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Preliminary characterization of two different crystal forms of acylphosphatase from the hyperthermophile archaeon *Sulfolobus solfataricus*

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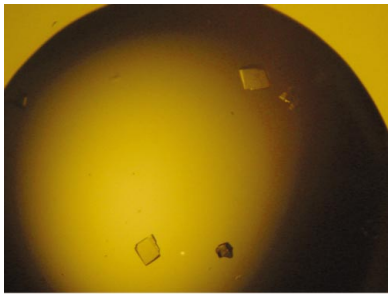


Acylphosphatase is a ubiquitous small enzyme that was first characterized in mammals. It is involved in the hydrolysis of carboxyl-phosphate bonds in several acylphosphate substrates, such as carbamoylphosphate and 1,3-biphosphoglycerate; however, a consensus on acylphosphatase action *in vivo* has not yet been reached. Recent investigations have focused on acylphosphatases from lower phyla, such as *Drosophila melanogaster* and *Escherichia coli*, in view of the application of these small proteins as models in the study of folding, misfolding and aggregation processes. An acylphosphatase from the hyperthermophilic archaeon *Sulfolobus solfataricus* has been cloned, expressed and purified. Here, the growth and characterization of a triclinic and a monoclinic crystal form of the hyperthermophilic enzyme are reported; X-ray diffraction data have been collected to 1.27 and 1.90 Å resolution, respectively.

1. Introduction

Acylphosphatase (AcP; EC 3.6.1.7) is a cytosolic enzyme (about 10 kDa) that is widespread in the eukaryotic and prokaryotic (both mesophilic and extremophilic) phyla. AcP catalyses the hydrolysis of carboxyl-phosphate bonds in acylphosphates, such as carbamoyl-phosphate, 1,3-biphosphoglycerate and the β-aspartylphosphate intermediate formed by the action of membrane pumps (Nassi *et al.*, 1993; Stefani & Ramponi, 1995). AcP can be found in many tissues of vertebrate species: in the skeletal muscle and in the heart as muscle-type AcP (MT-AcP) and in erythrocytes, brain and testis as (organ) common-type AcP (CT-AcP). These two isoforms share more than 50% amino-acid sequence identity, their sequences being highly conserved among vertebrates. The basic biological role of AcPs is still unclear, but studies of the hydrolysis of the phosphorylated intermediates of membrane Na^+/K^+ and Ca^{2+} ATPases suggest a possible role in controlling the flux of these ions through cell membranes (Stefani & Ramponi, 1995). An AcP-like prokaryotic domain in the hydrogenase factor HypF, the crystal structure of which has recently been reported (Rosano *et al.*, 2002), is involved in the binding of carbamoylphosphate and transfer of the carbamate moiety to another maturation factor, HypE, which provides the metal cluster of the hydrogenase with the metal ligands cyanide and carbon monoxide (Blokesch *et al.*, 2004).

The residues Arg23 and Asn41, which are conserved throughout the AcP family, are responsible for phosphate binding and catalytic activity (Stefani *et al.*, 1997). A nucleophilic water molecule is positioned close to the substrate and activated by Asn41; a substrate-assisted cleavage of the targeted carboxyl-phosphate bond can then take place (Pastore *et al.*, 1992; Thunnissen *et al.*, 1996; Stefani *et al.*, 1997; Rosano *et al.*, 2002; Zuccotti *et al.*, 2004).

The three-dimensional structures of various mesophilic AcPs have been elucidated. The structures of CT-AcP from bovine testis (Thunnissen *et al.*, 1996; PDB code 2acy) and of a novel *Drosphila melanogaster* AcP (AcPDro2; Zuccotti *et al.*, 2004; PDB code 1urr) have been determined by X-ray crystallography, while the structure of horse MT-AcP has been characterized by NMR (Pastore *et al.*, 1992; PDB code 1aps). Moreover, the same fold and catalytic residues are conserved in the AcP domain of HypF (Paschos *et al.*, 2001; Rosano *et al.*, 2002). Finally, preliminary crystallographic data on a

hyperthermophilic AcP from *Pyrococcus horikoshii* have recently been reported (Cheung *et al.*, 2004).

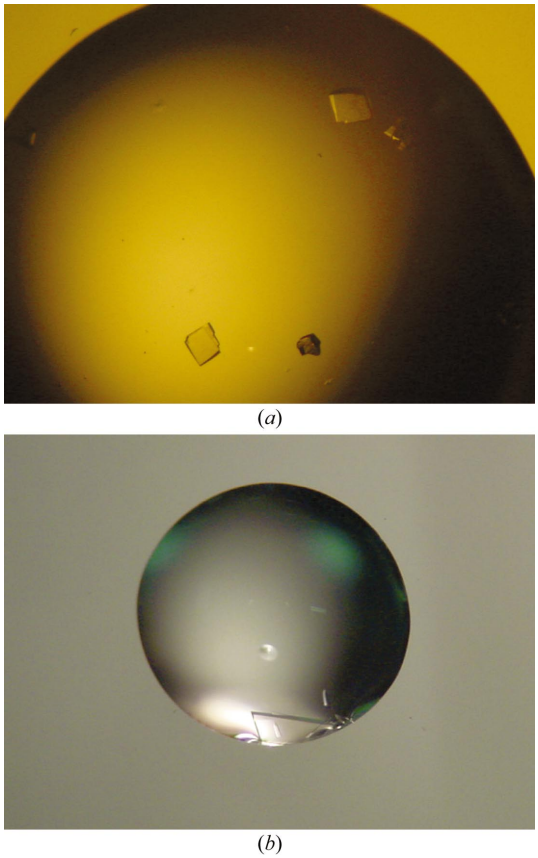
Here, we report the expression, purification, crystallization and preliminary X-ray diffraction data analysis of AcP from the hyperthermophile *Sulfolobus solfataricus*. We aim towards a thorough and comparative investigation on the molecular basis of thermostability and of processes related to folding and/or aggregation within the AcP family.

2. Materials and methods

2.1. Cloning, expression and purification

The gene encoding *S. solfataricus* AcP (Sso AcP) was initially inserted into a pEMBL plasmid. This DNA fragment was amplified by PCR using two primers (M-Medical) containing restriction sites for *Bam*HI and *Eco*RI. After purification and enzymatic digestion, the resulting fragment was ligated into pGEX-2T plasmid previously digested with the same enzymes. The plasmid was verified by DNA sequencing and trasformed into competent *Escherichia coli* DH5α cells. Expression and purification were carried out as previously described for human muscle acylphosphatase (Modesti *et al.*, 1995). The resulting sequence was GSMKKWSDTEVFEMLKRMYARV-YGLVQGVGFRKFVQIHAIRLGIKGYAKNLPDGSVEVVAEG-YEEALSKLLERIKQGPPAAEVEKVDYSFSEYKGEFEDFETY; the Gly-Ser dipeptide at the N-terminus results from the cloning in the pGEX2T plasmid.

The protein purity was checked by SDS–PAGE and ESI–MS. The expressed protein contained the full-length form together with truncated forms following proteolysis at several sites in the N-terminal region, which ended at residue 14. The N-terminal sequence contains the thrombin-cleavage consensus and a stretch starting with Met3 and terminating at Met14. This 13-residue tail probably does not belong to the Sso AcP sequence, whose gene is likely to start at the codon encoding the second methionine residue (Met14) of the sequence. Our investigation therefore focused on the structure of the truncated form starting at the second methionine.



(*a*)

(*b*)

Figure 1
A view of the two crystal forms of Sso AcP. (*a*) Plate-like monoclinic crystals. (*b*) Prismatic triclinic crystals.

Table 1
Data-collection and refinement statistics for Sso AcP.

Values in parentheses are for the last shell.

	Monoclinic $P2_1$ crystal form	Triclinic $P1$ crystal form
Beamline	ELETTRA XRD-1	ELETTRA XRD-1
Wavelength (Å)	0.931	1.200
Resolution range (Å)	29.54–1.90 (1.93–1.90)	30.00–1.27 (1.29–1.27)
Total reflections collected	132602	408775
Unique reflections	25338	43029
Redundancy	5.2	9.5
Completeness (%)	98.3 (95.7)	98.9 (98.1)
$R_{\rm merge}$ (%)	8.4 (33.1)	10.1 (39.5)
$\langle I/\sigma(I)\rangle$	9.7 (2.9)	5.2 (3.3)
Wilson plot B factor (Å^2)	26.8	12.4

2.2. Crystallization

The truncated Sso AcP was crystallized at 293 K in two different crystal forms using the vapour-diffusion method. In the first case, crystallization wells (600 µl reservoir solution containing 1.1 *M* lithium sulfate, 0.25 *M* ammonium sulfate, 0.1 *M* citrate pH 4.0) were equilibrated against droplets containing 1.0 µl reservoir solution and 1.0 µl Sso AcP solution (in 10 m*M* Tris, 10 m*M* NaCl pH 8.0), initially at a concentration of 7.3 mg ml^{-1}. Cryoprotectant solutions containing 1.3 *M* lithium sulfate, 0.5 *M* ammonium sulfate, 0.1 *M* citrate pH 4.0 and 15%(*v*/*v*) glycerol, or 15%(*v*/*v*) ethylene glycol, were used for data collection. Under these conditions, plate-shaped crystals (Fig. 1*a*) grew in about two weeks at 294 K to dimensions of about 0.07 × 0.05 × 0.03 mm.

A second crystal form was grown by equilibrating 600 µl reservoir solution containing 1.0 *M* sodium acetate, 0.05 *M* cadmium sulfate, 0.1 *M* HEPES pH 8.0, against a droplet containing 1.0 µl reservoir solution and 1.0 µl 7.3 mg ml^{-1} Sso AcP. The cryoprotectant solutions contained 1.3 *M* sodium acetate, 0.05 *M* cadmium sulfate, 0.1 *M* HEPES pH 8.0 and 15%(*v*/*v*) glycerol. Single prismatic crystals grew to dimensions of 0.3 × 0.2 × 0.2 mm after a few days, but with low yield and reproducibility.

2.3. Preliminary diffraction data analysis

X-ray diffraction data were collected using synchrotron radiation at ELETTRA (Trieste, Italy) beamline XRD-1 using wavelengths of 0.931 and 1.200 Å for the first and second crystal forms, respectively. The crystals grown at pH 4.0 were characterized as belonging to the monoclinic space group $P2_1$. Diffraction data were observed to a maximum resolution of 1.90 Å, with unit-cell parameters a = 48.98, b = 56.53, c = 60.81 Å, β = 103.7°; the crystals displayed very high mosaicity (1.2°). Estimation of the $V_{\rm M}$ packing parameter (2.1 Å^3 Da^{-1}) suggests the presence of four Sso AcP molecules in the asymmetric unit, with a solvent content of 39%. The X-ray diffraction data set was integrated and scaled to a maximum resolution of 1.90 Å using the programs *MOSFLM* (Leslie, 1992) and *SCALA* (Evans, 1997) from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The second crystal form was characterized

as belonging to the triclinic space group $P1$. The crystals diffracted to high resolution (1.27 Å), with unit-cell parameters $a = 26.19$, $b = 36.77$, $c = 45.98$ Å, $\alpha = 82.0$, $\beta = 77.4$, $\gamma = 72.4^\circ$, displaying two Sso AcP molecules in the asymmetric unit and a V_M packing parameter (2.1 Å^3 Da^{-1}) that is almost identical to that of the monoclinic crystal form. The diffraction data were integrated and scaled with *MOSFLM* and *SCALA* and truncated at 1.27 Å resolution, maintaining a sufficient signal-to-noise ratio in the last resolution shell. The relevant data-collection statistics are reported in Table 1; the relatively high R_{sym} measured in the case of the triclinic space group is likely to be related to the high data-set redundancy and to the remounting of the crystal during data collection that was required to achieve data completeness.

The amino-acid sequence conservation among Sso AcP and CT-AcPs (about 33% amino-acid identity) is expected to be sufficient to allow solution of the hyperthermophilic Sso AcP three-dimensional structure *via* molecular-replacement techniques. Because of the high-resolution data available for the triclinic crystal form and the six independent copies of the Sso AcP molecule present in the two crystal forms, high accuracy in the analysis of the thermostable protein structural parameters is anticipated.

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References

Blokesch, M., Paschos, A., Bauer, A., Reissmann, S., Drapal, N. & Bock, A. (2004). *Eur. J. Biochem.* **271**, 3428–3436.
Cheung, Y. Y., Allen, M. D., Bycroft, M. & Wong, K. B. (2004). *Acta Cryst.* D**60**, 1308–1310.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Evans, P. R. (1997). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **33**, 22–24.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **27**.
Modesti, A., Taddei, N., Bucciantini, M., Stefani, M., Colombini, B., Raugei, G. & Ramponi, G. (1995). *Protein Expr. Purif.* **6**, 799–805.
Nassi, P., Nerdiani, C., Liguri, G., Taddei, N. & Ramponi, G. (1993). *Biochim. Biophys. Acta*, **1147**, 19–26.
Paschos, A., Glass, R. S. & Bock, A. (2001). *FEBS Lett.* **488**, 9–12.
Pastore, A., Saudek, B., Ramponi, G. & Williams, R. J. P. (1992). *J. Mol. Biol.* **224**, 427–440.
Rosano, C., Zuccotti, S., Bucciantini, M., Stefani, M., Ramponi, G. & Bolognesi, M. (2002). *J. Mol. Biol.* **321**, 785–796.
Stefani, M. & Ramponi, G. (1995). *Life Chem. Rep.* **12**, 271–301.
Stefani, M., Taddei, N. & Ramponi, G. (1997). *Cell Mol. Life Sci.* **53**, 141–151.
Thunnissen, M. G. M., Taddei, N., Liguri, G., Ramponi, G. & Nordlund, P. (1996). *Structure*, **5**, 69–79.
Zuccotti, S., Rosano, C., Ramazzotti, M., Degl'Innocenti, D., Stefani, M., Manao, G. & Bolognesi, M. (2004). *Acta Cryst.* D**60**, 1177–1179.