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Preliminary crystallographic analysis of the cysteine desulfurase IscS from *Escherichia coli*

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IscS is a widely distributed cysteine desulfurase that catalyzes the pyridoxal phosphate dependent β-elimination of sulfur from L-cysteine and plays a central role in the delivery of sulfur to a variety of metabolic pathways. Crystals of *Escherichia coli* IscS have been obtained by the hanging-drop vapor-diffusion method using polyethylene glycol (PEG) as a precipitant. Initial seed crystals were obtained using PEG 6000 and sodium acetate, and diffraction-quality crystals were grown using a mixture of PEG 2000 and PEG 10 000 in the presence of sodium citrate. A complete native X-ray diffraction data set was collected from a single crystal at 103 K to a resolution of 2.1 Å. The crystals belong to space group $P2_12_12_1$ and have unit-cell parameters $a = 73.7086$, $b = 101.9741$, $c = 108.617$ Å ($\alpha = \beta = \gamma = 90^\circ$). Analysis of the Matthews equation and self-rotation function suggest two molecules per asymmetric unit, consistent with the presence of a single dimeric molecule.

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1. Introduction

IscS and the homologous enzyme NifS comprise a widely distributed class of cysteine desulfurases that play important roles in biological sulfur mobilization. IscS and NifS are each homodimers (~45 kDa subunits) and utilize pyridoxal 5′-phosphate to catalyze the β-elimination of sulfur from L-cysteine to yield alanine and either sulfane (S°) or, in the presence of a reducing agent, sulfide ($S^=$). The reaction mechanism, which involves formation of an enzyme-bound cysteine-persulfide intermediate, was initially elucidated in studies of NifS from *Azotobacter vinlandii* (Zheng *et al.*, 1993, 1994) and subsequently described for IscS from *Escherichia coli* (Flint, 1996). The sulfur made available by NifS and IscS is utilized in the biogenesis of iron–sulfur clusters (Zheng & Dean, 1994; Flint, 1996) and in the biosynthesis of thiamine (Lauhon & Kambapati, 2000), thionucleotides (Kambampati & Lauhon, 1999) and biotin (Bui *et al.*, 2000; Jarrett, 2001).

Recently, the crystal structure of NifS from the hyperthermophilic bacterium *Thermotoga maritima* was reported (Kaiser *et al.*, 2000). The general structure revealed that the protein is a homodimer, each subunit of which can be divided into a small and a large domain similar to that of α-family type IV aminotransferases (Mehta & Cristen, 2000. However, regions of the active site of the *T. maritima* NifS model, including the cysteine residue implicated in sulfur transfer (Zheng *et al.*, 1994), were disordered and omitted from the model. In addition, *T. maritima* NifS exhibits limited sequence similarity to NifS and IscS of higher organisms (<40% sequence identity) and is slightly smaller in size, lacking approximately 20 residues present at the C-terminus of proteins from higher organisms. In the case of *E. coli* IscS, the C-terminal region has been shown to be necessary for interactions with IscU, a sulfur acceptor involved in iron–sulfur cluster formation (Urbina *et al.*, 2001).

We have developed methods for high-level expression and purification of IscS from *E. coli*. The *E. coli* IscS protein sequence is 37% identical to that of *T. maritima* NifS and exhibits greater sequence similarity to IscS and NifS of higher organisms (*e.g.* 56% identity to human IscS). Analysis of the *E. coli* IscS structure may lead to a further understanding of the catalytic mechanism of IscS/NifS enzymes as well as providing information relevant to interactions with its sulfur-transfer partners. Herein, we describe the crystallization and preliminary X-ray diffraction analysis of *E. coli* IscS.

2. Experimental results

E. coli IscS was overexpressed and purified as previously described (Urbina *et al.*, 2001). Crystallization of *E. coli* IscS was achieved by the hanging-drop vapor-diffusion method (McPherson, 1999) at room temperature. Initial crystals with irregular shape were obtained using a mother liquor consisting of 13%(*w*/*v*) PEG 6000, 0.1 *M* Tris–HCl pH 9, 0.1 *M* sodium acetate and 5 m*M* dithiothreitol. Equal volumes of IscS ($16\ \mathrm{mg\ ml^{-1}}$) and

Table 1
Summary of crystallographic data.

Values in parentheses refer to the outermost shell (2.15–2.10 Å).

Unit-cell parameters (Å, °)	a = 73.709, b = 101.974, c = 108.617, $\alpha = \beta = \gamma = 90$
Space group	$P2_12_12_1$
Total No. of reflections	146645
No. of unique reflections	43267
Multiplicity	3.2 (3.1)
Completeness (%)	96.9 (93.3)
Intensity/error [$I/\sigma(I)$]	7.1 (2.7)
$R_{\rm sym}$† (%)	6.8 (24.5)

† $R_{\rm sym} = 100\sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the observed intensity and $\langle I\rangle$ is the statistically weighted average intensity of multiple observations of symmetry-related reflections.

mother liquor were mixed to form a 6 µl drop. Small crystals formed spontaneously over 24–48 h and these were used to produce a microseed stock. The final crystallization conditions consisted of 12%(w/v) PEG 10 000, 20%(w/v) PEG 2000, 0.1 M Tris–HCl pH 9 and 70 mM sodium citrate pH 6.5. The hanging drop consisted of 3 µl IscS (10 mg ml^{-1}) and 3 µl of mother liquor and was microseeded after a 48 h equilibration period. Using this crystallization procedure, crystals with cubic shape (of dimensions up to 100 µm) were generated and subsequently used for diffraction studies. Prior to data collection, crystals were transferred to a cryoprotectant solution consisting of the same mother-liquor solution containing ethylene glycol [20%(v/v)] and were flash-cooled in liquid nitrogen.

Diffraction data from the *E. coli* IscS crystals were collected at Stanford Synchrotron Radiation Laboratory (SSRL) beamline 9-2. A complete set of native data was obtained from a single crystal (approximately 0.1 × 0.1 × 0.05 mm in size) at 103 K. Diffraction data were reduced using *MOSFLM* (Leslie, 1998) and scaled with the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). A summary of the crystallographic data is presented in Table 1. Crystals were found to be orthorhombic and to belong to the $P2_12_12_1$ space group with unit-cell parameters a = 73.709, b = 101.974, c = 108.617 Å ($\alpha = \beta = \gamma = 90°$). The crystals diffracted to ∼2 Å with good completeness and $I/\sigma(I) > 2$ at 2.1 Å (see Table 1).

To determine the number of molecules in the asymmetric unit, the Matthews equation (Matthews, 1968) and self-rotation analysis (Brünger *et al.*, 1998) were applied to the diffraction data. Assuming two molecules of IscS per asymmetric unit, the volume-to-mass ratio ($V_{\rm M}$) was found to be 2.27 Å^3 Da^{-1} and the solvent content was calculated to be 45%; these values are in the range commonly observed for protein crystals (Matthews, 1968). Self-rotation analysis of the data indicated a strong peak at κ = 180°, indicating twofold non-crystallographic symmetry. Together, these results suggest that there are two molecules of IscS per asymmetric unit. Solution measurements indicate that IscS exists as a dimer (Flint, 1996), consistent with one dimeric molecule in the asymmetric unit.

E. coli IscS shares 37% sequence identity with *T. maritima* NifS, the X-ray structure of which has been reported to 2.0 Å (Kaiser *et al.*, 2000). The identification of a molecular-replacement solution using *T. maritima* NifS was pursued using *CNS* (Brünger *et al.*, 1998). A monomer of *T. maritima* NifS including residues conserved in *E. coli* IscS and alanine in place of non-identical residues was used as the search model. Rotation and translation searches gave a single best solution with a final packing value of 50% with two polypeptides in the asymmetric unit. The molecular-replacement solution yielded a crystallographic R factor of 42.9% ($R_{\rm free}$ = 46.5%) following a single round of simulated annealing in *CNS*. This model will be used to determine initial model phases and as the starting structure for model building. In addition, we are presently searching for heavy-atom derivatives to use for isomorphous replacement and multiple anomalous dispersion to improve the phases and reduce model bias.

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