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Expression, crystallization and preliminary crystallographic analysis of SufE (XAC2355) from *Xanthomonas axonopodis* pv. *citri*

Cristiane R. Guzzo,[a] Lucicleide R. Silva,[a] Leonor M. P. Galvão-Botton,[a] João A. R. G. Barbosa[b] and Chuck S. Farah[a]*

[a]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, CEP 05508-000, São Paulo, SP, Brazil, and [b]Centro de Biologia Molecular Estrutural, LNLS, CEP 13084-971, Campinas, SP, Brazil

Correspondence e-mail: chsfarah@iq.usp.br

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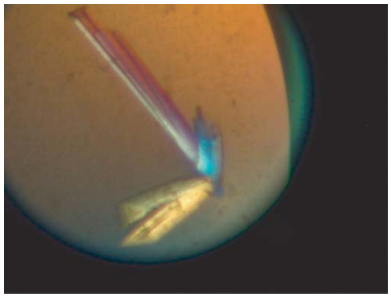
Xanthomonas axonopodis pv. *citri* (*Xac*) SufE (XAC2355) is a member of a family of bacterial proteins that are conserved in several pathogens and phytopathogens. The *Escherichia coli suf* operon is involved in iron–sulfur cluster biosynthesis under iron-limitation and stress conditions. It has recently been demonstrated that SufE and SufS form a novel two-component cysteine desulfarase in which SufS catalyses the conversion of L-cysteine to L-alanine, forming a protein-bound persulfide intermediate. The S atom is then transferred to SufE, from which it is subsequently transferred to target molecules or reduced to sulfide in solution. Here, the cloning, expression, crystallization and phase determination of *Xac* SufE crystals are described. Recombinant SufE was crystallized in space group $P2_12_12_1$ and diffracted to 1.9 Å resolution at a synchrotron source. The unit-cell parameters are a = 45.837, b = 58.507, c = 98.951 Å, $\alpha = \beta = \gamma = 90^\circ$. The calculated Matthews coefficient indicated the presence of two molecules in the asymmetric unit. Phasing was performed by molecular-replacement using *E. coli* SufE as a model (PDB code 1mzg) and an interpretable map was obtained.

1. Introduction

Xanthomonas axonopodis pv. *citri* (*Xac*) SufE (XAC2355) is a member of a family of proteins that are conserved in many prokaryotic pathogens and phytopathogens, including *Salmonella* ssp., *Escherichia coli*, *Pseudomonas* ssp., *Shigella* ssp., *Yersinia* ssp., *Leptospira interrogans*, *Erwinia carotovora*, *Vibrio* ssp., *Azotobacter vinelandii*, *Shewanella baltica*, *Xanthomonas* spp. and *Xylella* ssp. DNA sequences coding for SufE homologs have also been found in several eukaryotic genomes: *Plasmodium*, *Anopheles gambiae* and *Arabidopsis thaliana*. This protein was originally selected for structural analysis owing to its well folded and non-aggregated state in solution at a time when its function was unknown (Galvão Botton *et al.*, 2003).

In *E. coli*, the *sufABCDSE* cluster is expressed under conditions of iron limitation and oxidative stress, one of its functions being the restoration of damaged Fe–S clusters (Nachin *et al.*, 2001, 2003). In *E. coli*, SufA is a scaffold protein for the assembly of Fe–S clusters. SufB and SufD form a complex with SufC, a soluble cytoplasmic ABC-ATPase. SufBCD stimulates the cysteine desulfurase activity of the SufS–SufE complex (Outten *et al.*, 2003). In *Xac*, the cluster of genes *XAC2935-XAC2936-XAC2937-XAC2938* codes for homologues of SufB, SufC, SufD and SufS, respectively, although they were not annotated as such in the *Xac* genome (da Silva *et al.*, 2002). Genes coding for homologues of SufA (*XAC1619*) and SufE (*XAC2355*) are found elsewhere in the *Xac* genome.

Cysteine desulfurases are important pyridoxal 5′-phosphate (PLP) dependent enzymes that are involved in sulfur mobilization in many living organisms (Marquet, 2001; Mihara *et al.*, 2002; Fontecave *et al.*, 2003; Zheng *et al.*, 1994). The chemical steps involved in the cysteine desulfurase activity of the SufS–SufE complex consist of two stages. The first stage consists of the PLP-dependent transfer of sulfur from L-cysteine to a Cys residue on SufS, forming a persulfide linkage. The sulfur is then passed from SufS to a Cys residue on SufE, where it also forms a persulfide linkage (Loiseau *et al.*, 2003; Ollagnier-de-Choudens *et al.*, 2003). The cysteine desulfurase activity of SufS on its



own is approximately 30-fold to 50-fold less than that of the SufS–SufE complex (Loiseau *et al.*, 2003).

Recently, the structures of five SufE homologues have been determined: the NMR structures of *E. coli* YjdK (PDB code 1ni7; Liu *et al.*, 2005), *Mus musculus* IscU (PDB code 1wfz; unpublished data) and *Haemophilus influenzae* IscU (PDB codes 1r9p and 1q48; Ramelot *et al.*, 2004) and the crystal structures of *E. coli* SufE (PDB code1mzg; Goldsmith-Fischman *et al.*, 2004) and *Bacillus subtilis* IscU (PDB code 1xjs; unpublished data). In this report, we describe the cloning, expression, purification and crystallization of recombinant *Xac* SufE. An initial electron-density map of the *Xac* SufE crystal structure has been obtained by molecular replacement using the *E. coli* SufE structure as a model (PDB code 1mzg; Goldsmith-Fischman *et al.*, 2004).

2. Cloning and expression of *XAC2355*

The gene coding for SufE (*XAC2355*) was amplified by PCR from *Xac* genomic DNA using the following primers designed based on the published *Xac* genome sequence (da Silva *et al.*, 2002): forward, 5′-CATGCCATGGCTCATATGACCACCTCCCCCTTCC-3′; reverse, 5′-GGAATTCAAGCTTTCACTGCTGCGCGCG-3′). The PCR product was digested with *Hin*dIII and *Nde*I and subcloned into the pET-3a vector (Studier *et al.*, 1990) previously digested with the same endonucleases. SufE was expressed in *E. coli* strain BL21(DE3)pLysS (Studier *et al.*, 1990). The protein was produced by growing a culture in 2×TY medium to an optical density (600 nm) of 0.8, at which point heterologous protein expression was induced by the addition of 1 m*M* isopropyl *β*-D-thiogalactopyranoside and the cells were harvested after growing for 4 h and stored at 203 K.

3. Protein purification

Cells from 1 l culture were resuspended in 25 ml 50 m*M* Tris–HCl pH 8.0, 25% sucrose, 1 m*M* EDTA, 1 m*M* PMSF and lysed using a French press. The soluble fraction was applied onto a Q-Sepharose Fast Flow (FF) Hi-Load 16/10 column (Amersham Pharmacia) previously equilibrated with 50 m*M* Tris–HCl pH 7.0, 1 m*M* EDTA and 14 m*M* *β*-mercaptoethanol. Bound proteins were eluted using a 12 column volume 0–300 m*M* NaCl gradient. Fractions containing SufE were concentrated using an Amicon system with a 10 kDa molecular-weight cutoff membrane and then purified further by gel filtration on a Superdex 75 prep-grade column (Amersham Pharmacia) equilibrated with 5 m*M* Tris–HCl pH 7.0. The purity of the protein was judged by visualization of Coomassie-stained SDS–PAGE.

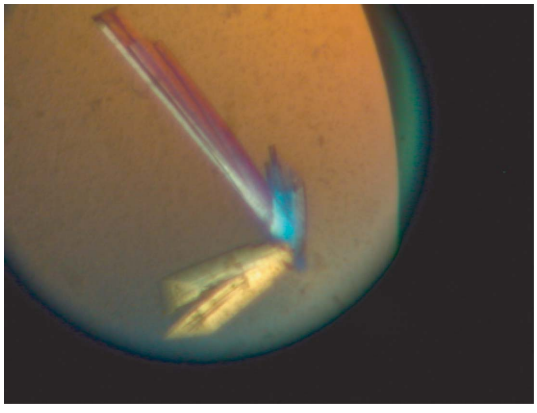


Figure 1
Crystals of SufE. The largest plates are approximately 400 µm in the longest dimension.

Table 1
Crystal parameters and data-reduction statistics.

The data sets were measured from a single crystal. Values in parentheses refer to the highest resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters	
a (Å)	45.837
b (Å)	58.507
c (Å)	98.951
No. of images	212
Resolution range (Å)	50.00–1.90 (1.97–1.90)
Wavelength (Å)	1.438
No. of observed reflections	128189
No. of unique reflections	20431 (2002)
$\langle I/\sigma(I)\rangle$	25.6 (4.4)
Multiplicity	6.3 (5.8)
Completeness (%)	93.5 (93.3)
R† (%)	5.8 (37.3)

† $R = \sum|I(h) - \langle I(h)\rangle|/\sum I(h)$.

4. Crystallization

Xac SufE crystals (Fig. 1) were grown using the hanging-drop vapour-diffusion technique at 291 K. Initial crystallization conditions that produced needle-like crystals were obtained by the sparse-matrix sampling approach using the Index crystallization screen kit from Hampton Research. Optimization was then pursued by varying the precipitant concentration and the buffer pH. Suitable crystals for diffraction experiments were obtained by mixing 1 µl of a 9.8 mg ml^{-1} protein solution (5 m*M* Tris–HCl pH 7.0) with 1 µl reservoir solution consisting of 17–21%(*w*/*v*) PEG 5000 and 0.1 *M* Tris–HCl pH 7.0, followed by equilibration against 0.4 ml reservoir solution. Rectangular crystals appeared within a few days and grew to mature size within a few weeks.

5. Data collection and preliminary structure analysis

X-ray diffraction data were collected at the D03B beamline of the Laboratório Nacional de Luz Síncrotron, Campinas, Brazil using a MAR CCD detector. Crystals equilibrated against 19% PEG 5000 and 0.1 *M* Tris–HCl pH 7.0 were transferred to solution containing

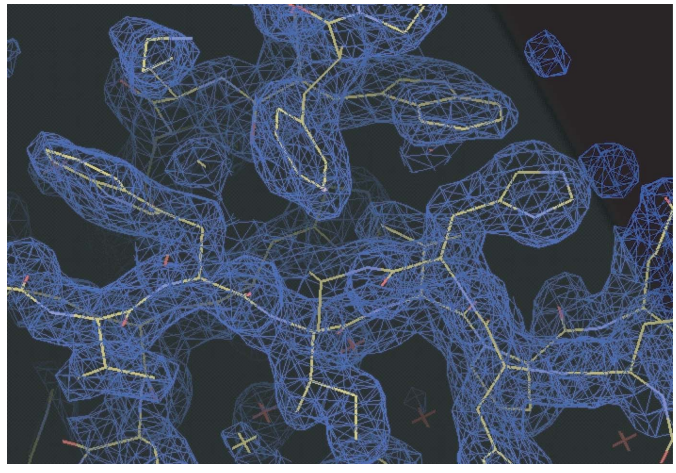


Figure 2
A section of the current SufE crystal $2F_o - F_c$ electron-density map contoured at 1.0σ.

26% PEG 400 for cryoprotection. The crystal was then flash-frozen in liquid nitrogen and maintained at 100 K in a nitrogen-gas stream during data acquisition. A single data set was collected at 1.438 Å using 1 min exposure and 1° oscillation from 1 to 212°. The diffraction patterns from this data set extended to approximately 1.9 Å resolution (Table 1). The crystal belongs to space group $P2_12_12_1$, with unit-cell parameters a = 45.837, b = 58.507, c = 98.951 Å, $\alpha = \beta = \gamma = 90^\circ$. There are two protein molecules per asymmetric unit, as suggested by the Matthews coefficient (V_M = 2.7 Å^3 Da^{-1}; solvent content 40.4%).

Diffraction data were indexed, integrated, scaled and merged using the *HKL*2000 package (Otwinowski & Minor, 1997). Details of data acquisition and data-processing statistics are shown in Table 1. Initial phases of the *Xac* SufE crystal were calculated by molecular replacement using *Phaser* (Storoni *et al.*, 2004; Read, 2001) from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) with the *E. coli* SufE structure 1mzg (Goldsmith-Fischman *et al.*, 2004) as the search model. The *E. coli* and *Xanthomonas* proteins share 34% sequence identity. The phases were improved by density-modification protocols using the programs *SOLOMON* (Abrahams, 1997) and *DM* (Collaborative Computational Project, Number 4, 1994; Cowtan, 1994). Interpretation of electron-density maps (Fig. 2) and model refinement are currently under way using the programs *COOT* Emsley & Cowtan, 2004), *REFMAC* (Murshudov *et al*., 1997) and *CNS* (Brünger *et al.*, 1998).

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