Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Expression, crystallization and preliminary crystallographic analysis of YciE, a stress protein from *Escherichia coli*

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Received 14 May 2004
Accepted 28 July 2004

The stress protein *Escherichia coli* YciE was overexpressed and purified in three chromatographic steps. Crystals were obtained using PEG 4000 as a precipitant. The YciE protein crystals diffracted to 3.0 Å resolution using a rotating-anode X-ray source. The lattice type is rhombohedral, with unit-cell parameters $a = b = 64.2$, $c = 167.9$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. The crystal belongs to space group $R32$.

1. Introduction

Organisms express a set of proteins to afford protection under a variety of stress conditions. Knowing the mechanism by which stress proteins protect cells is important for understanding the pathology of a broad set of diseases and for the development of therapeutics. *Escherichia coli* YciE protein is encoded by the gene *yciE* (Blattner *et al.*, 1997). YciE, a 19 kDa protein, was accumulated in a H-NS deletion strain, a genetic background that is known to derepress stress-gene expression (Yoshida *et al.*, 1993). Primary sequence alignment indicated that the YciE protein is conserved in a wide range of bacterial genomes. Interestingly, there is also sequence similarity with the *yciF* gene of *E. coli*, a member of the same operon that encodes another heat-shock protein of unknown function.

Most heat-shock proteins (HSPs) function as chaperones (GroEL, GroES *etc.*), proteases (ClpP, HslV *etc.*) or transcription regulators (PhoB, RseA *etc.*). To obtain a better understanding of the possible molecular function of the YciE stress protein, it was expressed in large quantities, purified to homogeneity and crystallized in preparation for X-ray crystallographic structure determination. Here, we report the crystallization and preliminary X-ray analysis of the *E. coli* YciE protein.

2. Materials and methods

2.1. Protein preparation

Genomic DNA was purified from *E. coli* K-12 strain MG1655 using a genomic DNA purification kit (Promega). The gene for the YciE protein was cloned by PCR using the oligonucleotide primers 5′-AGGGGCGC-CATGAATCGTATTGAACATTATC-3′ and 5′-CCGGAATTCTTATTTCTTCGCTTCTAC-GCCATC-3′ (Sigma-Genosys). The PCR product was digested with *Kas*I and *Eco*RI restriction enzymes (New England Biolabs) and ligated with pProEX-1 vector treated with the same restriction enzymes. The recombinant plasmid encoded a fusion protein with an N-terminal hexahistidine tag separated from the YciE protein by a tobacco etch virus (TEV) protease cleavage site and was designated p211 (Phan *et al.*, 2002). After confirmation of the plasmid's yciE DNA sequence at the UTMB recombinant DNA laboratory, p211 was transfected into DH10B cells for overexpression.

The cells were grown at 310 K in LB medium containing 50 µg ml^{-1} ampicillin. When the absorbance of the culture reached 0.6 at 600 nm, the culture was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.8 m*M*. 3 h after induction, cells were harvested and disrupted by sonication in buffer *A* (10 m*M* imidazole, 50 m*M* Tris pH 8.2, 300 m*M* NaCl, 2 m*M* 2-mercaptoethanol). Protein purification was performed at room temperature. After centrifugation of the homogenate at 20 000*g* for 30 min, the supernatant was applied to a nickel–nitrilotriacetic acid (Ni^{2+}–NTA) column (Qiagen). His-tagged YciE was eluted from the column with a gradient of 0–60% buffer *B* (1 *M* imidazole, 50 m*M* Tris pH 8.2, 300 m*M* NaCl, 2 m*M* 2-mercaptoethanol). Protein was dialyzed in 3 × 500 ml buffer *C* (25 m*M* Tris pH 8.0, 100 m*M* NaCl, 2 m*M* 2-mercaptoethanol) to remove imidazole. The protein concentration was measured by absorbance at 280 nm using an extinction coefficient of 19 060 M^{-1} cm^{-1}, assuming that all Cys residues are reduced. The N-terminal six-histidine tag was removed by overnight incubation with 0.1 mg of TEV protease per milligram of recombinant protein. The cleaved protein was loaded onto an Ni^{2+}–NTA column to remove the cleaved His tag and the TEV protease, which also contained a His tag (Kapust *et al.*, 2001). The flowthrough of the second Ni^{2+}–NTA column was concentrated using a Centricon YM-10 (Millipore) and further purified on a Superdex 75 column (Amersham

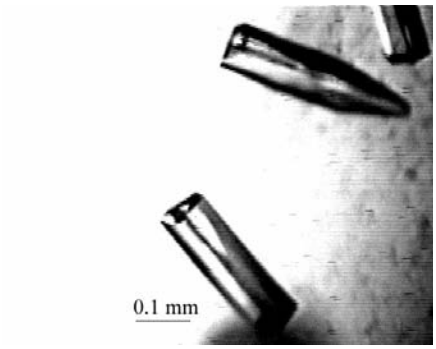

Figure 1
Typical crystals of YciE. The typical dimensions of diffraction-quality crystals are approximately 0.35 × 0.1 × 0.1 mm.

Pharmacia Biotech) equilibrated with buffer *C*. The homogeneity of the purified preparation was judged by SDS–PAGE and mass spectrometry. The typical yield of purified YciE protein was about 10 mg per litre of culture.

2.2. Crystallization and data collection

The purified YciE protein was concentrated to 7.0 mg ml^{-1} in sample buffer (25 m*M* Tris pH 8.0, 100 m*M* NaCl and 5 m*M* DTT) and crystallized using the hanging-drop vapour-diffusion technique. Preliminary screening was carried out using Hampton Research crystal screen kits (Crystal Screen, Crystal Screen II, Crystal Screen HT, Index HT and SaltRx HT) using both manual and robotic methods (HYDRA-PLUS-ONE Robotic System, Apogent Technologies Inc.). Several conditions (6 and 17 of Crystal Screen, B9 and B10 of SaltRx HT, A6, B5, B10, C8 and F2 of Crystal Screen HT and H1 of Index HT) yielded small crystals. After optimization of the crystallization conditions, crystals were obtained from 0.1 *M* Tris pH 7.5–8.5, 0.2 *M* magnesium chloride, 23–28% PEG 4000 (Fig. 1). A crystal was step-soaked into a cryoprotectant consisting of 0.1 *M* Tris pH 8.0, 0.2 *M* magnesium chloride, 30% PEG 4000 and 30%(*v*/*v*) glycerol, scooped up in a cryoloop, cooled in liquid nitrogen and mounted on the goniometer in a nitrogen stream at 100 K. X-ray diffraction was measured in-house on a MacScience X-ray rotating-anode generator with a DIP2030 imaging plate for macromolecular crystallography with a crystal-to-detector distance of 250 mm. Diffraction data were collected from a YciE crystal to 3.0 Å resolution and were indexed, integrated and scaled with *DENZO* and *SCALEPACK* from the *HKL* package (Otwinowski & Minor, 1997).

Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell (3.07–3.00 Å).

Space group	*R*32
Unit-cell parameters	
$a = b$ (Å)	64.2
c (Å)	167.9
Temperature (K)	100
Wavelength (Å)	1.54178
Resolution (Å)	90–3.0
Total No. reflections	125143
No. unique reflections	2782
Completeness (%)	96.9 (95.2)
$R_{\rm merge}$	0.127 (0.606)
Mean $I/\sigma(I)$	20.5 (2.8)

3. Results and discussion

The TEV-cleaved recombinant protein left two vector-derived residues Gly-Ala at the N-terminus of the endogenous YciE protein, with a calculated molecular weight of 19 089.5 Da (170 residues). YciE protein eluted on a Superdex 75 size-exclusion column as a homodimer. The purified YciE protein migrated as a single 19 kDa band with >95% homogeneity on 15% SDS–PAGE gel.

The YciE protein crystals diffracted to 3.0 Å resolution on a rotating-anode X-ray generator. The lattice type was rhombohedral, with unit-cell parameters $a = b = 64.2$, $c = 167.9$ Å. The crystal belongs to space group *R*32. Assuming the presence of one molecule per asymmetric unit, the calculated Matthews coefficient ($V_{\rm M}$) was 1.75 Å^3 Da^{-1}, with a solvent content of 29.5% (Matthews, 1968). Data-collection statistics are given in Table 1. The structure of the YciE protein is under investigation.

We thank Dr Mark A. White for maintaining the X-ray diffraction facility and for helpful discussions. We thank Dr Yousif Shamoo for access to the HYDRA-PLUS-ONE robotic system at Rice University. We thank Dr David Waugh for the TEV expression construct and Dr David Konkel for editing the manuscript. A Robert A. Welch Foundation award to ROF (H-1345) funded this work.

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