Chemistry & Biology, Vol. 9, 673–677, June, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S1074-5521(02)00156-4

Metallochaperones: Bind and Deliver

Minireview

Amy C. Rosenzweig[1]
Department of Biochemistry, Molecular Biology, and Cell Biology and
Department of Chemistry
Northwestern University
Evanston, Illinois, 60208

Summary

Metallochaperones deliver metal ions directly to target proteins via specific protein-protein interactions. Recent research has led to a molecular picture of how some metallochaperones bind metal ions, recognize their partner proteins, and accomplish metal ion transfer.

One-third of all proteins require metal cofactors for function. Metalloproteins play key roles in many biological processes, including respiration, photosynthesis, nerve transmission, and defense against toxic agents. In metalloenzymes, metal cofactors catalyze fundamental chemical reactions such as the conversions of oxygen to water, nitrogen to ammonia, and methane to methanol. The complexity of metalloenzyme active sites ranges from one metal ion with several protein ligands to polynuclear clusters including protein side chains, modified protein side chains, and exogenous ligands such as water or sulfide. Assembly of these metal centers is a complicated process, involving many accessory or helper proteins [1]. One recently identified class of accessory proteins, called metallochaperones, binds metal ions and delivers them directly to target enzymes via protein-protein interactions. How metallochaperones bind metal ions, recognize specific partner proteins, and facilitate metal transfer are important questions in bioinorganic chemistry. In the past few years, there has been significant progress toward understanding the structure, coordination chemistry, and molecular mechanisms of metallochaperones.

Overview of Copper Chaperones

The current picture of metallochaperone-mediated cofactor assembly derives primarily from studies of copper chaperones. The known copper chaperones are divided into three functional groups: the Atx1-like chaperones, the copper chaperones for superoxide dismutase, and the copper chaperones for cytochrome *c* oxidase (Figure 1). It is not clear how any of these chaperones initially acquire copper, but one possibility is through direct interactions with the Ctr family of membrane transporters. Atx1 is a cytosolic yeast copper chaperone that delivers copper to the transport ATPase Ccc2 in the trans-Golgi network. Ccc2 then translocates the copper into vesicles, where it is loaded into the multicopper oxidase Fet3. The human homolog of Atx1, Atox1, delivers copper to the Menkes and Wilson disease ATPases for ultimate incorporation into ceruloplasmin. A bacterial homolog of Atx1, called CopZ, has also been identified. The copper chaperones for superoxide dismutase, known as the CCS proteins, donate copper to the eukaryotic antioxidant enzyme copper,zinc superoxide dismutase (SOD1). Studies in yeast cells suggest that CCS is required because intracellular free copper concentrations are minimal [2]. Two proteins, Cox17 and Sco1, play a role in the assembly of cytochrome *c* oxidase. Cox17 is proposed to deliver copper to the inner mitochondrial membrane protein Sco1, which may then transfer the metal ion to the cytochrome *c* oxidase subunit 2 (Cox2) Cu_A site. Unlike the Atx1 and CCS chaperones, direct metal transfer between the cytochrome *c* oxidase chaperones and their putative partner proteins has not yet been demonstrated. All three types of copper chaperones bind Cu(I) with multiple cysteine ligands.

Atx1-Like Copper Chaperones

The Atx1 proteins and their target ATPases are characterized by a conserved CXXC metal binding motif (single-letter amino acid code where C is cysteine and X is any amino acid). The chaperones contain one such motif, whereas the target ATPases contain up to six repeating N-terminal domains with this motif. Crystal and solution structures of Atx1, Atox1, CopZ, and single domains of the Menkes protein and Ccc2 reveal a conserved $\beta\alpha\beta\beta\alpha\beta$ fold with the CXXC motif housed on a solvent-exposed loop [3]. In the X-ray structure of the Hg(II) form of Atx1, the Hg(II) ion is coordinated by the two conserved cysteines in a linear fashion ([3]; Figure 2A). By contrast, extended X-ray absorption fine-structure (EXAFS) data on the Cu(I) form of Atx1 indicate the presence of three sulfur ligands [4]. Two of these ligands probably derive from the CXXC motif, and the third could either be an exogenous thiol or a cysteine from a second Atx1 molecule. Interestingly, recent EXAFS data on the Cu(I) form of CopZ were also best fit with three sulfur ligands [5]. In the structures of the Cu(I), Hg(II), and Cd(II) forms of Atox1, CXXC motifs from two adjacent molecules coordinate the metal ions in either three- or four-coordinate geometries [6].

Structural and mutagenesis data have led to the proposal that protein-protein recognition involves positively charged residues on the chaperones and negatively charged residues on the target proteins [3, 5]. Recent NMR chemical-shift mapping studies of the interaction between Atx1 and Ccc2 are consistent with this model [7]. Metal delivery is predicted to involve a series of two- and three-coordinate intermediates in which the two CXXC motifs in the docked complex undergo ligand exchange reactions ([4]; Figure 1). The Atox1 structures demonstrate that the CXXC motifs from two separate domains can indeed interact and provide molecular models for the proposed metal exchange intermediates [6]. It is still unclear, however, whether Atx1 and Atox1 interact with all or just selected CXXC-containing domains from their respective target ATPases. The target domains are predicted to have different electrostatic

[1]Correspondence: amyr@northwestern.edu

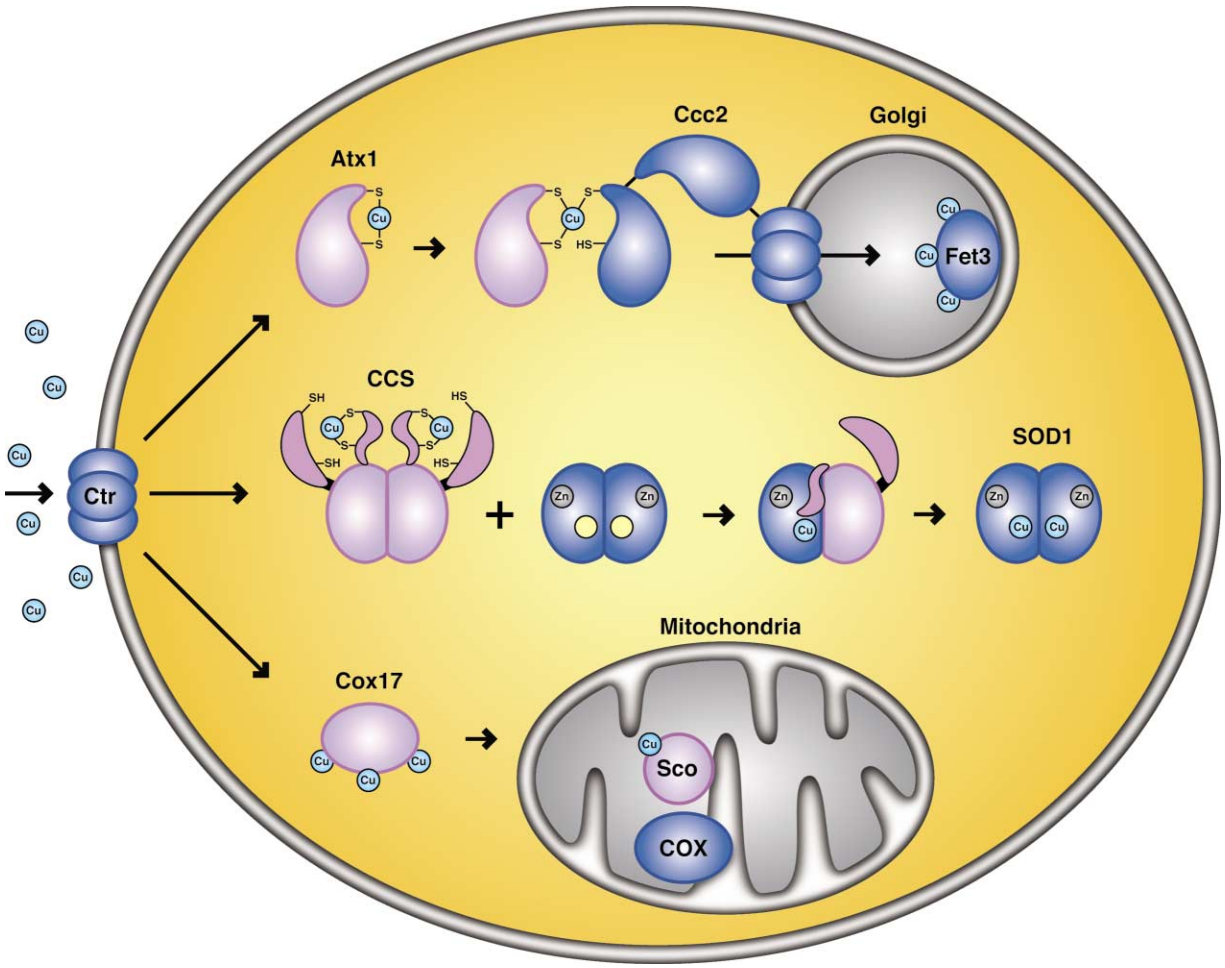

Figure 1. Copper Chaperone Pathways in Yeast

All three pathways are conserved in humans and other eukaryotes. Copper enters the cell through the Ctr membrane transporters. Copper delivery from Atx1 to the N-terminal domain of Ccc2 is proposed to occur by thiol exchange reactions and the formation of a three-coordinate intermediate. Copper delivery from CCS to SOD1 is proposed to occur via formation of a heterodimeric intermediate. The molecular details of copper delivery to COX have not been resolved.

surface characteristics, which would impact chaperone recognition. It is also not known how copper is inserted into Fet3 and ceruloplasmin once it is successfully translocated into the lumen of the Golgi.

Copper Chaperones for Superoxide Dismutase

The CCS chaperones are homodimers comprising three domains per monomer (Figure 1). The N-terminal domain (domain I) contains a CXXC sequence motif and structurally resembles the Atx1-like chaperones and their target domains. The middle domain (domain II) is structurally similar to SOD1, but lacks residues crucial for SOD1 activity. The CCS homodimer interface is formed exclusively by residues from domain II, and is very similar to the SOD1 homodimer interface [3]. The C-terminal 30 residues (domain III), which are required for metal transfer activity, contain a conserved CXC sequence motif and form an extended loop structure [8]. Although a structure of CCS with metal bound is not available, spectroscopic data suggest that the cysteines from both the domain I CXXC motif and the domain III CXC motif form the metal binding site [9].

The crystal structure of a complex between CCS and a mutant SOD1 that does not bind copper reveals a heterodimer comprising one monomer of each protein (Figure 3). The heterodimer interface is very similar to the CCS and SOD1 homodimer interfaces [8]. In the complex, the domain I CXXC motif is located ~35 Å from the SOD1 copper binding site, suggesting that a direct role in metal transfer is unlikely. This finding is consistent with the observations that domain I is not essential in vivo and that the CXXC motif is not conserved in all CCS homologs [8]. By contrast, the domain III CXC motif appears poised to deliver metal ions to the SOD1 active site. Although the details of metal insertion are still unclear, the structure suggests an intermediate involving cysteine ligands from the CXC motif and histidine ligands from the SOD1 copper site is possible. The oxidation state of the copper ion in this putative

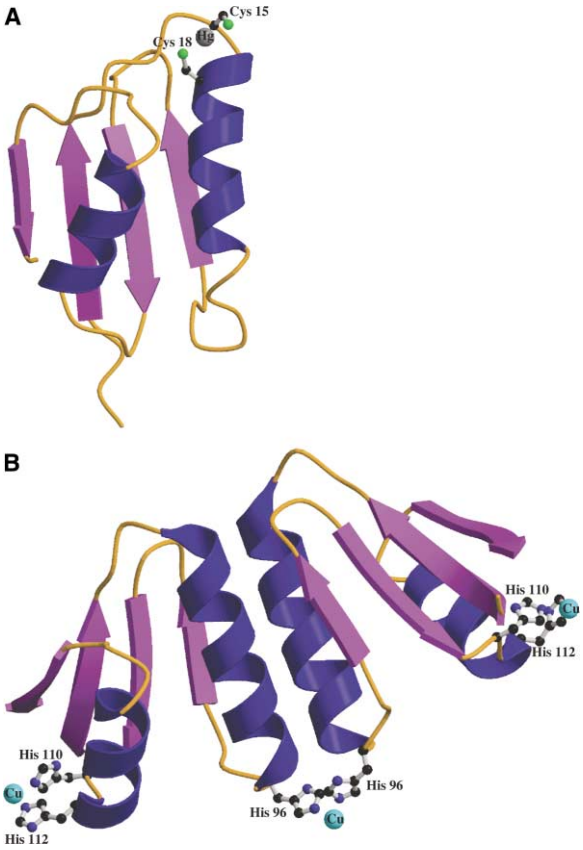

Figure 2. Structural Comparison of the Atx1 Copper Chaperone and the UreE Nickel Chaperone

Helices are shown in dark blue, β strands are shown in purple, and loop regions are shown in yellow.

(A) The Hg(II) form of Atx1 (PDB accession code 1CC8). The Hg(II) ion is coordinated by Cys 15 and Cys 18 from the conserved CXXC motif.

(B) The C-terminal domains of the Cu(II) form of the UreE dimer (PDB accession code 1GMW). Two copper ions are coordinated by His 110 and His 112 from each monomer, and a third copper ion is coordinated by the two His 96 residues at the dimer interface. Each site also includes several solvent ligands. The N-terminal domains (not shown) are not involved in dimerization or metal binding.

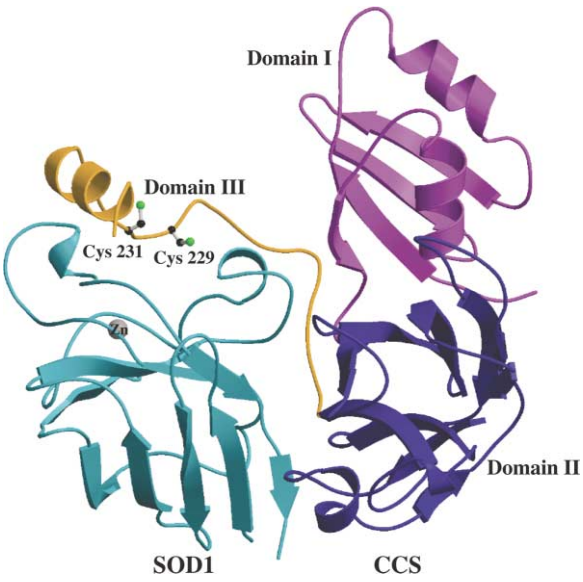

Figure 3. Heterodimeric Complex between CCS and SOD1

SOD1 is shown in light blue, and domains I, II, and III of CCS are shown in purple, dark blue, and yellow, respectively. The two cysteines in the CCS domain III CXC motif, Cys 229 and Cys 231, are well positioned to deliver copper ions to the SOD1 active site, which contains a single zinc ion but lacks copper because one histidine ligand has been mutated to phenylalanine to stabilize the complex (PDB accession code 1JK9).

intermediate remains an open question. Notably, the heterodimer structure also reveals an unexpected disulfide bond between CCS domain III and SOD1. Further studies are required to discern the exact role, if any, of disulfide formation in the metal transfer mechanism.

Copper Chaperones for Cytochrome c Oxidase

The chaperones for cytochrome *c* oxidase have not yet been structurally characterized, but mutagenesis and spectroscopic studies indicate that both Cox17 and Sco1 bind Cu(I) with cysteine ligands. Cox17 binds three copper ions by using cysteines in a conserved CCXC motif, in which all three cysteines are required to produce active cytochrome *c* oxidase. According to optical, luminescence, and X-ray absorption spectroscopic data, the three copper ions are arranged in a polynuclear cluster. Determining the nuclearity of this cluster is difficult because Cox17 exists as both dimers and tetramers. The oligomerization state is proposed to play a role in metallochaperone function [10]. Sco1 binds one Cu(I) ion, and EXAFS data indicate the presence of one nitrogen and two sulfur ligands, which are derived from the two cysteines in a conserved CXXXC motif. Mutation of these cysteines and a conserved histidine abolish Sco1 copper binding and result in a nonfunctional cytochrome *c* oxidase [11].

Stable complexes between Cox 17 and Sco1 or between Sco1 and Cox2 are not formed [11], but interactions between Sco1 and Cox2 have been detected by affinity chromatography and immunoprecipitation experiments [12], suggesting that metal transfer via transient complex formation is possible. For Atx1 and CCS, structural similarities between chaperone and target protein play an important role in recognition and docking. Although the Sco1 CXXXC metal binding motif is conserved in Cox2 and provides the Cu_A cysteine ligands, structural similarity is not predicted from the sequences. Two additional issues further differentiate this system from the Atx1 and CCS pathways. First, the Cu_A site requires two copper ions, and Sco1 only binds one copper ion. Oligomerization might therefore be important for delivering multiple copper ions [11]. Second, Cox17 is localized to both the cytosol and the mitochondrial intermembrane space [13]. An oligomeric, copper bound Cox17 is likely to require unfolding for mitochondrial import, complicating the question of where and when Cox17 obtains copper.

Nickel Chaperones

Metallochaperones are also believed to deliver nickel ions to enzymes such as urease, hydrogenase, and CO dehydrogenase. For assembly of the urease dinuclear

nickel site, apo urease forms a complex with three proteins, UreD, UreF, and UreG, and is then activated by the addition of nickel, bicarbonate, GTP, and a putative metallochaperone called UreE [1]. UreE binds six Ni(II) ions, but a truncated protein that binds two Ni(II) ions is fully active, suggesting that just two sites are involved in delivery to urease. These two spectroscopically distinct sites are five or six coordinate with 2–4 histidine ligands [14]. Efforts to crystallize the Ni(II) form of the protein have been unsuccessful, but crystal structures of UreE substituted with Cu(II) and Zn(II) have been determined. The structure of the Cu(II)-containing protein from *Klebsiella aerogenes* reveals a dimer in which each monomer comprises two domains. The C-terminal metal binding domain structurally resembles the copper chaperone Atx1, but it binds metals in a completely different fashion (Figure 2B). Three Cu(II) binding sites are present, one at the dimer interface and one on the surface of each monomer. Each Cu(II) ion is coordinated by two histidine residues and several water molecules. The site at the dimer interface is proposed to deliver Ni(II) ions, whereas the other two sites are postulated to serve as auxiliary Ni(II) reservoirs [15]. The dimer interface site is also present in the structure of the Zn(II)-containing protein from *Bacillus pasteurii*. In these crystals, the Zn(II) ion can be replaced with Ni(II) [16].

The UreE structures also provide insight into possible mechanisms of metal ion delivery. The N-terminal domain is structurally similar to molecular chaperones and possesses an extended hydrophobic region that might facilitate docking with urease and the other assembly proteins [15, 16]. Urease is a trimer of trimers, but a lack of structural similarity between UreE and any of the urease polypeptides suggests that a subunit exchange mechanism like that employed by CCS is unlikely. The details of metal transfer are further expected to differ from the mechanisms proposed for Atx1 and CCS because two Ni(II) ions must be transferred between histidine ligands rather than one metal ion being transferred either by thiol exchange or from cysteine to histidine coordination environments. Future studies of this system are likely to yield new paradigms of metallochaperone function.

Other Metallochaperones

The recent advances in understanding copper and nickel chaperones underscore the possibility that additional metallochaperones exist for biologically relevant metal ions. Although genetic and biochemical data indicate that accessory proteins are required for assembling many other metal cofactors, a metallochaperone function has not been assigned definitively to any of these proteins. For copper, several proteins have been implicated in assembly of the nitrous oxide reductase catalytic Cu_Z cluster [17]. For iron, the bacterial IscU and IscA proteins, which are conserved in eukaryotes [18], serve as scaffolds for the formation of [2Fe-2S] and [4Fe-4S] clusters [19]. Synthesis of the nitrogenase iron-molybdenum cofactor (FeMoco) also involves a number of helper proteins. It is yet not known how clusters assembled on any of these proteins are delivered to the appropriate target enzymes. A recent crystal structure of FeMoco-deficient nitrogenase reveals the pathway for cofactor delivery, however. Conformational changes result in the presence of a positively charged funnel that could accommodate insertion of the negatively charged cofactor [20]. A metallochaperone that would dock near this funnel has not yet been identified.

Conclusions

The current understanding of metallochaperone structure and function reveals several general themes. Metallochaperones bind metal ions in exposed sites readily accessible for metal ion exchange. Docking between metallochaperones and their partner proteins is facilitated by specific protein-protein interactions. In the Atx1 and CCS systems, structural similarities between chaperone and target protein promote complex formation and metal ion transfer. This close resemblance may not apply to other metallochaperones, including Cox17, Sco1, UreE, and the yet-to-be-identified FeMoco chaperone. Nevertheless, all metallochaperones probably possess some type of docking surface tuned to recognize particular target proteins. Numerous gene products involved in assembly of other metal cofactors have been identified and may function as metallochaperones. As more biochemical, spectroscopic, and structural data become available, additional mechanisms of metallochaperone-mediated metal cofactor assembly are likely to emerge.

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