basis of Zn−S chemistry.

Perspective

The Zn−S interaction is a partnership in which sulfur imparts mobility on zinc and zinc modulates the chemical properties of sulfur. In this sense, its functional potential adds up to more than the sum of its parts. The inconspicuous nature of the zinc ion with only one valence state in proteins and the apparent simplicity of tetrahedral coordination in zinc finger proteins somewhat belies the functional significance of this essential element. The convergence of zinc and redox metabolism in Zn−S coordination environments establishes multiple pathways for the dynamic regulation of protein structure and function, and zinc metabolism. The fact that zinc proteins can be redox proteins comes at a price, however. Redox stress can damage the Zn−S sites and release zinc. Increases in the availability of cellular zinc can induce apoptosis and are a major, and perhaps critical, factor in the progression of many diseases (*122*). The topics discussed in this article raise expectations for major developments in several disciplines of research. Identification of both the signaling pathways that control zinc redistribution and the proteins that are regulated by zinc will likely demonstrate that the redox-inert zinc ion stands side by side with magnesium and calcium with regard to its significance in cellular signaling.

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