Crystal Structure of the Copper Chaperone for the Menkes and Wilson Disease Proteins

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Introduction

The Menkes and Wilson disease proteins belong to a subfamily of P-type ATPases that functions in translocating heavy-metal ions across cell membranes. Mutations in the genes for the Menkes and Wilson ATPases lead to genetic disorders of copper metabolism. Both the Menkes and Wilson disease proteins are thought to acquire copper via a metallochaperone protein called Hah1 (or Atox1).[1] Hah1 is a member of an emerging class of proteins that deliver metal ion cofactors to specific target proteins.[2] The mechanism of copper transfer between Hah1 and the Menkes and Wilson disease proteins is unknown, but is proposed to involve conserved MT/HCXXC sequence motifs present in both the chaperone and in the N-terminal soluble domains of the target ATPases. According to biochemical and spectroscopic data, both chaperone and target proteins bind Cu(I), which is coordinated by the two cysteine residues in the MT/HCXXC motif.[2] Based on the spectroscopic studies of the yeast homolog of Hah1, Atx1, it has been proposed that facile copper transfer occurs by docking of the chaperone and target proteins with the MT/HCXXC metal binding sites in close proximity and the subsequent formation of two- and three-coordinate intermediates in which the metal ion is ligated simultaneously by both proteins.[3] To investigate the plausibility of this mechanism, we have determined the crystal structures of Hah1 in the presence of Cu(I) (CuHah1), Hg(II) (HgHah1), and Cd(II) (CdHah1).

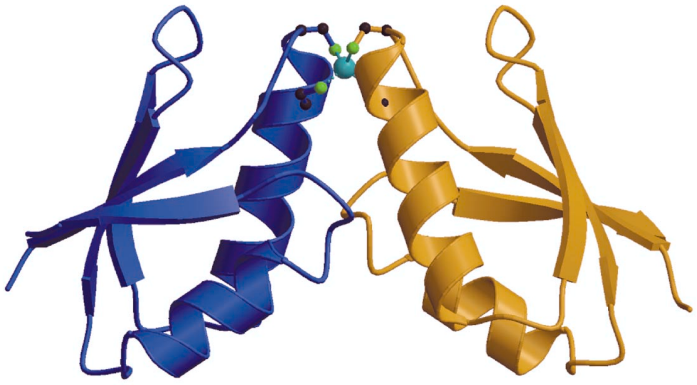

FIG. 1. Structure of CuHah1. Monomer A is shown in blue and monomer B is shown in yellow. The copper ion is shown as a cyan sphere, and the four cysteine residues in the two MT/HCXXC motifs are shown as ball-and-stick representations.

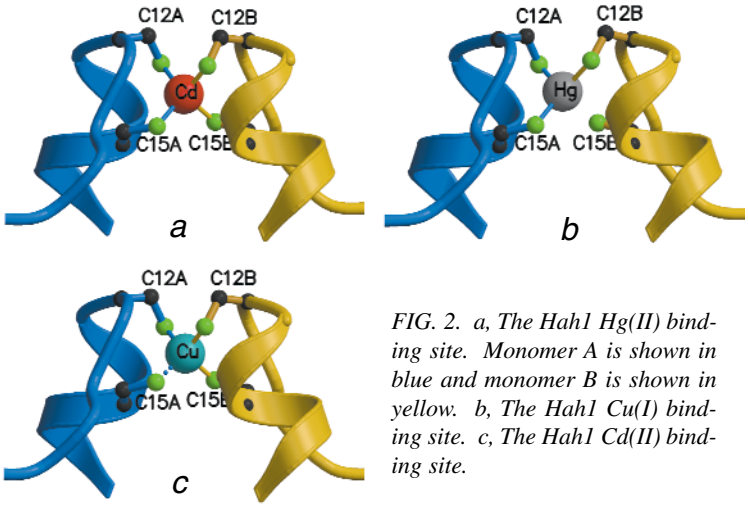

FIG. 2. a, The Hah1 Hg(II) binding site. Monomer A is shown in blue and monomer B is shown in yellow. b, The Hah1 Cu(I) binding site. c, The Hah1 Cd(II) binding site.

Methods and Materials

Hah1 was purified, reconstituted with metal ions, and crystallized as described.[4] The structure was solved by a combination of molecular replacement (MR) and multiple isomorphous replacement (MIR). Crystals for data collection were frozen in appropriate cryosolvents and transported to DND-CAT (APS) and to SSRL for data collection. Six different heavy atom derivatives were obtained by soaking the crystals in $UO_2(OAc)_2$, $K_2Pt(CN)_4$, EMTS, $Pb(OAc)_2$, $YbCl_3$, and $SmCl_3$. Data sets were processed with the programs DENZO and SCALEPACK,5 and MIR phasing was performed with CNS.6 The resultant electron density map was interpretable and led to the successful structure determinations of CuHah1, HgHah1, and CdHah1.

Results and Discussion

In the crystallographic asymmetric unit, two Hah1 molecules are linked by a metal ion (Fig. 1). Hah1 exhibits a βαββαβ fold, similar to that observed for its yeast homolog Atx1[7] as well as for one metal binding domain of its target protein, the Menkes ATPase.[8] Hah1 is the first of these domains to be structurally characterized with a metal ion coordinated by more than one protein molecule. The metal binding sites in the three structures are shown in Fig. 2. In CdHah1 (Fig. 2a), the Cd(II) ion is coordinated in a tetrahedral fashion by four cysteine residues, Cys A12 and Cys A15 from monomer A and Cys B12 and Cys B15 from monomer B. In HgHah1, the metal binding site exhibits distorted tetrahedral geometry with Hg···S distances of 2.3 Å for Cys A12, 2.5 Å for Cys B12, 2.5 Å for Cys A15, and 2.8 Å for Cys B15 (Fig. 2b). The first three distances are well within the expected range for three-coordinate Hg(II) whereas the 2.8 Å Hg···S distance for Cys B15 is too long for a covalent bond.

The CuHah1 structure is the only structure of a MT/HCXXC-containing metallochaperone or target domain with copper present in the metal binding site. The cysteine sulfur atoms form a distorted tetrahedral array with Cu···S distances of 2.3 Å for Cys A12, Cys B12, and Cys B15, and 2.4 Å for Cys A15 (Fig. 2c). The first three distances are very similar to those observed in both mononuclear and polynuclear three-coordinate Cu(I)-thiolate model complexes. The fourth Cu···S distance in CuHah1, 2.4 Å, is outside the primary bonding distance for known Cu(I) com-

plexes with either S, N, or O ligands. The metal binding sites in all three Hah1 structures are stabilized in part by an extended hydrogen bonding network. In each structure, the cysteine sulfur atom of Cys 12 is hydrogen bonded to the side chain oxygen atom of Thr 11 on the opposite protein molecule. Taken together, the structures provide models for three-coordinate intermediates in metal ion transfer and suggest a detailed molecular mechanism for protein recognition and metal ion exchange between MT/HCXXC-containing domains.[4]

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