Structure of the ATP Binding Domain from the 
Archaeoglobus fulgidus Cu⁺-ATPase

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The P-type ATPases translocate cations across membranes using the energy provided by ATP hydrolysis. CopA from Archaeoglobus fulgidus is a hyperthermophilic ATPase responsible for the cellular export of Cu⁺ and is a member of the heavy metal P₁B-type ATPase subfamily, which includes the related Wilson and Menkes diseases proteins. The Cu⁺-ATPases are distinct from their P-type counterparts in ion binding sequences, membrane topology, and the presence of cytoplasmic metal binding domains, suggesting that they employ alternate forms of regulation and novel mechanisms of ion transport. To gain insight into Cu⁺-ATPase function, the structure of the CopA ATP binding domain (ATPBD) was determined to 2.3 Å resolution. Similar to other P-type ATPases, the ATPBD includes nucleotide binding (N-domain) and phosphorylation (P-domain) domains. The ATPBD adopts a closed conformation similar to the nucleotide-bound forms of the Ca²⁺-ATPase. The CopA ATPBD is much smaller and more compact, however, revealing the minimal elements required for ATP binding, hydrolysis, and enzyme phosphorylation. Structural comparisons to the AMP-PNP-bound form of the Escherichia coli K⁺-transporting Kdp-ATPase and to the Wilson disease protein N-domain indicate that the five conserved N-domain residues found in P₁₉-type ATPases, but not in the other families, most likely participate in ATP binding. By contrast, the P-domain includes several residues conserved among all P-type ATPases. Finally, the CopA ATPBD structure provides a basis for understanding the likely structural and functional effects of various mutations that lead to Wilson and Menkes diseases.

The P-type ATPases encompass a large family of integral membrane proteins that couple ATP hydrolysis with the transport of cations across cell membranes (1, 2). Within this protein family, members of the P₁B subfamily specifically transport metal ions including Cu⁺, Ag⁺, or Zn²⁺/Cd²⁺/Pb²⁺ (3, 4). Widely distributed in nature, the P₁B-type ATPases confer heavy metal tolerance to microorganisms (5, 6) and are essential for the absorption, distribution, and bioaccumulation of metal micronutrients by multicellular eukaryotes (7, 8).

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tectural differences between heavy metal transporting and P₇₀-type ATPases, homology modeling based on the SERCA1 structures is of limited utility in understanding P₁₆₉-type ATPase function and transport mechanisms at the molecular level. We have determined the 2.3 Å resolution crystal structure of the ATPBD from CopA from the hyperthermophile *Archaeoglobus fulgidus,* a well characterized Cu²⁺-ATPase that contains all of the key elements present in eukaryotic Cu²⁺-ATPases (13, 15, 30). The CopA ATPBD structure, because of its small size and compactness, constitutes a minimal ATP binding-phosphorylation unit. As such, the structure allows for the identification of the central catalytic features independently of the secondary structural elements likely associated with trafficking, targeting, and regulatory functions.

**EXPERIMENTAL PROCEDURES**

**Cloning of the CopA ATPBD**—The CopA ATPBD (residues Lys⁴⁰⁷-Lys⁶⁷¹) was PCR-amplified from CopA cDNA (30) by using the primers 5'-GCCCTTGTTCTCTATGAAATGCGGAGGCT-C-CTGAA-3' and 5'-CGGGAAGGCTCTGGCTGTTTGGCAT- GTTCTCTGCT-3', which introduce a Bsa1 restriction site at the 5'- and 3'-ends of the PCR product. The PCR product was digested with Bsa1 and cloned into the pPR-IBA1 vector (IBA), which introduces an 8-amino-acid (WSHPQFEK) streptactin tag at the C terminus of the protein. The accuracy of the insert was confirmed by DNA sequencing. BL21 Star(DE3)pLysS E. coli cells carrying the plasmid pSJS1240 encoding for rare tRNAs (tRNA arg AGA/AGG and tRNA ile AUA) (31) were transformed with the pATPBD construct.

**Expression and Purification of the CopA ATPBD**—E. coli cells containing the pATPBD plasmid were grown at 37 °C in Luria-Bertani medium containing 100 mg/liter ampicillin and 30 mg/liter chloramphenicol. ATPBD expression was induced with 250 μM isopropyl β-D-thiogalactopyranoside at an A₆₀₀ of 0.7. The cells were harvested by centrifugation at 6000 × g for 5 min 3–4 h after induction. The pellet was frozen in liquid nitrogen and stored at −80 °C.

For purification, the frozen cells were resuspended in 100 mM Tris, pH 7.5, 150 mM NaCl (Buffer W), to which DNase, 5 mM MgCl₂, and 1 mM phenylmethylsulfonyl fluoride were added. The cell suspension was sonicated for 10 min (30-s pulses with a 30-s rest) and centrifuged at 163,000 × g for 1 h. The supernatant was then loaded onto a 10-ml streptactin column (IBA) conditioned in buffer W, and the ATPBD was purified according to the manufacturer’s protocol. SDS-PAGE of the eluted protein indicated it was greater than 95% pure. The ATPBD was exchanged into 20 mM MOPS, pH 7.0, 150 mM NaCl, and 5% glycerol solution and crystallized by the hanging drop method at room temperature (22–24 °C). Equal volumes of protein and a precipitant solution containing 100 mM sodium acetate, pH 4.6, 5–8% polyethylene glycol 4000, and 10–20% glycerol were combined. Large rectangular crystals grew within 2–3 days. Prior to data collection, the ATPBD crystals were quickly exchanged into a cryosolution containing 50 mM sodium acetate, pH 4.6, 5% polyethylene glycol 4000, and 25% glycerol and flash frozen in liquid nitrogen.

**Data Collection and Structure Determination**—A SAD data set was collected at 100 K at the sector 32 beamline at the Advanced Photon Source (see Table 1). The crystals diffracted to 2.3 Å resolution, belong to the space group P₃₂₁, and have unit cell dimensions of a = b = 80.78 Å and c = 105.96 Å. The data sets were integrated by using MOSFLM (33) and scaled with SCALA (34). Both SOLVE (35) and CNS (36) were used to find nine heavy atom sites corresponding to three selenium positions in each of the three ATPBD molecules in the asymmetric unit. CNS yielded the most interpretable electron density maps after density modification. The three ATPBDs comprising the asymmetric unit were built using XtalView (37), and the model was refined with CNS (Table 1). Non-crystallographic symmetry restraints were imposed throughout the refinement. Most of the residues in the three asymmetric units were observed in the electron density maps except for residues 407–410 at the N terminus, 670–671 at the C terminus, and 637–645 in the P-domain. A Ramachandran plot calculation with PROCHECK (38) indicated that 91% of the residues had the most favored geometry with the rest in additionally allowed regions. All figures were generated with PyMOL (39).

**RESULTS AND DISCUSSION**

**Overall Structure**—The *A. fulgidus* CopA ATPBD spanning residues 407–671 was expressed and purified to homogeneity. This isolated domain was able to bind and hydrolyze ATP at a measurable rate (4.2 nmol Pᵢ/mg/min) when incubated at 70 °C, the growth temperature of the source organism. The *A. fulgidus* ATPBD exhibits a kidney bean-like topology with a cleft for ATP binding at the interface between the N- and P-domains (Fig. 1a). Two 5–6-amino-acid loops (residues 429–
434 between β1 and β2 and residues 547–552 between β7 and α6) form a hinge region connecting the N- and P-domains. Notably, the CopA ATPBD adopts a closed conformation similar to the SERCA1 structures with bound AMP-PCP or AlF4− and ADP, which both mimic the E1P-ADP state of the protein (27, 29) (Fig. 1b). This closed conformation is characterized by a quasiparallel orientation of helices α5 and α7, similar to helices α7 and α10 in the nucleotide-bound SERCA1 structures (Fig. 1b). By contrast, these helices adopt a perpendicular orientation in the open forms of P2-type ATPases (25) (Fig. 1c). Among the different P-type ATPases, and within the specific P1,A and P2 subfamilies, there is little sequence conservation in these helices (Fig. 2 and supplemental Fig. S1). Nevertheless, mutations in the corresponding MNK and WND helices are known to lead to Wilson and Menkes diseases (Fig. 2) (40). A crystal structure with a nucleotide analogue bound to the CopA ATPBD could not be obtained despite several cocrystallization and soaking efforts. The ATPBD may be locked in this closed state either by the crystallization conditions or by intrinsic interactions that confer thermostability to CopA.

Hinge Region—The two short loops linking the N- and P-domains likely function as a hinge, allowing the two domains to undergo structural rearrangements concomitant with nucleotide binding and phosphoester intermediate formation (25, 27, 29). Several highly conserved residues are present in the hinge region. These include Thr430 and Asp548, which appear to be universally conserved among all ATPases, and Gly432, which is conserved only among the P1,A family members. The Thr430 and Asp548 side chains interact via hydrogen bonding. In addition, the Gly432 amide nitrogen interacts weakly with the Asp548 side chain. A similar pattern is observed in SERCA1 (supplemental Fig. S2) (25–29). These interactions may be critical for stabilizing the conformation of the phosphorylation loop (D424KTGT), which is just upstream of Thr430 in the P-domain, and are the focal point about which the hinge flexes. In support of a key role for these residues, mutations in Thr430 and Gly432 in WND are linked to disease states (Fig. 2) (40).

The N-domain—The N-domain consists of a six stranded antiparallel β-sheet sandwiched between two α-helices on each side of the sheet (Fig. 3a). Structures of N-domains from SERCA1 (25), the Na+/K+-ATPase (41), the Kdp-ATPase (KdpB) (42) and more recently WND (5) all exhibit this overall fold despite a lack of sequence homology (Fig. 3 and supplemental Fig. S1). Moreover, SERCA1, the Na+/K+-ATPase, and WND differ significantly from CopA in that their sequences include several insertions ranging from 10 to 60 residues in

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length. The WND N-domain has an unstructured 39-amino-acid loop between β4 and β5 that extends into the cytosol away from the N-domain (Fig. 3, b and c). This loop, which spans 56 amino acids in MNK, is not essential for catalysis and comprises just four residues in CopA. The MNK and WND loops do not bear any sequence resemblance to each other, suggesting that these elements may specifically target the two proteins to different cellular compartments. Like WND, the Ca	extsuperscript{2+} and Na	extsuperscript{+},K	extsuperscript{+}-ATPases have additional loops and structural elements, such as a seventh strand on the β-sheet (Fig. 1 and supplemental Fig. S1). Some of these inserts in SERCA1 participate in interactions with the A-domain (25–29). The additional structural elements found in the various P-type ATPases may therefore be specific for their function and help to promote domain-domain interactions as well as proper cellular localization.

The CopA N-domain is quite similar to the KdpB N-domain with bound AMP-PNP. There are no extra insertions in the KdpB sequence, and the root mean square deviation for Ca atoms between KdpB and CopA is 1.54 Å (Fig. 3d).

Sequence analysis of the different P	extsubscript{1}β-type ATPase N-domains reveals that five amino acids, Glu	extsuperscript{457}, His	extsuperscript{462}, Gly	extsuperscript{490}, Gly	extsuperscript{492}, and Gly	extsuperscript{501}, are universally conserved (Fig. 2). All of these residues are predicted to interact with the docked nucleotide in CopA on the basis of the superimposed KdpB structure. Residues Glu	extsuperscript{457}, His	extsuperscript{462}, and Gly	extsuperscript{492} may function to orient and bind the adenine ring through hydrogen bonding, whereas Gly	extsuperscript{490} and Gly	extsuperscript{501} likely interact primarily with the ribose moiety and α-phosphate.

The SEHP loop is likely to be important for protein function. The P-domain comprises a six-stranded parallel β-sheet sandwiched between six α-helices, three on each side of the sheet (Fig. 4a). The SERCA1 P-domain is the only other known P-domain structure of a P-domain, has a similar fold (root mean square deviation for Ca atoms = 1.7 Å) with the exception of a 50-amino-acid insert between α10 and β14 (Fig. 4, b and c). In CopA, both the invariant D	extsuperscript{424}TKGT and O	extsuperscript{572}GD loops are positioned in the back of the ATPBD crevice near the hinge region (Fig. 1a). These loops, which are critical for enzyme phosphorylation during catalysis, adopt similar conformations to those observed in the different SERCA1 structures (Fig. 4, c and d) (25–29). The loop between β10 and α9 containing the sequence A	extsuperscript{614}XGDDGXXND rests at the edge of the crevice marking the interface between the N- and P-domains. In the SERCA1 structures, the equivalent loop interacts with the A-domain and adopts different orientations. Finally, residues 637–645, for which no electron density was observed, would be located on a loop between β11 and β12 far from the phosphorylation site on the protein exterior (Figs. 1a and 4a). The analogous region in SERCA1 forms a small helix (Fig. 1b) and interacts with the A-domain in several different ways depending upon conformation of SERCA1. This loop in CopA would likely become more ordered in the presence of other cytosolic domains.

The similarities in the core fold of the P-domain are not surprising considering that the majority of the conserved residues among the different P-type ATPase ATPBDs are located in this domain (Figs. 2, 4d, and supplemental Fig. S1). The analogous region in SERCA1 forms a small helix (Fig. 1b) and interacts with the A-domain in several different ways depending upon conformation of SERCA1. This loop in CopA would likely become more ordered in the presence of other cytosolic domains.
likely coordinate Mg$^{2+}$, and Asp$^{574}$ hydrogen bonds to the nucleotide ribose moiety. The remaining conserved residues likely participate in second coordination sphere hydrogen bonding or serve a specific structural role to preserve the active site geometry.

**Domain Interactions**—In P-type ATPases, transmembrane ion transport is coupled to ATP hydrolysis via key structural and conformational changes (1, 2). These various movements include transient domain-domain interactions such as those described for the P- and N-domains of P$_2$-type ATPases (25–28). Electrostatic surface maps of the CopA ATPBD (Fig. 5a) do not reveal any obvious patches of positive or negative charge suggestive of a domain interaction site. Comparisons to the SERCA1 structures provide some insight into potential interaction surfaces, however. Mapping the known A-domain interaction sites in SERCA1 (28) onto the surface of the CopA ATPBD identifies specific parts of the P-domain as likely to interact with the A-domain (Fig. 5, b and c). These sites correspond to the slightly positively charged surface at the crest of the P-domain ATP-binding cleft (Fig. 5a). Notably, interactions between the A-domain and the N-domain in the SERCA1 structures (26–29) involve regions of secondary structure not present in P$_2$-ATPases. This observation does not preclude possible interactions between the A- and N-domains in CopA but could be related to the additional requirement for metal-dependent interactions between the N-MBDs and the ATPBD (17, 44) and between the N-MBDs and metallochaperones (46).

**Structural Significance of WND and MNK Mutations**—A number of mutations in the ATPBDs of WND and MNK have been identified in Wilson and Menkes diseases patients (Fig. 2) (40). The structure of the CopA ATPBD provides new insight into the probable effects of these mutations on enzyme function. For example, mutations T1031S, T1033A, G1035V, and R1038K occur in the WND hinge region and are
predicted to alter P- and N-domain movement. Other mutations might affect ligand binding by direct participation in ligand-protein interactions or by proximity to interacting residues. These mutations not only include the well characterized mutations in the SEHP region like H1043P but also in L1083P, L1232P, V1252I, and D1296N. Such mutations may affect the folding and stability of the protein.

In sum, the CopA ATPBD structure reveals the key components required for ATP binding and hydrolysis by P_{1B}^+ ATPases. The overall fold, which resembles that observed in P_{2}^- ATPases, represents the basic scaffold capable of performing key steps in the classical P-type ATPase E1/E2 mechanism. The structure additionally serves as a basis to explore domain-domain interactions specific to heavy metal transport. Finally, the CopA ATPBD structure provides a new framework for the structural analysis of many mutations leading to Wilson and Menkes diseases.

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