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A Tale of Two Metals

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DOI 10.1016/j.chembiol.2010.01.004

A recent study by Thomas O'Halloran and his group at Northwestern University (Avarez et al. *Sciencexpress*, www.scienceexpress.org, 26 November 2009) has provided insights into how tetrathiomolybdate inhibits copper-trafficking proteins through metal cluster formation. The broader context of the work provides an attractive explanation of the long recognized but poorly understood antagonistic effects of Mo and Cu in living organisms.

The relationship between copper and molybdenum as trace nutrients goes back many decades and was of particular importance to sheep farmers in Scotland, where the high molybdenum content of the soils and peats in the Scottish highlands led to severe copper deficiency in sheep. Solutions to such questions usually emerge incrementally but in their recent *Science* paper, the O'Halloran laboratory presents the results of a structural study of the interaction of tetrathiomolybdate with a copper chaperone Atx1 that provides a comprehensive explanation for many of the observed interactions between copper and molybdenum in living cells.

When tetrathiomolybdate (TM) was reacted with copper-loaded Atx1, a purple complex was formed that gave rise to crystals diffracting to 2.3 Å. Solution of the structure revealed an unusual multinuclear cluster formed by the interaction of the thio groups of TM with each of three Atx1 Cu(I) centers (Figure 1). TM did not remove copper from the chaperone as the researchers had anticipated, but rather it recruited copper-loaded chaperone molecules into an oligomeric complex. Since the overall stoichiometry was $S_6Cu_4MoS_4$, the cluster must recruit one additional copper atom, perhaps from a fourth Atx1 molecule that is converted to its apo form. They showed further that TM efficiently inhibited the transfer of copper from Atx1 to its physiological partner, the Ccc2 ATPase. TM is a promising candidate for the treatment of Wilson disease, in which copper accumulates in liver, kidney ,and brain as the result of a defect in the efflux pump ATP7B. The authors were quick to recognize that the EXAFS spectra at both the Cu and Mo K edges closely resemble those published in 2009 by Zhang et al. on complexes formed in the livers of LP rats (an animal model of Wilson disease) who had been treated with TM. The new crystal structure

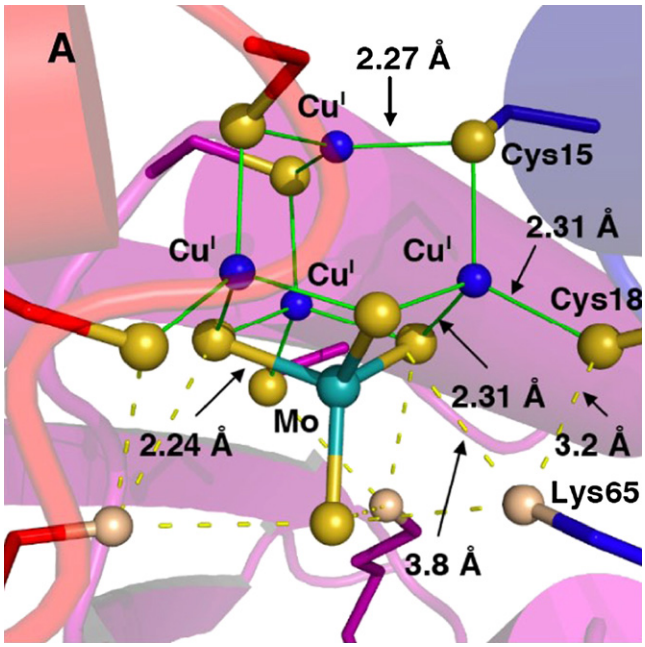

Figure 1. Structure of the Novel $S_6Cu_4MoS_4$ Cluster Formed between Tetrathiomolybdate and Three Molecules of the Copper Chaperone Atx1
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suggests a compelling explanation for the efficacy of TM in chelation therapy and the effects of Mo in general in causing cellular copper deficiency. Since one molecule of TM sequesters three chaperones and inactivates a fourth by copper removal, it would be a powerful cellular antagonist of copper transport and trafficking.

A remarkable feature of this cluster is its apparent ability to self assemble. It is also remarkable that the Atx1 trimer interface is almost entirely stabilized by the Cu-TM cluster. If we ignore the effects of hydration of the TM ion, the process is strongly entropically negative, implying a strong enthalpic driving force for cluster formation. This is not the only example in Cu(I) transport and regulatory systems where copper clusters appear to self-assemble into thermodynamically stable entities. Transcription factors such as ACE1 and MAC1 in yeast, CopY from *Enterococcus hirae*, the C terminus of yeast CTR1, and the cytochrome oxidase assembly factors Cox11, 17, and 19 all exhibit characteristic features of cuprous thiolate clusters. Almost no crystallographic information is available for any of these regulatory or assembly factors. They have, however, been well studied by EXAFS and show remarkable similarities, all containing a 2.7 A Cu-Cu interaction suggestive of some common core structure. A close look at the Atx1-TM cluster reveals the presence of two fused Cu_3S_3 hexameric rings, which are structural elements often found in inorganic cuprous thiolate model complexes that exhibit 2.7 Å Cu-Cu distances. Formation of these seemingly exceptionally stable Cu_3S_3 fragments may be a major factor in the self assembly of all these complexes.

Similarities also exist to the Cu(I) clusters proposed to form in the copper chaperone for superoxide dismutase (CCS), a three domain protein that binds copper at two cysteine-containing motifs,CXXC in domain 1 and CXC in domain 3. Using a variant in which the D1 CXXC site has been eliminated, our laboratory has identified multinuclear Cu(I) clusters that form at the interface of domains 2 and 3 of the human CCS. EXAFS studies on a selenocysteine-substituted derivative at both the Cu and Se edges suggested a Cu_4S_6 structure containing three fused Cu_3S_3 six-membered rings of alternating Cu and S (or Se) atoms (Barry et al., 2008). Although the CCS monomers are expected to bind only a single Cu atom at the D3 CXC motif, these clusters recruit additional thiols from domain 2 of the protein and additional Cu(I) ions to complete the Cu_4S_6 stoichiometry within a CCS dimer in a manner not dissimilar to the Atx1-TM complex chemistry. This suggests a possible general mechanism for the formation of Cu(I) clusters in which stable Cu_3S_3 hexameric rings are built on a scaffold that can be a protein site, an oligomeric interface, or a small molecule scaffold such as TM.

Further crystal structures will be necessary to confirm these ideas, but the present structure offers much insight into how these complex clusters are assembled. It also remains to be determined if the Cu-Mo cluster described in the present article, or the multinuclear clusters characterized spectroscopically in other systems persist under physiological condidtions within the cell. Notwithstanding these obvious caveats, it is clear that nature continues to exploit the beautiful and complex chemistry of transition metals to regulate metal ion homeostasis, providing new and exciting intersections between bioinorganic chemistry and cell biology.

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