Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystals of GlpE, a 12 kDa sulfurtransferase from *Escherichia coli*, display 1.06 Å resolution diffraction: a preliminary report

Domenico Bordo,[a,b]* Timothy J. Larson,[c] Janet L. Donahue,[c] Andrea Spallarossa[a,d] and Martino Bolognesi[a,b]

[a]National Institute for Cancer Research c/o Advanced Biotechnology Center, Largo R. Benzi 10, 16132 Genova, Italy, [b]Department of Physics – INFM, University of Genova, Via Dodecaneso 33, 16146 Genova, Italy, [c]Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA, and [d]Department of Pharmaceutical Sciences, University of Genova, Via Benedetto XV 3, 16132 Genova, Italy

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

Received 6 October 2000
Accepted 25 October 2000

The *Escherichia coli sn*-glycerol 3-phosphate regulon contains the *glpE* gene coding for a 12 kDa protein which displays a sequence and a thiosulfate:cyanide sulfurtransferase activity similar to those of rhodanese enzymes. The GlpE protein was overexpressed, purified to homogeneity and crystallized in the trigonal space group $P3_1$ (or $P3_2$). The unit-cell parameters are $a = b = 53.87$, $c = 30.52$ Å, $\gamma = 120^\circ$. Evaluation of the crystal packing parameter establishes the presence of one molecule per asymmetric unit, with a solvent content of 42%. The GlpE crystals display very high resolution diffraction; a 1.06 Å data set was collected using synchrotron radiation ($\lambda = 0.9102$ Å) with an overall completeness of 99.6%.

1. Introduction

Sulfurtransferases (E.C. 2.8.1.*x*) are a class of enzymes that catalyze the formation, interconversion and transport of compounds containing sulfane S atoms. Rhodanese (E.C. 2.8.1.1), which catalyzes the formation of thiocyanate from thiosulfate and cyanide by way of a double-displacement mechanism, is the best characterized enzyme in the family. According to the accepted mechanism, in the presence of both thiosulfate and cyanide rhodanese cycles between a sulfur-free (Rhod) and a sulfur-substituted (Rhod-S) state (1). Rhodanese enzymes are generally composed of two domains (each of about 120 amino acids), henceforth called rhodanese domains or modules, which display very similar three-dimensional structure in the absence of substantial sequence homology. The C-terminal domain contains the active-site Cys residue, while the N-terminal domain mostly plays a structural role.

$$\begin{aligned} \text{Rhod} + S_2O_3^{2-} &\rightarrow \text{Rhod-S} + SO_3^{2-} \\ \text{Rhod-S} + CN^- &\rightarrow \text{Rhod} + SCN^- \end{aligned} \qquad (1)$$

Two rhodanese three-dimensional structures have been solved to date. Bovine (Ploegman *et al.*, 1978) and *Azotobacter vinelandii* rhodanese (Bordo *et al.*, 2000) display similar three-dimensional structures in spite of the low sequence homology (24% identical residues). In both cases, the catalytic Cys residue is located at the bottom of a cradle-like pocket delimitated by a five-residue loop (the catalytic loop) which, however, displays a different amino-acid composition (Bordo *et al.*, 2000). Maintenance of the sulfurtransferase activity in both proteins indicates that the catalytic mechanism is largely based on the conserved main-chain conformation of the catalytic loop, while substrate specificity is modulated in a more complex way by the amino acids surrounding the catalytic centre.

A number of physiological roles have been proposed for rhodanese. The *in vitro* catalyzed reaction and the enzyme abundance in bovine liver mitochondria led to the proposition that this enzyme is, at least in mammals, involved in cyanide detoxification (Sörbo, 1975; Westley *et al.*, 1983). Other roles in sulfur metabolism (Donadio *et al.*, 1990; Klimmek *et al.*, 1998) and biosynthesis or repair of iron–sulfur clusters (Bonomi *et al.*, 1977; Pagani *et al.*, 1983) have been proposed. More recently, extensive genome sequencing has shown that rhodanese or rhodanese-like proteins are present in the major phyla of living organisms. Furthermore, in several bacteria (*e.g. Mycobacterium tuberculosis* and *Synechocystis* sp.) more genes coding for distinct rhodanese-like proteins have been found. These proteins are mostly formed by two consecutive rhodanese modules as observed in rhodanese enzymes. In some cases, however, predicted proteins are comprised of a single rhodanese domain. Examples of proteins with a single rhodanese domain are the senescence-associated protein Sen1 from *Arabidopsis thaliana* (Oh *et al.*, 1996) and the sulfide dehydrogenase from *Wolinella succinogenes* (Krafft *et al.*, 1995). Rhodanese modules are also found covalently bound to other enzyme domains with distinct (putative) functions. Examples with single C-terminally bound rhodanese modules include ThiI from *E. coli* (Palenchar *et al.*, 2000), MoeB from *M. tuberculosis* and NADH-oxidase (NoxA-3) from *Archaeoglobus fulgidus*. These observations indicate that rhodanese domains are likely to be involved in several distinct biological functions.

Table 1
Data-collection statistics.

Values for the last shell (1.08–1.06–Å) are given in parentheses.

Source	X-11 beamline, DESY, Hamburg
Wavelength (Å)	0.9102
Temperature (K)	100
Resolution (Å)	1.06
Number of observations	138403
Unique reflections	44832
Data completeness (%)	99.6 (96.4)
Average redundancy	3.1
$\langle I/\sigma(I)\rangle$	42.8 (2.7)
$R_{\rm merge}$ (%)	2.3 (28.1)

Eight genes coding for rhodanese modules have been identified in *E. coli*. One of them, *glpE*, is found in the *glp* regulon that codes for proteins responsible for the metabolism of *sn*-glycerol 3-phosphate (Ray *et al.*, 2000). The corresponding GlpE protein (108 amino acids, 12 kDa) is comprised of a single rhodanese domain and displays weak but significant sequence homology (17% identical residues) with the C-terminal domain of *A. vinelandii* rhodanese. The catalytic Cys residue (Cys230 in *A. vinelandii* rhodanese), is conserved in GlpE. Notably, the amino-acid sequence (CYHGNS) of the catalytic loop is distinct from that of either the bovine (CRKGVT) or *A. vinelandii* (CQTHHR) enzymes. An *in vitro* colorimetric assay (Sörbo, 1955) has shown that GlpE displays thiosulfate: cyanide sulfurtransferase (*i.e.* rhodanese) activity (Ray *et al.*, 2000). However, the rather high K_m for either thiosulfate or cyanide suggests that these compounds are not the physiological substrates of the enzyme. Further characterization of GlpE suggests that thioredoxin 1 or related dithiol proteins could instead be the physiological sulfur-acceptor substrate of GlpE (Ray *et al.*, 2000).

Determination of the three-dimensional structure of GlpE will help elucidate the catalytic mechanisms and substrate specificity of GlpE and of related domains or proteins within the sulfurtransferase molecular family. We describe here the purification and crystallization of GlpE 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 GlpE protein was purified from *E. coli* strain BL21(DE3)(pGZ105)(pMS421) essentially as described previously (Ray *et al.*, 2000), with minor modifications. In this strain, *glpE* expression from pGZ105 is controlled by a phage T7 promoter. Overexpression of GlpE results following induction of T7 RNA polymerase with isopropylthio-β-D-galactopyranoside (IPTG) [pMS421 (Waldburger & Susskind, 1994) carries *lacI*^{q}, ensuring low uninduced T7 RNA polymerase expression]. Cells from a 1 l culture (induced with IPTG for 4.5 h) were harvested by centrifugation, washed with 500 ml of 25 m*M* Tris acetate pH 8.6, 10 m*M* ammonium thiosulfate and frozen at 203 K. GlpE was released from the cells by three cycles of extraction into 20 ml of 50 m*M* Tris–HCl pH 7.2, 3 m*M* ethylenediamine tetraacetic acid (EDTA) using the freeze–thaw treatment described by Ray *et al.* (2000). Two cycles of chromatography on a Waters quaternary methylamine (Q)-polymethacrylate anion-exchange column (10 × 100 mm; Protein-Pak Q15HR 1000 Å) yielded >30 mg of highly purified GlpE. Peak fractions that contained no detectable impurities as assessed by Coomassie blue staining of a 15% SDS polyacrylamide gel (3 µg GlpE per lane of a minigel) were pooled and stored at 203 K in the presence of 10% glycerol. The rhodanese activity of the purified preparation was ∼200 U mg^{-1}, where 1 U is the amount of enzyme which will catalyse the transformation of 1 µmol of substrate per minute.

2.2. Crystallization

For crystallization purposes, the protein was brought to a final concentration of 8 mg ml^{-1} in a buffer containing 50 m*M* Tris pH 7.0, 3 m*M* EDTA, 100 m*M* NaCl and 10%(*v*/*v*) glycerol. Crystallization conditions were determined using the sparse-matrix method (Jancarik & Kim, 1991), as implemented in Hampton Crystal Screens (Hampton Research, Laguna Hills, CA). Crystals were grown by hanging-drop vapour diffusion employing 2 µl drops containing equal volumes of protein and reservoir solution. Crystals suitable for X-ray diffraction analysis grew over a period of 3–5 d after equilibration against a reservoir solution containing 0.1 *M* $CaCl_2$, 0.1 *M* CH_3COONa pH 4.6 and 20%(*v*/*v*) 2-propanol. The crystallization experiments were carried out at 277 K.

The GlpE crystals had a rather irregular and compact shape, often without clear edges. Their typical dimensions were about 0.2 × 0.2 × 0.2 mm. After removal from the crystallization drop, crystals suffered from instability. Therefore, data collection was carried out within 48 h.

Prior to data collection, crystals could be frozen at 100 K following immersion in a cryoprotectant solution identical to the crystallization solution but containing an additional 20%(*v*/*v*) ethanediol.

2.3. Data collection, processing and analysis

X-ray data collection on a native protein crystal was carried out at 100 K on an MSC R-AXIS II image-plate system coupled to a Rigaku RU-200 rotating-anode generator (Cu $K\alpha$ radiation). The crystal diffracted beyond 2.0 Å resolution and displayed a mosaic spread of 0.3° [completeness, 97.8%; completeness in last shell, 97.0%; $\langle I/\sigma(I)\rangle$, 58; last shell $\langle I/\sigma(I)\rangle$, 5]. Subsequently, synchrotron data collection was carried out on the same crystal (kept frozen in liquid nitrogen) at the X-11 beamline at DESY, Hamburg. A data set was collected to a resolution limit of 1.06 Å. To also achieve a good $I/\sigma(I)$ in high-resolution shells, a rather fine slicing along the spindle axis (0.25° per frame) was adopted. Remarkably, the diffracting power of the GlpE crystal was not affected during data collection.

Analysis of the diffraction pattern showed that the GlpE crystals belong to the trigonal space group $P3_1$ (or $P3_2$), with unit-cell parameters $a = b = 53.87$, $c = 30.52$ Å, $\gamma = 120°$. Estimate of the crystal packing parameter (Matthews, 1968) showed that the lattice accommodates one GlpE molecule per asymmetric unit ($V_{\rm M}$ = 2.13 Å^3 Da^{-1}), with a solvent content of 42%(*v*/*v*).

Data were indexed and reduced with the program *DENZO* (Otwinowski, 1993) and scaled with the program *SCALEPACK* (Otwinowski & Minor, 1997).

The method of molecular replacement, as implemented in the program *AMoRe* (Navaza, 1994), was used in an attempt to solve the structure of GlpE. Several search models were derived from either the high-resolution bovine rhodanese (Gliubich *et al.*, 1998) or from *A. vinelandii* rhodanese (Bordo *et al.*, 2000), but no solution could be found to correctly locate the template molecule in the GlpE unit cell.

The quality of the diffraction data observed in the outermost resolution shell (1.08–1.06 Å, see Table 1) indicates that the diffraction limit of GlpE crystals extends beyond 1.0 Å. Optimization of the exposure time and resolution limit should allow collection of diffraction data beyond 1.0 Å resolution in the near future, possibly rendering GlpE eligible for direct-methods structure solution. The elucidation of the three-dimensional structure of GlpE is in the meantime being pursued through multiple

isomorphous replacement methods by way of heavy-atom screening.

We are grateful to Ehmke Pohl for technical assistance at the X11 EMBL-DESY beamline and for attempting the structure solution of GlpE by direct methods using an unpublished version of *SHELXD*. This research was supported in part by the grant from MURST 'Solfotransferasi procariotiche' (1999–2001) and ASI grant 99-22. Data collection at EMBL-Hamburg was supported by EU funding.

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