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Purification, crystallization and preliminary X-ray diffraction analysis of adenosine triphosphate sulfurylase (ATPS) from the sulfate-reducing bacterium *Desulfovibrio desulfuricans* ATCC 27774

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Native zinc/cobalt-containing ATP sulfurylase (ATPS; EC 2.7.7.4; MgATP: sulfate adenylyltransferase) from *Desulfovibrio desulfuricans* ATCC 27774 was purified to homogeneity and crystallized. The orthorhombic crystals diffracted to beyond 2.5 Å resolution and the X-ray data collected should allow the determination of the structure of the zinc-bound form of this ATPS. Although previous biochemical studies of this protein indicated the presence of a homotrimer in solution, a dimer was found in the asymmetric unit. Elucidation of this structure will permit a better understanding of the role of the metal in the activity and stability of this family of enzymes.

1. Introduction

ATP sulfurylases (ATPS; EC 2.7.7.4; MgATP:sulfate adenylyltransferases) are ubiquitous enzymes that catalyze the transfer of the adenylyl group from ATP to inorganic sulfate, yielding adenosine-5′-phosphosulfate (APS) and pyrophosphate (PP_i),

$$\mathrm{MgATP} + \mathrm{SO_4^{2-}} \longleftrightarrow \mathrm{MgPP_i} + \mathrm{APS}.$$

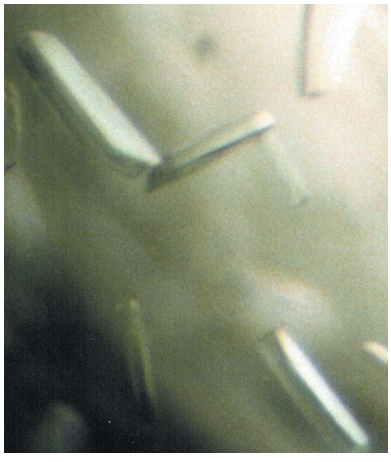
The APS produced in this reaction has a high-energy mixed phospho–sulfo anhydride bond that is used for sulfate activation and reduction in the cell.

ATPS are widely distributed in nature and have been found in virtually all types of organism (Leyh, 1993; Mulder, 1981) since they were first characterized (Robbins & Lipmann, 1958*a*,*b*). Several different physiological roles have been proposed for ATPS in different species (Hanna *et al.*, 2002, 2004), including sulfate assimilation, sulfate reduction and pyrophosphate recycling.

Two completely different unrelated types of ATPS have been distinguished: heterodimeric (Leyh, 1993) and monomeric or homo-oligomeric ATPS, with molecular weights ranging from 38 to 69 kDa per monomer (Kappler & Dahl, 2001; Sperling *et al.*, 2001). The size variations arise from APS kinase or PAPS-binding allosteric domains residing on the same polypeptide chain (Foster *et al.*, 1994; Kappler & Dahl, 2001; Lansdon *et al.*, 2004; Sperling *et al.*, 2001; Ullrich *et al.*, 2001).

The ATPS from *Desulfovibrio desulfuricans* (*Dd*ATPS) and *D. gigas* (*Dg*ATPS) were identified for the first time as cobalt/zinc-containing metalloproteins (Gavel *et al.*, 1998). Both enzymes were purified as homotrimers, with monomeric molecular weights of ∼47 kDa (423 residues) and ∼49 kDa, respectively.

Several crystal structures of ATPS have been reported, including those of hexameric ATPS from *Pseudomonas chrysogenum* (MacRae *et al.*, 2001, 2002) and *Saccharomyces cerevisiae* (Lalor *et al.*, 2003; Ullrich *et al.*, 2001; Ullrich & Huber, 2001), dimeric ATPS from *Riftia pachyptila* (Beynon *et al.*, 2001) and *Thermus thermophilus* HB8 (Taguchi *et al.*, 2003, 2004) and the human dimeric ATPS–APS kinase complex (PAPS synthetase 1; Harjes *et al.*, 2005). All these ATPS, with the exception of that of *S. cerevisiae* (Ullrich *et al.*, 2001; Ullrich & Huber, 2001), were crystallized from proteins cloned and expressed in *Escherichia coli*.



The structure of the dimeric ATPS from *T. thermophilus* HB8 (*Tt*ATPS) showed the presence of one zinc ion per subunit (Taguchi *et al.*, 2004). The structure revealed that the zinc is distant from the active site and is close to the dimer interface, suggesting that it plays a structural role, possibly in multimerization of the enzyme. Sequence alignment of ATPS from *D. desulfuricans*, *D. gigas* and *T. thermophilus* suggests a similar role for the metal in these proteins. This would indicate that *Dd*ATPS and *Dg*ATPS could also exist and function as dimers. In *Tt*ATPS, the metal is coordinated by three cysteine residues and a histidine, all of which are conserved in the *Desulfovibrio* strains.

Here, we report the purification, crystallization and X-ray characterization of the native zinc-containing form of ATPS from *D. desulfuricans* ATCC 27774. Diffraction data were collected to 2.5 Å resolution. These data will allow the determination of the structure of the zinc-bound form of ATPS. The structure will help to elucidate the role of the metal, as well as provide some insights on how cobalt/zinc selection might affect the activity and stability of the protein.

2. Materials and methods

2.1. Purification

D. desulfuricans ATCC 27774 cells were grown under anaerobic conditions in the medium described by Liu & Peck (1981) using nitrate as a terminal electron acceptor. Cells were harvested at the beginning of the stationary phase and were resuspended in 10 m*M* Tris–HCl buffer pH 7.6 containing 1 m*M* PMSF at a ratio of 1:1(*w*:*v*). The cells were lysed using a French press (62 MPa). The extract was centrifuged at 15 000*g* for 65 min and then at 180 000*g* for 75 min in order to eliminate the membrane fraction. The purification procedure was adapted from that published previously (Gavel *et al.*, 1998) and included four chromatographic steps: ion exchange on DEAE-52 cellulose and Source 15Q (Pharmacia), molecular-weight exclusion on Superdex 200 (Pharmacia) and adsorption chromatography on hydroxyapatite (BioRad). All purification procedures were performed aerobically at 277 K. Specific activities were determined at each step in the purification process (Gavel *et al.*, 1998). The purity of the final preparation of ATPS and subunit identification were confirmed by SDS–PAGE and N-terminal sequencing. The active ATPS exhibited specific activities of 22.5 ± 0.8 units mg^{-1} in the reverse direction and 28.9 ± 0.8 units mg^{-1} in the forward direction (as determined by molybdolysis). Metal analysis [determined by ICP analysis performed on a Jobin–Yvon (Ultima) instrument] indicated the presence of approximately one metal atom per subunit. ATPS was aliquoted at a final protein concentration of 10 mg ml^{-1} in 10 m*M* Tris–HCl buffer pH 7.5 and was stored at 190 K for initial crystallization trials.

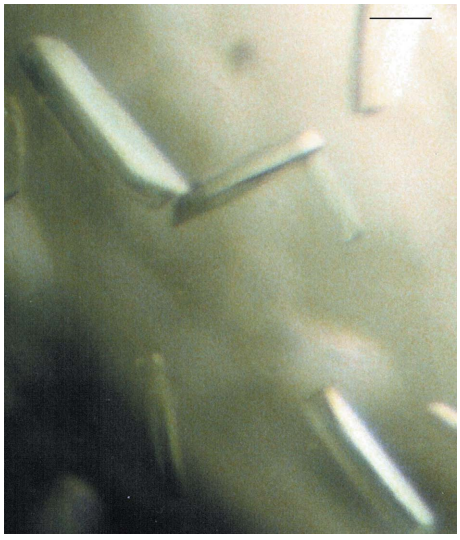


Figure 1
Crystals of *D. desulfuricans* ATPS (the scale bar is 0.1 mm in length).

2.2. Crystallization, data collection and processing

Crystallization trials of the ATPS were performed using the hanging-drop vapour-diffusion method using 2 µl drops (with a protein:well solution ratio of 1:1) over a well containing 700 µl well solution. Initial crystallization conditions were screened using an in-house modified version of the sparse-matrix method of Jancarik & Kim (1991) in combination with the commercial Crystal Screen and Crystal Screen 2 from Hampton Research (California, USA) at 277 and 293 K. Crystallization conditions were improved by screening additives and varying the protein and precipitant concentrations.

Multiple data sets were collected either on an in-house Cu $K\alpha$ rotating-anode generator or at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). Crystals were flash-cooled directly in liquid nitrogen and stored or transferred to a gaseous nitrogen stream (100 K) using Paratone oil as a cryoprotectant. The best data set was collected on beamline ID14-3 at the ESRF using an ADSC Quantum-4R detector. The data were processed using *MOSFLM* v.7.0.1 and *SCALA* from the *CCP*4 package v.6.0.2 (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

ATPS from *D. desulfuricans* ATCC 27774 was purified to homogeneity and crystallized.

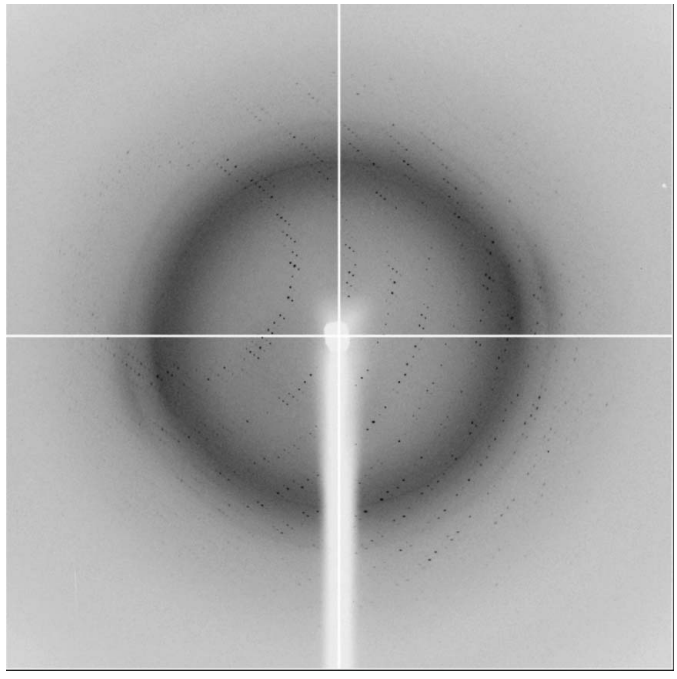


Figure 2
Diffraction pattern of the ATPS crystal (the resolution at the edge is 2.45 Å) obtained on beamline ID14-3 (ESRF).

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	ID14-3 (ESRF, Grenoble)
Crystal data	
Crystal system	Orthorhombic
Unit-cell parameters (Å)	a = 110.1, b = 126.2, c = 155.0
Maximum resolution (Å)	2.8
Mosaicity (°)	1.1
Molecules per ASU	2
Matthews coefficient (Å^3 Da^{-1})	2.83
Data collection and processing	
Space group	$C222_1$
Resolution limits (Å)	30–2.8 (2.95–2.8)
Wavelength (Å)	0.934
No. of observed reflections	189690 (27399)
No. of unique reflections	26876 (3853)
Redundancy	7.1
$R_{\rm merge}$† (%)	0.154 (0.541)
Completeness (%)	99.9 (99.9)
$\langle I/\sigma(I)\rangle$	4.9 (1.4)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the measurement of reflection hkl and $\langle I(hkl)\rangle$ is the mean value of $I(hkl)$ for all i measurements.

The best crystallization conditions were 3 *M* ammonium sulfate, 100 m*M* Tris–HCl pH 7.5 with 10%(*v*/*v*) MPD as an additive (the protein:well solution:MPD ratio was 1:1:0.2 and the final drop volume was 2.4 µl) using a protein concentration of ∼10 mg ml^{-1} at 277 K. Crystals grew to dimensions of about 0.2 × 0.1 × 0.05 mm (Fig. 1). The crystals diffracted to beyond 2.5 Å resolution (Fig. 2) and belong to space group $C222_1$, with unit-cell parameters a = 110.1, b = 126.2, c = 155.0 Å. A full 180° data set was collected, with 1° oscillation per image. The X-ray data collected on beamline ID14-3 (ESRF) were almost 100% complete to 2.45 Å but had to be cut to 2.8 Å in order to ensure high data quality (data-collection statistics are presented in Table 1).

Although the early biochemical studies indicated the presence of a homotrimer in solution (Gavel *et al.*, 1998), the calculated Matthews coefficient is 2.83 Å^3 Da^{-1} (Matthews, 1968), suggesting the presence of a dimer in the asymmetric unit, as predicted by sequence comparison with *Tt*ATPS and the structure of this enzyme. The self-rotation function (calculated using *POLARRFN*; Collaborative Computational Project, Number 4, 1994) exhibited clear peaks at κ = 180°, whereas no peaks were observed at κ = 120° (data not shown), further supporting the presence of a dimer.

A preliminary structure solution has been obtained using *BALBES* (Long *et al.*, 2008), which used PDB entries 1jhd (*R. pachyptila*; Beynon *et al.*, 2001), 1v47 (*T. thermophilus* HB8; Taguchi *et al.*, 2004) and 1r6x (*S. cerevisiae*; Lalor *et al.*, 2003) as search models (1v47 yielded the best solution). The solution found indicated the presence of a dimer in the asymmetric unit and the phases obtained yielded an interpretable map for at least 60% of the protein. The remainder of the protein still has poor density. A model is being built manually using *Coot* (Emsley & Cowtan, 2004).

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