

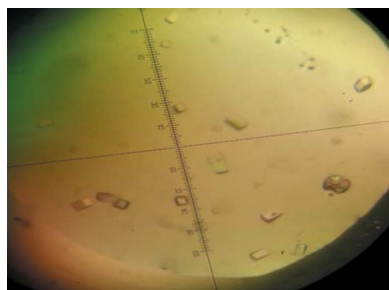
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## Crystallization and preliminary X-ray diffraction analysis of UspE from *Escherichia coli*

Universal stress proteins (Usps) are among the most highly induced genes when bacteria are subjected to several stress conditions such as heat shock, nutrient starvation or the presence of oxidants or other stress agents. *Escherichia coli* has five small Usps and one tandem-type Usp. UspE (or YdaA) is the tandem-type Usp and consists of two Usp domains arranged in tandem. To date, the structure of UspE remains to be elucidated. To contribute to the molecular understanding of the function of the tandem-type UspE, UspE from *E. coli* was overexpressed and the recombinant protein was purified using Ni-NTA affinity, Q anion-exchange and gel-filtration chromatography. Crystals of UspE were obtained by sitting-drop vapour diffusion. A diffraction data set was collected to a resolution of 3.2 Å from flash-cooled crystals. The crystals belonged to the tetragonal space group  $I4_122$  or  $I4_322$ , with unit-cell parameters  $a = b = 121.1$ ,  $c = 241.7$  Å.

### 1. Introduction

Universal stress proteins (Usps) are widespread proteins in the genomes of bacteria, archaea, fungi, flies and plants (Siegele, 2005; Gustavsson *et al.*, 2002). The expression of Usps in bacteria is triggered in response to a large variety of adverse environmental factors such as heat and the presence of uncouplers, polymyxin, cycloserine, oxidants, metals, ethanol, nutrient starvation or DNA-damaging agents (Siegele, 2005; Tkaczuk *et al.*, 2013). The overproduced Usps have been shown to play an important role in stress resistance and to aid survival through various mechanisms in several bacterial species (Nyström & Neidhardt, 1994). Usp-containing organisms are usually equipped with several *usp* genes (Kvint *et al.*, 2003). The *usp* genes usually encode either small Usps (around 14–15 kDa) harbouring one Usp domain or a large version (around 30 kDa) consisting of two Usp domains (Pfam accession No. PF0582) in tandem. Usp domains are also found in combination with other various domains, such as transporters, permeases, channels and protein kinases, in different organisms (Kvint *et al.*, 2003).

*Escherichia coli* has six Usp proteins. Five of them have one Usp domain: UspA, UspC (YecG), UspD (YiiT), UspF (YnaF) and UspG (YbdQ). UspE (YdaA) is a tandem-type Usp that contains two Usp domains (Sousa & McKay, 2001). Based on structural and amino-acid sequence analyses, *E. coli* Usps or Usp domains have been divided into four different classes. UspA, UspC and UspD belong to class I, whereas UspF and UspG belong to class II. The first Usp domain of UspE belongs to class III and the second domain to class IV (Zarembinski *et al.*, 1998). The Usps have evolved different physiological functions. For example, the class I UspA and UspD are required for defence against superoxide-generating agents, while the class I UspC and the class II UspF and UspG are instead mainly related to cellular adhesion and motility. In addition, the role of the unique Usp protein of class III/IV, UspE, overlaps with the functions of all of the other classes (Nachin *et al.*, 2005; Diez *et al.*, 2000). These similarities and differences in expression patterns correlate with the biological roles defined by the analysis of *usp* mutants. However, the molecular function and the mechanism of action of the Usp proteins mostly remains unclear.

Many Usp protein structures have been solved using X-ray crystallography. However, the structure of UspE has not been published to date, although the crystal structure of UspE from *Proteus mirabilis* is available in the PDB. The structure of the UspE from *E. coli* is required to understand the function of this tandem-type Usp, as the Usp proteins from *E. coli* have been the most extensively studied. Here, we report the purification, crystallization and preliminary X-ray analysis of the intact form of UspE from *E. coli*.

## 2. Materials and methods

### 2.1. Construction of the UspE expression plasmid

To construct the plasmid expressing UspE, the DNA fragment encoding *E. coli* O157:H7 UspE (residues 1–316) was amplified from the genomic DNA of *E. coli* O157:H7 strain by PCR using standard methods and the following oligonucleotides: 5'-GGGCCATGGAC-ATGGCTATGTATCAGAAC-3' and 5'-GGGCTCGAGTAAATC-GTCTTCTTCGTC-3'. The PCR product for the fragment was digested and ligated into the *Nco*I and *Xho*I sites of pPROEX-HTA (Invitrogen, USA) vector, which contains a hexahistidine tag-containing region (Met-Ser-Tyr-Tyr-His-His-His-His-His-Asp-Tyr-Asp-Ile-Pro-Thr-Thr) and a TEV protease cleavage site (Glu-Asn-Leu-Tyr-Phe-Gln) at the N-terminus. The resulting plasmid (pPROEXHTA-UspE) expressed a hexahistidine tag and a TEV protease cleavage site at the N-terminus of UspE.

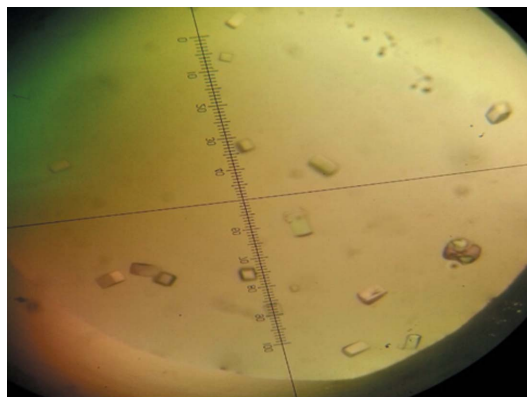
### 2.2. Expression and purification of the recombinant UspE protein

The recombinant plasmid pPROEXHTA-UspE was transformed into *E. coli* BL21(DE3) cells to produce the UspE protein. The cells were grown in 1.5 l LB broth with 50  $\mu\text{g ml}^{-1}$  ampicillin at 310 K until an  $\text{OD}_{600}$  of 0.6–0.8 was reached. Protein expression was induced by adding 0.5 mM isopropyl  $\beta$ -D-1-thiogalactopyranoside to the culture at 303 K. Cells were harvested by centrifugation 6 h after induction and were then stored at 193 K until use. The harvested cells were suspended in a lysis buffer consisting of 20 mM Tris pH 8.0, 150 mM NaCl, 2 mM  $\beta$ -mercaptoethanol and were disrupted by sonication. The lysate was centrifuged at 45 000g for 30 min at 277 K. The resulting supernatant was mixed with Ni-NTA affinity resin (Qiagen, Netherlands) that had been pre-incubated with Tris buffer; the mixture was then stirred for 30 min at 4°C. After the slurry had been loaded into the column, unbound proteins were washed out with lysis buffer supplemented with 20 mM imidazole. Recombinant UspE with the hexahistidine tag was eluted with 30 ml Tris buffer supplemented

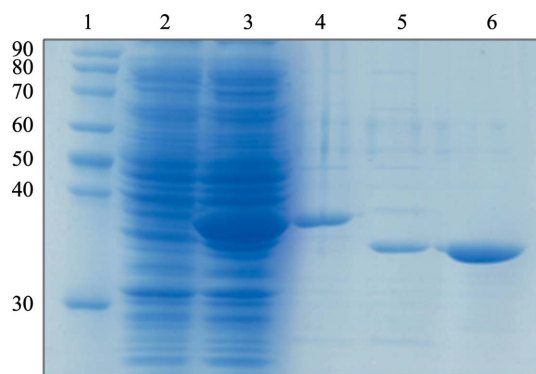
with 200 mM imidazole. The eluted fractions were analyzed by SDS-PAGE. Fractions containing UspE were pooled and  $\beta$ -mercaptoethanol was added to a final concentration of 10 mM. This solution was incubated with recombinant TEV protease overnight at 298 K to remove the hexahistidine tag. The UspE was diluted threefold with 20 mM Tris pH 8.0 buffer and then loaded onto a HiTrap Q column (GE Healthcare, USA). The protein was eluted from the column using a linear gradient of 0–1 M NaCl in 20 mM Tris buffer pH 8.0. The collected fractions containing the UspE protein were concentrated using Centrprep (Millipore, USA) and separated on a HiLoad Superdex 200 gel-filtration column (GE Healthcare, USA) pre-equilibrated with lysis buffer. Total proteins were analyzed by SDS-PAGE on a 15% gel and stained with Coomassie Blue.

### 2.3. Crystallization and crystallographic data collection

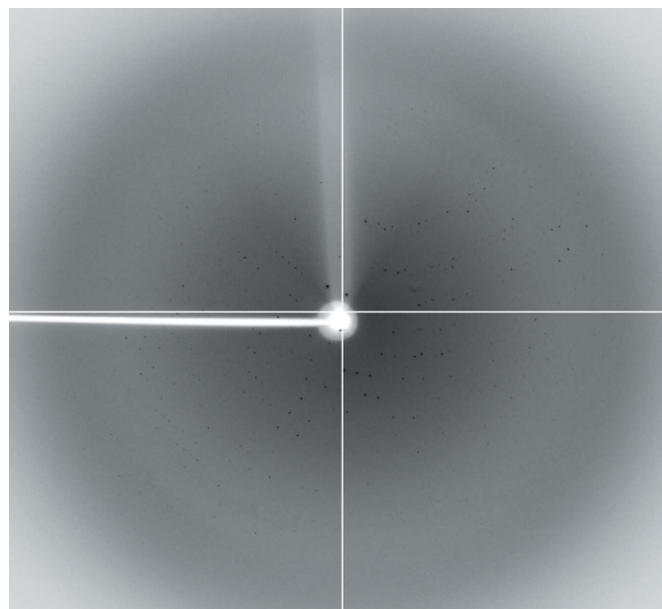
Prior to crystallization, the pooled sample of purified UspE was concentrated to 10 mg  $\text{ml}^{-1}$  using a Vivaspin centrifugal concentrator



**Figure 2**  
Crystals of *E. coli* UspE. The approximate dimensions of the crystals are  $0.09 \times 0.09 \times 0.2$  mm.



**Figure 1**  
SDS-PAGE of purified UspE. Lane 1, molecular-weight markers (labelled in kDa); lane 2, uninduced cell supernatant; lane 3, 0.5 mM IPTG-induced cell supernatant; lane 4, pooled fractions eluted from a nickel-ion affinity column; lane 5, after treatment with TEV protease; lane 6, final sample.



**Figure 3**  
Representative diffraction pattern from a crystal of UspE. The diffraction pattern was displayed using *MOSFLM* (Battye *et al.*, 2011). The resolution at the edge of the image is 3.0 Å.

**Table 1**

Diffraction statistics.

X-ray source	Beamline 7A, PLS
Space group	$I4_122$ or $I4_322$
Wavelength (Å)	1.000
Resolution (Å)	38–3.2
Unit-cell parameters (Å, °)	$a = b = 121.1$ , $c = 241.7$ , $\alpha = \beta = \gamma = 90$
Completeness (%)	92.7 (90.2)
$R_{\text{merge}}^{\dagger}$ (%)	15.3 (33.4)
Multiplicity	5.3 (3.6)
Average $I/\sigma(I)$	9.2 (3.3)

$\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$ , where  $I_i(hkl)$  is the intensity of the  $i$ th measurement of reflection  $hkl$ ,  $\langle I(hkl) \rangle$  is the sum over all reflections and  $\sum_i$  is the sum over  $i$  measurements of reflection  $hkl$ .

fitted with a 10 kDa molecular-weight cutoff filter (Millipore, USA). Initial crystallization of UspE was performed with Crystal Screen HT, a high-throughput sparse-matrix screening kit (Hampton Research, USA), using the microbatch method at various temperatures (277, 279, 288 and 295 K). Crystals of the recombinant UspE protein were obtained by vapour diffusion, but only at 288 K. X-ray diffraction data were collected using an ADSC Q310 CCD detector on beamline 7A of Pohang Light Source (PLS), Republic of Korea.

### 3. Results and discussion

The recombinant full-length UspE protein was successfully expressed at 30°C in the cytosol of *E. coli* and the protein was purified to a sufficient level for crystallization using the protein-purification methods described in §2. The purity of the protein was determined by Coomassie-stained SDS-PAGE (Fig. 1). The protein sample successfully crystallized under several conditions in the initial crystallization screening trials using the sitting-drop vapour-diffusion method. The crystallization conditions were optimized to produce shiny single crystals (Fig. 2) in droplets consisting of 1 µl protein solution (10 mg ml<sup>-1</sup> protein in 20 mM Tris buffer pH 8.0 containing 150 mM NaCl) and 1 µl precipitant solution consisting of 10% 1,4-dioxane, 1.6 M ammonium sulfate, 0.1 M MES pH 6.5. The droplets were equilibrated by the sitting-drop vapour-diffusion method against 1 ml precipitant solution at 288 K for 7 d. To collect the diffraction data set, the crystals were transferred into a cryo-protectant solution consisting of reservoir solution supplemented

with 25% (v/v) glycerol. The best crystals diffracted to approximately 3.0 Å resolution (Fig. 3) and belonged to the tetragonal space group  $I4_122$  or  $I4_322$ , with unit-cell parameters  $a = b = 121.1$ ,  $c = 241.7$  Å, and diffracted to a maximum resolution of 3.2 Å. In particular, analysis of the diffraction along the  $h$ ,  $k$ ,  $l$  axes clearly demonstrates that the  $c$  axis of the crystal is the  $4_1$  or  $4_3$  axis. The diffraction data set had a resolution range of 38–3.2 Å with 92.7% completeness and an  $R_{\text{merge}}$  of 15.3 at the remote wavelength (Table 1). Complete data statistics are given in Table 1. In order to solve the structure, molecular replacement was attempted with *MOLREP* (Winn *et al.*, 2011) using the structure of *P. mirabilis* PM11202 (78.5% sequence identity; PDB entry 3olq; Midwest Center for Structural Genomics, unpublished work) as the search model. There were two molecules in the asymmetric unit, and the corresponding Matthews coefficient (Matthews, 1968) and solvent content are 3.10 Å<sup>3</sup> Da<sup>-1</sup> and 60.37%, respectively. Model building and structure refinement are under way.

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### References

- Battye, T. G. G., Kontogiannis, L., Johnson, O., Powell, H. R. & Leslie, A. G. W. (2011). *Acta Cryst.* **D67**, 271–281.
- Diez, A., Gustavsson, N. & Nyström, T. (2000). *Mol. Microbiol.* **36**, 1494–1503.
- Gustavsson, N., Diez, A. & Nyström, T. (2002). *Mol. Microbiol.* **43**, 107–117.
- Kvint, K., Nachin, L., Diez, A. & Nyström, T. (2003). *Curr. Opin. Microbiol.* **6**, 140–145.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 481–497.
- Nachin, L., Nannmark, U. & Nyström, T. (2005). *J. Bacteriol.* **187**, 6265–6272.
- Nyström, T. & Neidhardt, F. C. (1994). *Mol. Microbiol.* **11**, 537–544.
- Siegele, D. A. (2005). *J. Bacteriol.* **187**, 6253–6254.
- Sousa, M. C. & McKay, D. B. (2001). *Structure*, **9**, 1135–1141.
- Tkaczuk, K. L., Shumilin, I. A., Chruszcz, M., Evdokimova, E., Savchenko, A. & Minor, W. (2013). *Evol. Appl.* **6**, 434–449.
- Zarembinski, T. I., Hung, L.-W., Mueller-Dieckmann, H.-J., Kim, K.-K., Yokota, H., Kim, R. & Kim, S.-H. (1998). *Proc. Natl Acad. Sci. USA*, **95**, 15189–15193.
- Winn, M. D. *et al.* (2011). *Acta Cryst.* **D67**, 235–242.