Structure of the ATPase subunit CysA of the putative sulfate ATP-binding cassette (ABC) transporter from *Alicyclobacillus acidocaldarius*

Frank Scheffel[a], Ulrike Demmer[b], Eberhard Warkentin[b], Anja Hülsmann[a], Erwin Schneider[a,*], Ulrich Ermler[b,*]

[a] *Humboldt-Universität zu Berlin, Mathematisch-Naturwissenschaftliche Fakultät I, Institut für Biologie, Berlin, Germany*
[b] *Max-Planck-Institut für Biophysik, Max-von-Laue-Strasse 3, 60438 Frankfurt, Germany*

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Abstract CysA, the ATPase subunit of a putative sulfate ATP-binding cassette transport system of the gram-positive thermoacidophilic bacterium *Alicyclobacillus acidocaldarius*, was structurally characterized at a resolution of 2.0 Å in the absence of nucleotides. In line with previous findings on ABC-ATPases the structures of the two monomers (called CysA-1 and CysA-2) in the asymmetric unit differ substantially in the arrangement of their individual (sub)domains. CysA-2 was found as a physiological dimer composed of two crystallographically related monomers that are arranged in an open state. Interestingly, while the regulatory domain of CysA-2 packs against its opposing domain that of CysA-1 undergoes a conformational change and, in the dimer, would interfere with the opposing monomer thereby preventing solute translocation. Whether this conformational state is used for regulatory purposes will be discussed.

Keywords: Sulfate ABC transporter; Regulation; Conformational change

1. Introduction

The family of ABC transport systems comprises a diverse class of transport proteins that couple the translocation of solutes across biological membranes to the free energy of ATP hydrolysis. Members of the family have been identified in organisms belonging to each of the three major kingdoms, some of them with medical relevance [1].

ABC transporters share a common architecture comprising two variable hydrophobic transmembrane domains (TMDs) that form the translocation pathway and two conserved hydrophilic ABC-ATPases that hydrolyze ATP. Each ABC-ATPase basically consists of a nucleotide-binding domain that is built up of a catalytic subdomain harboring the Walker A, Walker B and H motifs and a helical subdomain that contains the ABC signature motif 'LSGGQ' and the so-called Q-loop [2] (Fig. 1(a)).

The ATP driven translocation mechanism of this protein class is still not well understood at an atomic level despite multiple biochemical and structural investigations [2,3]. X-ray structures are available from three complete ABC transporters, BtuCD of *Escherichia coli* [4], MsbA of *E. coli* [5] and *Vibrio cholerae* [6] as well as from several nucleotide-binding (ABC) domains (ABC-ATPases) [2,3]. ABC-ATPases of members of the CUT1 and MOI subfamilies [7] contain an additional C-terminal (regulatory) domain that might be involved in regulatory processes. MalK [8] and *Sulfolobus solfataricus* glucose ABC-ATPase (GlcV) [9] belong to this group. ABC-ATPases revealed a pronounced conformational variability concerning the arrangement of the (sub)domains within one monomer and how the two monomers are assembled to form the physiologically relevant dimer.

We have focused on a putative sulfate ABC transporter of the gram-positive acidothermophilic bacterium *Alicyclobacillus acidocaldarius* [10]. The heterotetrameric CysTWA protein complex belongs to the MOI (mineral and inorganic ions) subfamily [7] and is composed of the transmembrane subunits, CysT (29 kDa) and CysW (30 kDa), and two copies of the ATP-binding subunit, CysA (40 kDa). CysA (putative *Alicyclobacillus acidocaldarius* sulfate ABC transporter) displays highest sequence identity to putative sulfate ABC transport proteins of *Mesorhizobium loti* (43%), *Geobacter sulfurreducens* (41%), and *Bacillus clausii* (41%). In the presented work we describe the structure of CysA in comparison to other members of the ABC-ATPase family and discuss its implication for a potential regulatory process.

2. Materials and methods

2.1. Overproduction and purification of CysA

The *cysA* gene (Accession No. CAB65650) was cloned from genomic DNA. For overproduction *cysA* was inserted as a *Bsp*HI–*Sal*I fragment into expression vector pQE9 (providing an N-terminal His-tag; Quiagen, Germany) previously linearized by digestion with *Nco*I (partial) and *Sal*I. The resulting plasmid pFSA13 was transferred into the *E. coli* strain JM109. Cells were grown in LB (Luria–Bertani) medium at 30 °C to $A_{650} = 0.3$, then IPTG (0.5 mM) was added to induce *cysA* expression and growth continued for 4 h. Subsequently cells were

*Corresponding authors.
E-mail addresses: erwin.schneider@rz.hu-berlin.de (E. Schneider), ermler@mpibp-frankfurt.mpg.de (U. Ermler).

Abbreviations: CysA, putative *Alicyclobacillus acidocaldarius* sulfate ABC transporter; $MalK_{eco}$, *E. coli* maltose ABC-ATPase; $MalK_{tli}$, *Thermococcus litoralis* maltose ABC-ATPase; GlcV, *Sulfolobus solfataricus* glucose ABC-ATPase

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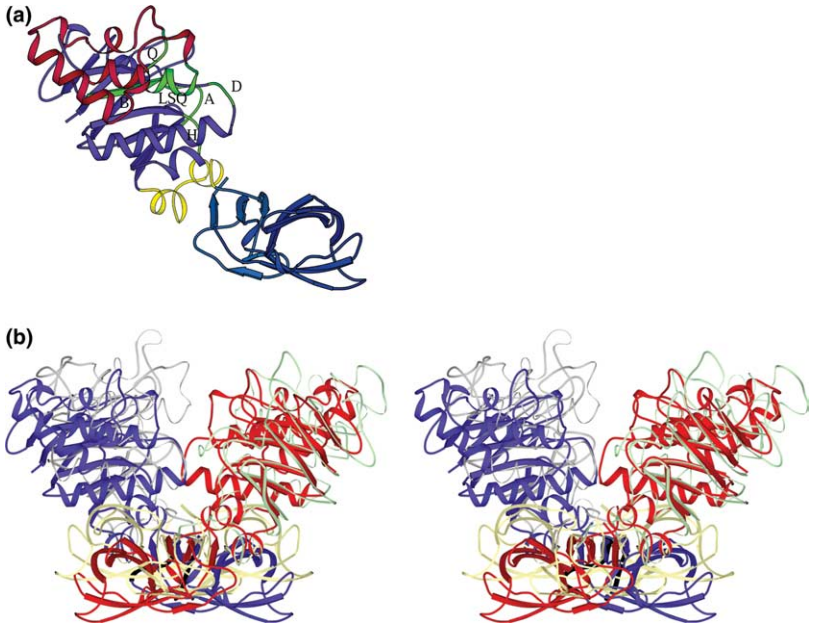

Fig. 1. Structure of CysA: (a) Ribbon diagram of the CysA monomer. The catalytic subdomain of the nucleotide-binding domain is shown in purple, the helical subdomain in red, the linker region in yellow and the regulatory domain in blue (distal β-sandwich) and royal blue (proximal β-sandwich). The location of conserved sequence motifs is indicated by capital letters: 'Walker' sites (A, B), D-loop, Q-loop, ABC signature (LSQ), and H motif. (b) Stereo representation of the CysA-2 dimer found in an open state. The monomers were shown in blue and red. For comparison CysA-2 was superimposed with the $MalK_{eco}$(open) structure at the front side (red) and with the $MalK_{eco}$(close) structure at the back side (green). In addition, the regulatory domains of the CysA-1 monomers are shown in yellow after superimposing with the catalytic domain. Figs. 1 and 2 have the same orientation and were generated using BOBSCRIPT [32].

harvested and disruptured by passage through a French Press. After ultracentrifugation the soluble fraction containing CysA was bound to a cation exchange resin (SP–Sepharose fast flow, Amersham–Pharmacia) and eluted with 0.1 M NaCl. The pooled fractions were then applied to Ni-NTA chromatography yielding about 20 mg of purified protein per 1 l culture. The protein was concentrated up to 20 mg/ml in 50 mM Tris–HCl, pH 7.5, 250 mM NaCl, 5 mM $MgCl_2$ and 5% glycerol by ultrafiltration (Amicon, YM30, Millipore, Bedford) and centrifugation through VivaSpin columns (cut off: 30 kDa; Vivascience, Hannover, Germany). Selenomethionine-labeled protein was produced using the method of metabolic inhibition [11]. Functionality of CysA was investigated by determining its intrinsic ATPase activity [12]. Maximal velocity of ATP hydrolysis of 11 μmol P_i/min/mg with a $K_{m,ATP}$ of 1.4 mM was found at a temperature of 60 °C, as expected, and at pH 7.5 [9].

2.2. Crystallization and X-ray structure determination

Native and selenomethionine-labeled CysA could be crystallized with the hanging-drop vapor diffusion method at a temperature of 18 °C. Most suitable crystals grew from 1 μl protein solution (7.5 mg/ml) and 1 μl reservoir solution containing 5–7% PEG 4000, 5% isopropanol and 0.1 M sodium citrate, pH 5.6. The crystal reached a size up to $0.08 \times 0.25 \times 0.35$ mm^3. Data of native and selenomethionine-labeled CysA were collected at the ID14-4 and ID29 beamlines in Grenoble and processed with the HKL suite [13]. Crystallographic data are listed in Table 1.

The positions of the selenium atoms in the asymmetric unit were detected from the peak data set of the selenomethionine-labeled protein using SHELXD [14] and refined using SHARP [15]. The derived phases (SHARP) were improved by standard solvent flattening and molecular averaging procedures [16]. The resulting electron density map at 2.5 Å resolution was of sufficient qualitiy to build about 50% of the model automatically using MAID [17] and the remaining residues manually using O [18]. The obtained model was refined within CNS [19] (Table 1). Model errors were assessed with CNS. The coordinates of the putative sulfate ABC-ATPase of *A. acidocaldarius* have been deposited at the RCSB as entry 1Z47.

3. Results

3.1. Structure determination and description

Since molecular replacement trials with various ABC-ATPases as models failed, the structure of CysA was determined with the multiple anomalous dispersion method (Table 1). During structure determination, we recognized that one molecule (CysA-1) in the asymmetric unit is being well defined while the other (CysA-2) is fairly flexible. The average temperature factors of CysA-1 and CysA-2 are 33 and 49 Å^2, respectively. In particular, the distal β-sandwich of the regulatory domain and the antiparallel β-sheet of the catalytic subdomain are highly disordered which might explain the relatively high R_{free} value.

CysA is composed of the nucleotide-binding domain that is subdivided into a catalytic and a helical subdomain [20] and of a regulatory domain [8] (Fig. 1(a)). The fold of the nucleotide-binding domain essentially corresponds to that of other ABC-ATPases [2]. According to O [18] the rms deviation between the individual catalytic and helical subdomains of CysA and those of the most related structurally known ABC-ATPases ranges from 1.0 to 1.5 Å. While the conformation of the Walker A site and the ABC signature motif are highly conserved between CysA-1, CysA-2 and other ABC- ATPases, substantial differences were observed in the Q-loop, the Walker B site and the D-loop. These segments are highly disordered in CysA-2 and might be rigidified upon ATP binding by an induced-fit process. Nucleotide-binding and regulatory domains are connected by a linker segment (residues 229–248) that consists of two helices being oriented perpendicularly relative to each other (Fig. 2). The regulatory domain of CysA is built