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Crystallization and preliminary X-ray diffraction studies of phospho-adenylylsulfate (PAPS) reductase from *E. coli*

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Abstract

PAPS reductase from *E. coli* is involved in sulfur metabolism and catalyses the reduction of phospho-adenylyl-sulfate (PAPS) to sulfite. The protein has been cloned, overexpressed and purified from *E. coli*. Crystallization experiments resulted in crystals suitable for X-ray diffraction. The crystals belong to the orthorhombic space group $C222_1$ with cell dimensions a = 81.9, b = 97.4, c = 109.5 Å, and contain one molecule per asymmetric unit. At cryogenic (100 K) temperatures the crystals diffract to a resolution limit of 2.7 Å using a rotating anode and to 2.0 Å at a synchrotron source.

1. Introduction

Assimilatory sulfate reduction is used by prototrophic organisms as a source for reduced sulfur as required for the biosynthesis of all sulfur-containing metabolites (Cole & Ferguson, 1988). This metabolic pathway is driven by a number of enzymes, including ATP-sulfurylase (E.C. 2.7.7.4), adenylylsulfate (APS) kinase (E.C. 2.7.1.25), phospho-adenylylsulfate (PAPS) reductase and sulfite reductase (E.C. 1.8.1.2 NADH or E.C. 1.8.7.1 ferredoxin dependent). In the terminal step of this pathway sulfite is reduced to sulfide which is used for cysteine biosynthesis. In *E. coli* the genes coding for this group of enzymes are clustered in one region of the genome. The gene coding for the PAPS reductase, *cys*H, is located together with the genes *cys*I and *cis*J that code for the hemoprotein and for the flavoprotein subunits of the bacterial sulfite reductase (Ostrowski *et al.*, 1989*a*,*b*). Recently, the structure of the hemoprotein subunit of sulfite reductase from *E. coli* has been determined as the first member of this metabolic pathway (Crane *et al.*, 1995). PAPS reductase acts as a dimer in solution and has a molecular weight of 27 927 per monomer (244 amino acids) (Krone *et al.*, 1991). The enyzme can be expressed in *E. coli* and has been characterized by site-directed mutagenesis (Behrendt *et al.*, 1995). The enzyme is reduced by thioredoxin by a still unknown mechanism that releases sulfite (Russel *et al.*, 1990). A ping-pong mechanism is favoured in which reduced thioredoxin binds to PAPS reductase and leaves before the substrate (PAPS) is bound (Holmgren, 1989). From steady-state kinetics it was implied that thioredoxin reduces the enzyme first, before it could transfer two electrons to PAPS, yielding PAP and sulfite. How these two electrons are stored in the enzyme without a prosthetic group is not clear at present. The enzyme contains a single cysteine near the C terminus which is conserved between cyanobacteria, enterobacteria and purple sulfur bacteria (Holmgren, 1989; Schwenn, 1994). Whether this cysteine is involved in the enzymatic mechanism, *e.g.* by the formation of a disulfide bond in the oxidized form, is still not known.

In this paper we describe the crystallization and preliminary X-ray diffraction experiments of PAPS reductase in its reduced form as the first step towards an understanding of the complex mechanism of PAPS reduction.

2. Materials and methods

2.1. *Expression and purification*

The protein was overexpressed using *E. coli* strain JM96FAK harbouring the plasmid pCH10 and purified as described previously (Krone *et al.*, 1991; Behrendt *et al.*, 1995). The appropriate fractions were pooled and then the sample was concentrated and checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). The purity of the protein sample was checked by mass spectrometry. Gel-filtration experiments carried out with a Superose 12 column (Pharmacia) confirmed that the protein is a dimer in solution.

2.2. *Crystallization*

After purification 0.1% β-octyl-glucoside was added to the protein, which was then dialyzed against 10 m*M* Tris–HCl pH = 8.00 plus 0.1 m*M* EDTA for 1 h at 277 K. The sample was concentrated up to 8 (mg protein) ml^{-1} at 277 K using a centricon with 10 kDa cutoff. The protein concentration was measured using the method of Bradford (Bradford, 1976). Crystallization screenings were carried out both at 277 and 293 K. Some flake-like crystals and small needles were obtained at 293 K. The best results were obtained at 277 K and the crystals appeared after 2–3 d. Orthogonal crystals grew up to a maximum size of 800 × 50 × 50 µm from 50 m*M* Bis–Tris pH = 6.00, 100 m*M* Ca^{2+} acetate, 9–11% methyl-PEG 5000 and 5 m*M* DTT (Fig. 1*a*).

2.3. *Data collection and reduction*

At room temperature the PAPS reductase crystals diffract to 2.7 Å resolution using a rotating anode. The crystals last for several hours and although a full data set could be collected in this way, a clear decay of the diffraction intensities was observed. Therefore, cryo-cooling conditions (100 K) have been developed. The quality of the data was greatly improved, but the resolution stayed the same. The crystals were soaked in cryobuffer containing 20% PEG 400 for a couple of minutes and then flash-frozen in an Oxford Cryosystems Cryostream. The cryoconditions were easy to reproduce. In-house data collection was carried out with 10 min exposure time using a rotating anode with a MAR image plate. Data collection at the EMBL Outstation in Hamburg was performed at the X11 beamline. At X11 the crystals diffract to about 2.0 Å and we used the data to 2.3 Å. At the ESRF (BL14) the crystals diffract

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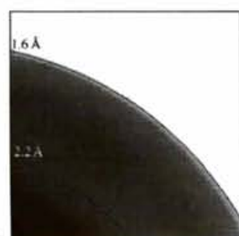
to 1.9 Å resolution (Fig. 1*b*). All the data were processed with the programs *DENZO* and *SCALEPACK* (Otwinowski, 1993).

3. Results and discussion

PAPS reductase can be expressed in *E. coli* routinely with yields of about 10 mg l^{-1}. The protein has been purified in the reduced

(*a*)



(*b*)

Fig. 1. (*a*) Orthorhombic crystals of PAPS reductase grown from 50 m*M* Bis–Tris pH = 6.00, 100 m*M* Ca^{2+} acetate, 9–11% methyl-PEG 5000 and 5 m*M* DTT to a size of 800 × 50 × 50 µm. (*b*) Diffraction pattern from PAPS reductase at BL4 (ESRF Grenoble). The resolution is indicated.

Table 1. *In-house data collection statistics (native)*

Resolution limits (Å)	I/σ	$R_{\rm sym}$†	Completeness (%)
20.0–7.22	25.32	0.041	96.6
7.22–5.77	15.51	0.074	97.6
5.77–5.06	17.40	0.071	98.8
5.06–4.60	20.96	0.062	98.4
4.60–4.28	21.75	0.059	97.1
4.28–4.03	20.49	0.060	97.9
4.03–3.83	18.00	0.071	98.5
3.83–3.66	17.37	0.077	98.2
3.66–3.52	15.39	0.079	97.8
3.52–3.40	14.34	0.083	97.9
3.40–3.29	12.96	0.095	98.5
3.29–3.20	11.71	0.104	98.1
3.20–3.12	10.13	0.118	97.6
3.12–3.04	8.41	0.143	98.1
3.04–2.97	7.34	0.157	98.0
2.97–2.91	6.66	0.168	98.0
2.91–2.85	6.01	0.186	97.5
2.85–2.80	5.19	0.190	97.7
2.80–2.75	3.77	0.174	60.5
2.75–2.70	2.57	0.206	0.50
Average values	14.44	0.085	91.2

† $R_{\rm sym} = \sum_{hkl} |I_i - I_{\rm avg}| / \sum_{hkl} \sum |I_i|$.

Table 2. *Data collection statistics at beamline X11 (Hamburg)*

Resolution limits (Å)	I/σ	$R_{\rm sym}$†	Completeness (%)
50.00–6.24	35.24	0.052	100.0
6.24–4.95	35.89	0.056	100.0
4.95–4.33	36.59	0.030	100.0
4.33–3.93	34.59	0.035	100.0
3.93–3.65	29.61	0.045	100.0
3.65–3.44	23.68	0.052	99.90
3.44–3.26	22.40	0.061	99.90
3.26–3.12	18.53	0.078	100.0
3.12–3.00	14.78	0.106	100.0
3.00–2.90	11.87	0.136	100.0
2.90–2.81	9.88	0.160	100.0
2.81–2.73	8.36	0.190	100.0
2.73–2.66	7.39	0.209	100.0
2.66–2.59	6.46	0.240	100.0
2.59–2.53	5.78	0.261	100.0
2.53–2.48	5.47	0.277	99.90
2.48–2.43	4.65	0.317	99.90
2.43–2.38	4.04	0.377	99.90
2.38–2.34	3.68	0.395	100.0
2.34–2.30	3.18	0.430	96.40
Average values	19.09	0.075	99.80

† $R_{\rm sym} = \sum_{hkl} |I_i - I_{\rm avg}| / \sum_{hkl} \sum |I_i|$.

form. Mass spectrometry of protein from re-dissolved crystals revealed a discrepancy between the protein and its theoretical mass of about 1590 Da, which corresponds to a loss of 13 amino-acid residues. This might be due to proteolytic degradation or to an autocatalytic cleavage like in ribonucleotide reductase (Mao *et al.*, 1992). Orthorhombic crystals of the protein have been obtained which belong to the space group $C222_1$ with cell dimensions of a = 81.9, b = 97.4 and c = 109.5 Å (Fig. 1). At the beginning the crystals were obtained at low pH (about 4.3) in 0.5 *M* citrate. However, in order to avoid possible problems with heavy-atom soaks due to low pH and complexation, the initial crystallization conditions have been

modified. The crystals which are now routinely used for heavy-atom soaks are obtained from Bis–Tris and Ca^{2+} acetate and are isomorphous with the crystals obtained previously from citrate. Tables 1 and 2 show the statistics for data collection with PAPS reductase crystals (Bis–Tris) both at home (native) and at the EMBL Outstation, Hamburg (native-X11). Assuming a monomer of the protein in the asymmetric unit the crystal volume per protein mass (V_m) is 3.8 Å^3 Da^{-1}, corresponding to a solvent content of about 65%. This is still within the range commonly observed for protein crystals (Mathews, 1968). The protein is always a dimer in solution and also active as such. Therefore, we also expected a dimer in the crystal. It was found from a self-rotation analysis that the dimer axis coincides with a crystallographic twofold. Heavy-metal soaks are in progress in order to solve the structure by multiple isomorphous replacement.

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