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SseA, a 3-mercaptopyruvate sulfurtransferase from *Escherichia coli*: crystallization and preliminary crystallographic data

Andrea Spallarossa,[a,b] Aristodemo Carpen,[c] Fabio Forlani,[c] Silvia Pagani,[c] Martino Bolognesi[a] and Domenico Bordo[d]*

[a]Dipartimento di Fisica, INFM, Centro di Eccellenza per la Ricerca Biomedica, Universita' di Genova, Via Dodecaneso 33, 16146 Genova, Italy, [b]Dipartimento di Scienze Farmaceutiche, Universita' di Genova, Viale Benedetto XV 3, 16132 Genova, Italy, [c]Dipartimento di Scienze Molecolari Agroalimentari, Universita' di Milano, Via Celoria 2, 20133 Milano, Italy, and [d]Laboratory of Structural Biology, Istituto Nazionale Ricerca sul Cancro, Largo R. Benzi 10, 16132 Genova, Italy

Correspondence e-mail: domenico@alcor.ge.infm.it

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SseA, the translation product of the *Escherichia coli sseA* gene, is a 31 kDa protein endowed with 3-mercaptopyruvate:cyanide sulfurtransferase activity *in vitro*. As such, SseA is the prototype of a sulfurtransferase subfamily distinguished from the better known rhodanese sulfurtransferases, which display thiosulfate:cyanide sulfurtransferase activity. The physiological role of the two homologous enzyme families, whose catalytic activity is centred on a reactive invariant cysteine, is a matter of debate. In this framework, the forthcoming crystal structure analysis of SseA will be based on the tetragonal crystal form (space group $P4_1$ or $P4_3$) reported here, with unit-cell parameters $a = b = 150.2$, $c = 37.9$ Å.

1. Introduction

Sulfurtransferases (EC 2.8.1.*x*) are a class of enzymes widely distributed among living organisms that catalyze the *in vitro* transfer of a sulfane S atom from a suitable donor molecule to a nucleophilic acceptor (Westley, 1977; Westley *et al.*, 1983). Of the sulfurtransferases, bovine rhodanese (thiosulfate:cyanide sulfurtransferase; TST; EC 2.8.1.1) is the best characterized enzyme (Lang, 1933; Sörbo, 1960; Ploegman *et al.*, 1978; Gliubich *et al.*, 1998). Rhodanese catalyzes the transfer of a sulfane S atom from thiosulfate to cyanide. The reaction occurs *via* formation of a covalent enzyme–sulfur intermediate, with the persulfide S atom covalently bound to the thiol group of the catalytic Cys residue. The physiological role of rhodanese is still under debate, since it appears unlikely that such a ubiquitous enzyme is uniquely involved in cyanide detoxification, as originally proposed. In this regard, the enzyme displays rather a wide substrate specificity. Various thiosulfonates or polythionates may act as sulfur donors, while sulfite and thiol compounds may replace cyanide as the sulfane sulfur acceptor (Ploegman, 1977).

A related sulfurtransferase enzyme, 3-mercaptopyruvate:cyanide sulfurtransferase (MST; EC 2.8.1.2), catalyzes a reaction similar to rhodanese in which thiosulfate is replaced by 3-mercaptopyruvate (1).

$$\mathrm{HSCH_2COCOO^- + CN^- \rightarrow CH_3COCOO^- + CNS^-}. \quad (1)$$

MST activity was identified quite a long time ago (Meister *et al.*, 1954); it was also shown that, similar to rhodanese, nucleophilic compounds other than cyanide may act as sulfur acceptors in the same reaction (Kun & Fanshier, 1959). Even although MST activity has been found to be widely distributed in prokaryotes and eukaryotes (Wood & Fiedler, 1953; Jarabak & Westley, 1978; Westley *et al.*, 1983), only eukaryotic MST enzymes from rat and *Arabidopsis thaliana* have been biochemically characterized to date (Nagahara *et al.*, 1995; Nagahara & Nishino, 1996; Papenbrock & Schmidt, 2000*a*,*b*; Nakamura *et al.*, 2000). Probably owing to intrinsic enzyme instability, little has been reported to date about the chemical mechanism of MST catalysis (Jarabak & Westley, 1978, 1980; Nagahara *et al.*, 1999).

Significant sequence similarity is found between MST and TST enzymes (rat MST and TST have a sequence identity of 59%; Nagahara *et al.*, 1995), including a conserved (putatively) catalytic Cys residue at equivalent locations in the respective amino-acid sequences. From a phylogenetic viewpoint, this observation indicates that the two enzymes share a common ancestor and are likely to adopt similar three-dimensional folds (Nagahara *et al.*, 1995; Bordo & Bork, 2002).

Structural studies carried out on bovine and *Azotobacter vinelandii* rhodanese (Ploegman *et al.*, 1978; Bordo *et al.*, 2000) have shown that TSTs are composed of two similarly folded domains, each about 120 amino acids long, with the invariant catalytic Cys residue located in the C-terminal domain. The catalytic Cys is the first residue of a six-amino-acid loop that folds in a cradle-like structure around the thiol group of the essential Cys. Although the residues surrounding the active site are deemed to be important for substrate specificity and enzyme activity, bovine and *A. vinelandii* TSTs have widely differing sequences in the active-site loop (CRKGVT compared with CQTHHR, respectively). In contrast, the characterized MST enzymes display the

conserved active-site loop sequence CG[S/T]GVT ([S/T] indicates the occurrence of either Ser or Thr), suggesting a stronger evolutionary pressure for active-site-loop residue conservation (Bordo & Bork, 2002).

The identification and cloning of the *Escherichia coli sseA* gene (Hama *et al.*, 1994) and the recent biochemical characterization of the corresponding MST protein, SseA (31 kDa, 280 amino acids), allowed the identification of the first prokaryotic protein to show 3-mercaptopyruvate:cyanide sulfurtransferase activity *in vitro* (Colnaghi *et al.*, 2001). In SseA the active-site loop composition is also identical to that observed in eukaryotic enzymes (CGSGVT). As expected, SseA displays clear sequence similarity with bovine and *A. vinelandii* TSTs (35% and 27% identical residues, respectively; Fig. 1), indicating a similarly arranged tandem domain conformation.

The determination of the three-dimensional structure of SseA, the first member of the MST enzyme subfamily to be analyzed by X-ray crystallography, will help to elucidate the catalytic mechanism and the structural determinants involved in MST substrate specificity. Here, we describe the crystallization of SseA from *E. coli*, together with a preliminary analysis of the X-ray diffraction data.

2. Methods and results

2.1. Protein expression and purification

The *sseA* gene (SWISS-PROT sequence accession No. P31142) was cloned and overexpressed as described by Colnaghi *et al.* (2001). The extract (8 ml, 20 mg ml^{-1} protein concentration) from a BL21(DE3) (pEN1) induced culture was fractionated overnight on a column of Sephacryl HR S-200 (Pharmacia Biotech, Sweden) equilibrated in 50 m*M* Tris–HCl buffer pH 8.0 containing 0.3 *M* NaCl. The fractions displaying 3-mercaptopyruvate:cyanide sulfurtransferase activity were pooled and brought to 75% saturation with ammonium sulfate. Further purification of *E. coli* SseA was achieved by HPLC gel filtration of the ammonium sulfate precipitate solubilized in 15 ml 50 m*M* Tris–HCl buffer pH 8.0 containing 0.1 *M* NaCl (3 mg ml^{-1} protein concentration). 200 µl aliquots of this material were chromatographed on a Superdex 75HR column (Pharmacia) previously equilibrated in the solubilization buffer. MST activity was assessed using the discontinuous method (Jarabak, 1981) that quantitates the product thiocyanate based on the absorption of the ferric thiocyanate complex at 460 nm. Assays lasted 1–2 min; one unit (U) of enzyme is defined as the amount of enzyme that produces 1 µ*M* thiocyanate per minute at 310 K. Protein concentration was determined by a dye-binding colorimetric assay (Bradford, 1976).

2.2. Crystallization

For crystallization purposes, the protein was concentrated to 13.6 mg ml^{-1} in a buffer containing 50 m*M* Tris–HCl pH 8.0 and 50 m*M* NaCl. $Na_2S_2O_3$ was added to the protein to a final concentration of 5 m*M*. Crystals were grown by the hanging-drop vapor-diffusion method by mixing an equal amount (1 µl) of protein and reservoir solutions. Initial crystallization conditions were determined by the sparse-matrix method (Jancarik & Kim, 1991) as implemented in Hampton Crystal Screens I and II (Hampton Research, CA, USA). The optimization of the preliminary crystallization conditions resulted in crystals of optimal size, having square rod shapes and typical dimensions of 0.07 × 0.07 × 0.75 mm, which grew after 10 d equilibration against a reservoir solution containing 1.9 *M* $(NH_4)_2SO_4$, 40 m*M* MES pH 6.2, 2%(*v*/*v*) PEG 400 and 10 m*M* $MnCl_2$ at 294 K (Fig. 2). The presence of 5 m*M* $Na_2S_2O_3$ in the protein solution was essential to avoid the formation of amorphous precipitates preventing crystal formation. This is likely to be ascribable to the intrinsic propensity of the reduced thiol group of Cys237 to form interchain disulfide bridges. Sulfane sulfur-donor compounds such as $S_2O_3^{2-}$ keep the enzyme in the persulfurated form, preventing aggregation/unfolding (Bordo *et al.*, 2001).

2.3. Data collection and processing

X-ray diffraction data were collected at 100 K after immersion of SseA crystals in paraffin oil for cryoprotection. Data collections were carried out on the ID14-4 and BW7A synchrotron beamlines at ESRF, Grenoble and DESY, Hamburg, respectively, to a resolution limit of 2.8 Å (the X-ray detector types used were ADSC Q4R and MAR CCD, respectively). Data were indexed and processed using *DENZO* and *SCALEPACK*, respectively (Otwinowski, 1993; Otwinowski & Minor, 1997); further data manipulations were performed using programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). SseA crystals belong to the tetragonal space group $P4_1$ (or $P4_3$), with unit-cell parameters $a = b = 150.2$, $c = 37.9$ Å, $\alpha = \beta = \gamma = 90^\circ$. Calculation of the crystal packing parameter (Matthews, 1968) indicates that the crystal lattice accommodates either two or three molecules per asymmetric unit (V_M = 3.5 or 2.3 Å^3 Da^{-1}; solvent contents are 65% or 47%, respectively). The $\chi = 120^\circ$ section of the SseA

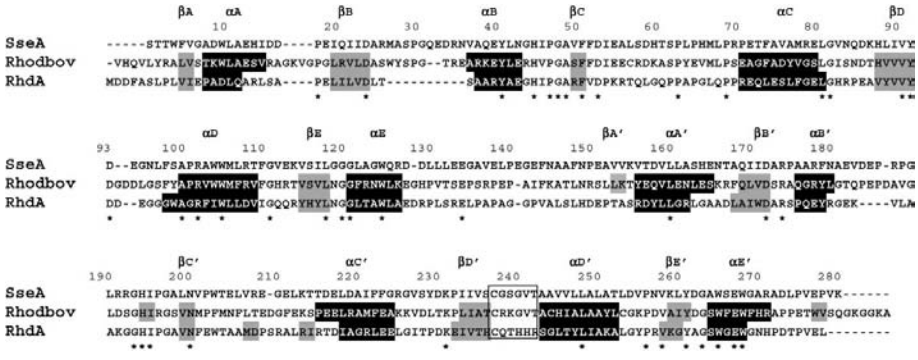

Figure 1
Multiple sequence alignment of SseA, bovine and *A. vinelandii* rhodanese enzymes (accession Nos. P31142, P00586 and P52197, respectively). Residues assuming β-strand and α-helix conformation in the two TST structures (PDB entries 1rhs and 1e0c, respectively) are shown with gray and black backgrounds, respectively. Asterisks indicate conserved amino acids. Active-site loop residues are included in a box. Conventional labels for secondary-structure elements (Bordo *et al.*, 2000) and SseA amino-acid numbering are shown on the top lines. Sequences were aligned with the program *CLUSTAL_W* (Thomson *et al.*, 1994).

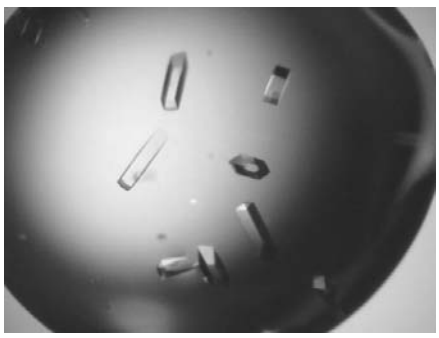

Figure 2
Crystals of SseA. Typical crystal dimensions are 0.75 × 0.07 × 0.07 mm.

Table 1
X-ray data-collection statistics.

Data in parentheses are for the last resolution shell (2.85–2.80 Å).

Resolution limit (Å)	2.8
X-ray source	ID14-4, ESRF, Grenoble
Wavelength (Å)	0.9393
Space group	$P4_1/P4_3$
Unit-cell parameters (Å, °)	$a = b = 150.2$, $c = 37.9$, $\alpha = \beta = \gamma = 90$
Mosaicity (°)	0.66
No. of observations	312079
Unique reflections	21524
Data completeness (%)	95.6 (97.9)
$\langle I/\sigma(I)\rangle$	15.9 (2.2)
$R_{\rm sym}$† (%)	6.6 (48.3)

† $R_{\rm sym} = \sum_{i,h} |(I_h - \langle I_h\rangle)|/\sum(I)_i$, where I is the observed intensity of the hth reflection.

self-rotation function, calculated in the 10.0–3.5 Å resolution range, does not display any significant peak. On the other hand, the $\chi = 180^\circ$ section shows peaks at the 4σ level compatible with the location of non-crystallographic twofold axes in the unit-cell *ab* plane, suggesting that a SseA dimer is present in the asymmetric unit. Data-collection statistics are given in Table 1.

Elucidation of the SseA structure is presently under way *via* molecular-replacement methods (Navaza, 1994; Kissinger *et al.*, 2001; Vagin & Teplyakov, 1997). For this purpose, the sequence alignment of SseA with bovine and *A. vinelandii* TSTs (Gliubich *et al.*, 1998; Bordo *et al.*, 2000) (Fig. 1) is being used to deduce suitable search models.

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