Published in final edited form as:

Nat Chem Biol. 2008 February ; 4(2): 107–109. doi:10.1038/nchembio.2007.57.

Cu(I) recognition via cation- π and methionine interactions in CusF

Yi Xue^{1,6}, Anna V Davis^{1,6}, Gurusamy Balakrishnan², Jay P Stasser³, Benjamin M Staehlin¹, Pamela Focia⁴, Thomas G Spiro², James E Penner-Hahn³, and Thomas V O'Halloran^{1,5}

¹Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, USA.

²Department of Chemistry, University of Washington, Box 351700, Seattle, Washington 98195, USA.

³Department of Chemistry and Biophysics Program, University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109, USA.

⁴Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, Illinois 60611, USA.

⁵Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2205 Tech Drive, Evanston, Illinois 60208, USA.

Abstract

Methionine-rich motifs have an important role in copper trafficking factors, including the CusF protein. Here we show that CusF uses a new metal recognition site wherein Cu(I) is tetragonally displaced from a Met₂His ligand plane toward a conserved tryptophan. Spectroscopic studies demonstrate that both thioether ligation and strong cation- π interactions with tryptophan stabilize metal binding. This novel active site chemistry affords mechanisms for control of adventitious metal redox and substitution chemistry.

In recent years, metal-specific gene regulatory and cation-trafficking proteins have been isolated and demonstrate metal binding motifs with unprecedented coordination chemistry tailored to their function¹. For example, the CXXC sequence, found in cytosolic copper chaperones and trafficking proteins, provides for facile Cu(I) transfer via low-coordination-number anionic intermediates^{1,2}. Extracellular or periplasmic copper trafficking domains, however, function in environments that are more oxidizing than the cytosol and frequently have less well understood methionine-rich sequences^{3–8}. The *cus* operon encodes a bacterial copper homeostasis system with several methionine-motif proteins^{5,9,10}, including the periplasmic protein CusF, which is thought to serve as copper chaperone or regulator^{5,6}. CusF binds Cu(I) *in vitro*¹¹, and a methionine-rich Cu(I) site was proposed⁶ based on an apo-CusF structure and NMR chemical shift data. Here we show that metal recognition in CusF involves a strong interaction between a cationic Cu(I)-thioether/imidazole center and the aromatic ring of tryptophan. To our knowledge, such cation- π interactions have not been reported for transition metal receptors or metalloenzyme active sites.

Correspondence should be addressed to T.V.O. (t-ohalloran@northwestern.edu).

⁶These authors contributed equally to this work.

Published online at <http://www.nature.com/naturechemicalbiology>

Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions>

Anaerobic titration of Cu(I) into solutions of the CusF protein produces a pronounced red shift of the Trp44 absorption at 280 nm and a concomitant, and nearly complete, quenching of the tryptophan emission at 350 nm as 1:1 Cu(I):protein stoichiometry is achieved (Supplementary Fig. 1 and Supplementary Methods online). The extent of the metal-induced quenching in this case is particularly notable and is consistent with close proximity of the Cu(I) ion to the indole side chain. We also generated a truncated form of the protein, TrCusF (CusF₈₋₈₈), which excludes N-terminal histidine residues previously implicated in CusF Cu(II) interactions. The spectral properties of Cu(I)-TrCusF are identical to those of the full-length protein (Supplementary Fig. 1), which indicates that the N-terminal residues do not affect CusF Cu(I) coordination.

The crystal structures of Cu(I)-CusF and Ag(I)-CusF (Supplementary Fig. 2 and Supplementary Table 1 online) provide more insight into this unique copper-binding protein. Both metal-bound forms of CusF are defined by a small barrel with two orthogonally packed β -sheets (an OB-fold) and are almost identical to the apo form of CusF (ref. 6) (Fig. 1a). In both Cu(I)-CusF and Ag(I)-CusF structures, the metal ion is located at the top of the β -barrel at the base of loop 2 and is coordinated to His36, Met47 and Met49. The coordination geometry in both structures is close to trigonal planar, with the copper and silver ions displaced by 0.5 Å from the plane defined by the coordinating atoms of His36, Met47 and Met49 toward Trp44 (Fig. 1b,c). The trigonal planar coordination geometry is prevalent for Cu(I) metal centers, and the bond distances agree well with the established distances from small-molecule analogs in the Cambridge Structure Database.

Comparison of the metal binding region (residues 36–49) of the apo-CusF and Cu(I)-CusF structures shows that upon metal binding, the Met47 and Met49 side chains reorient to make favorable bonds between the thioether sulfur and the metal center, whereas His36 and Trp44 shift only slightly (Fig. 1c). Space-filling representations reveal that the methionine sulfurs are substantially more exposed to solvent in the apo structure, perhaps facilitating metal capture (Fig. 1d). Upon metal binding, the thioether side chains rotate to tuck the metal ion into a coordination pocket pressed up against Trp44, with His36 at the base of this pocket. This site differs from those reported for methionine-rich coordination sites in the periplasmic CopC protein, in which one Cu(I) coordinates via three methionines and a histidine (ref. 3), or two Cu(I) ions each bind to four methionines across a dimer interface⁸. In contrast, the Cu(I)-CusF and Ag(I)-CusF described here are discrete mononuclear 1:1 protein-metal complexes with protected trigonal Met₂His copper coordination still poised at the protein surface.

In both the copper and silver structures, Trp44 is surprisingly close to the metal center: the C ϵ 3 and C ζ 3 atoms of the indole ring are 2.67 Å and 2.86 Å from Cu(I), and 2.99 Å and 3.29 Å from Ag(I), respectively. Comparison of these interactions with small-molecule η^2 Cu(I)-arene and Ag(I)-arene complexes in the Cambridge Structure Database indicates that the Cu(I)-CusF and Ag(I)-CusF metal-Trp distances are at the long end of characterized η^2 arene complexes. It is of note that in about a quarter of the CusF sequences in the databases, the conserved tryptophan is substituted by the metal-binding residue methionine.

X-ray absorption spectroscopy on Cu(I)-TrCusF samples confirmed the Cu(I) oxidation state and provided higher resolution metal-ligand distances (Supplementary Fig. 3 online). The X-ray absorption near-edge structure (XANES) edge energy of 8,984.5 eV and spectral shape demonstrate trigonally coordinated Cu(I), and the extended X-ray absorption fine structure (EXAFS) corroborates Met₂His coordination, with the best fit defining a Cu-N shell with one nitrogen at 1.97 Å from the copper and a Cu-S shell with two sulfurs at 2.26 Å. These distances were used as restraints in final refinement of the crystallographic data. Fits using a rigid imidazole to predict the imidazole multiple scattering consistently show a small (~14%) improvement when a shell of two carbons is added. This apparent Cu-C distance (2.73 Å, σ^2

$= 8.8 \times 10^{-3} \text{ \AA}^2$) agrees with the crystallographic Cu-Trp carbon distances; however, the improvement is too small for unambiguous identification of a Cu-C interaction from EXAFS alone.

To further probe a possible metal-tryptophan interaction, CusF complexes were studied by UV resonance Raman (UVR) spectroscopy, which is very sensitive to changes in the local environment of tryptophan residues¹², including cation- π interactions¹³. The interaction of cations with the negatively charged electron distribution found in the center of aromatic side chains (especially tryptophan) is known to be a significant noncovalent force in biological systems¹⁴. Raman excitation wavelengths for apo-TrCusF and Cu(I)-TrCusF samples were selected from spectral regions in which the apo and holo proteins show the greatest difference in UV absorption; that is, at the negative (220 and 229 nm) and positive (238 and 244 nm) bands of the Cu(I)-TrCusF minus apo-TrCusF difference spectra (Supplementary Fig. 4 online). Raman spectra excited at the negative region of the absorption band showed decreases in tryptophan intensity (~47% for 220 nm and 23% for 229 nm) for Cu(I)-TrCusF relative to apo-TrCusF (Fig. 2). Excitation in the positive region showed tryptophan intensity increases of about 100% and 200% for 238 nm and 244 nm, respectively. In addition, modes specific to the tryptophan six-membered ring, W16, W13 and W7 ($1,345 \text{ cm}^{-1}$), undergo frequency shifts of -2, -4 and +3 respectively upon metal complex formation. A similar pattern of intensity changes and intensity inversion has been previously characterized for cation-tryptophan π interactions in both protein and small-molecule model systems¹³. It is notable that the magnitude of the intensity changes observed for CusF upon metal coordination is considerably greater than what has been previously reported for cation- π interactions and that these changes are accompanied by small tryptophan wavelength shifts. Though intensity changes might be anticipated for significant changes in the hydrophobic or hydrogen bonding environment of Trp44, we are confident in ruling out these possibilities based on analysis of other tryptophan UVR markers. Finally, Ag(I)-CusF and Cu(I)-CusF samples produced almost identical UVR spectra (Supplementary Fig. 4). Thus, the wavelength-dependent inversion of Raman intensities in addition to the reported tryptophan mode frequency shifts suggest a strong cation-tryptophan π interaction for both the Cu(I) and Ag(I) forms of the protein.

The close proximity of Trp44 is an unprecedented feature in metalloprotein chemistry, and we considered that it might be an organometallic complex with an η^2 -coordinated tryptophan. However, the crystallographically determined Cu-C distances are long compared with similar small-molecule Cu(I) species, and at present we have no spectroscopic support to address the degree of covalency of the Cu-C interactions. Therefore, given the data in hand, the simplest description of the Cu(I)-tryptophan site is a cation- π interaction. Examination of the Cation-Aromatic Database (CAD, <http://203.199.182.73/gnsmmg/databases/cad/>) demonstrates that the Cu(I)-arene interaction in CusF is exceptional in its Cu-C distances relative to protein structures reported so far (Supplementary Table 2 online). The extent to which Cu(I)-arene interactions play a role in other metalloproteins remains to be seen. The structure of a truncated form of CusF bound to silver ion, published while this paper was under revision, shows a Met₂His coordination to Ag(I) with a nearby tryptophan but provides no evidence for cation- π interactions¹⁵.

Molecular recognition in this methionine-rich site is quite different from the Cu(I) coordination chemistry in other copper chaperones and metalloregulatory proteins. For example, this methionine-rich Cu(I) site has no negatively charged ligands, and Cu(I) binding leads to a cationic coordination site—in contrast to CXXC Cu(I) complexes, which confer an overall anionic character to the coordination site. These local electrostatic features are frequently counterbalanced by other protein moieties, such as the ammonium cation of Lys65 that interacts with the anionic Cu(Cys)₂ center in Atx1 (refs. 1-2), or the Trp44 interaction with the cationic Cu(I)-Met₂His center in CusF described here. Given that a thioether moiety of methionine is

more stable than a thiol with respect to physiological oxidation, methionine ligation may provide a preferred strategy over cysteine ligation for handling redox-active Cu(I) in oxidizing environments such as the periplasm. Preliminary studies of the tryptophan-to-methionine mutant (W44M TrCusF) indicate that replacement of the indole side chain with a thioether leads to an increase of the Cu(I) affinity of the protein by approximately two orders of magnitude, as might be anticipated by the addition of a coordinate covalent bond to the Cu(I) sphere (Supplementary Fig. 5 online). This variation between a weaker cation- π interaction versus a coordinate covalent bond affords a precise thermodynamic tuning of metal-transfer sites and thus has functional implications in a number of metal trafficking pathways. It is possible that tryptophan-for-methionine substitution might expand the defined methionine-rich motifs. The CusF active site chemistry affords a distinct class of copper receptor proteins with a means to both control metal exchange and prevent adventitious redox reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Research was supported by US National Institutes of Health (NIH) grants GM 38784 (to T.V.O.), GM 38047 (to J.E.P.-H.) and GM 25158 (to T.G.S.) and by National Research Service Award Postdoctoral Training Grant GM 071129 (to A.V.D.). Support from the R.H. Lurie Comprehensive Cancer Center (P30 CA060553) of Northwestern University to the Structural Biology Facility and Keck Biophysics Facility is acknowledged. Portions of this work were performed at the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT, beamline 17-ID-B) at the Advanced Photon Source (APS) and at the Stanford Synchrotron Radiation Laboratory (SSRL). Use of the APS is supported by the US Department of Energy (DOE). SSRL is operated by the DOE, Office of Basic Energy Sciences. The SSRL Biotechnology Program is supported by the NIH and the DOE.

References

1. Finney LA, O'Halloran TV. *Science* 2003;300:931–936. [PubMed: 12738850]
2. Arnesano F, et al. *Genome Res* 2002;12:255–271. [PubMed: 11827945]
3. Arnesano F, Banci L, Bertini I, Mangani S, Thompsett AR. *Proc. Natl. Acad. Sci. USA* 2003;100:3814–3819. [PubMed: 12651950]
4. Peariso K, Huffman DL, Penner-Hahn JE, O'Halloran TV. *J. Am. Chem. Soc* 2003;125:342–343. [PubMed: 12517140]
5. Franke S, Grass G, Rensing C, Nies DH. *J. Bacteriol* 2003;185:3804–3812. [PubMed: 12813074]
6. Loftin IR, et al. *Biochemistry* 2005;44:10533–10540. [PubMed: 16060662]
7. Banci L, et al. *Proc. Natl. Acad. Sci. USA* 2005;102:3994–3999. [PubMed: 15753304]
8. Zhang L, Koay M, Mahert MJ, Xiao Z, Wedd AG. *J. Am. Chem. Soc* 2006;128:5834–5850. [PubMed: 16637653]
9. Outten FW, Huffman DL, Hale JA, O'Halloran TV. *J. Biol. Chem* 2001;276:30670–30677. [PubMed: 11399769]
10. Munson GP, Lam DL, Outten FW, O'Halloran TV. *J. Bacteriol* 2000;182:5864–5871. [PubMed: 11004187]
11. Kittleson JT, et al. *Biochemistry* 2006;45:11096–11102. [PubMed: 16964970]
12. Harada, I.; Takeuchi, H. *Spectroscopy of Biological Systems*. Clark, RJH.; Hester, RE., editors. New York: John Wiley & Sons; 1986. p. 113-175.
13. Okada A, Miura T, Takeuchi H. *Biochemistry* 2001;40:6053–6060. [PubMed: 11352741]
14. Ma JC, Dougherty DA. *Chem. Rev* 1997;97:1303–1324. [PubMed: 11851453]
15. Loftin IR, Franke S, Blackburn NJ, McEvoy MM. *Protein Sci* 2007;16:2287–2293. [PubMed: 17893365]

Figure 1.

Crystal structure of Cu(I)-CusF. Amino acids 13–87 are resolved in the structure, and the Cu (I) occupancy is 70%. **(a)** Overlay of the protein backbones of the apo-CusF (blue)⁶, Cu(I)-CusF (bronze) and Ag(I)-CusF (silver) structures. **(b)** Electron density map of the CusF copper complex, including His36, Met47, Met49 and Trp44. The distance from copper to the centroid of the indole six-member ring is 3.3 Å. **(c)** Overlay of the apo-CusF (blue) and Cu(I)-CusF structures. Met47 and Met49 clearly twist to accommodate copper complexation. **(d)** Space-filling representation of the apo metal site with the tryptophan carbons in green, Trp44 and His36 nitrogens in blue, and Met47 and Met49 sulfurs in dark yellow (top). In the Cu(I)-CusF structure, Met47 and Met49 reorient to tuck the metal ion into the binding pocket (bottom). The coordinates and structure factors have been deposited in the Protein Data Bank under codes 2VB2 (Cu(I)-CusF) and 2VB3 (Ag(I)-CusF).

Figure 2.

UVRR spectra. Shown are apo-TrCusF (black), Cu(I)-TrCusF (red), and the Cu(I)-CusF minus apo-CusF difference (blue) spectra excited at 220, 229, 238 and 244 nm, as labeled.

Wavenumber shifts in the Cu(I) spectra are indicated in red. Tryptophan (W) and phenylalanine (F) modes are labeled according to standard conventions. Four of the tryptophan modes discussed are represented at the right (adapted from ref. 12). W13 corresponds to in-plane C-H bending vibration of the benzene ring, with the low frequency (1,345 cm⁻¹) of the W7 doublet reported to originate from the combination of out-of-plane C-H vibrations (W25 + W33) of the benzene ring. W16 is the benzene ring breathing vibration.