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Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of an ASCH domain-containing protein from *Zymomonas mobilis* ZM4

The human activating signal cointegrator 1 (ASC-1) homology (ASCH) domain is frequently observed in many organisms, although its function has not yet been clearly defined. In *Zymomonas mobilis* ZM4, the ZMO0922 gene encodes a polypeptide that includes an ASCH domain (zmASCH). To provide a better structural background for the probable role of ASCH domain-containing proteins, the ZMO0922 gene was cloned and expressed. The purified protein was crystallized from 30%(*w*/*v*) polyethylene glycol 400, 0.1 *M* cacodylic acid pH 6.5 and 0.2 *M* lithium sulfate. Diffraction data were collected to 2.1 Å resolution using synchrotron radiation. The crystal belonged to the primitive trigonal space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 51.67$, $c = 207.30$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. Assuming the presence of one molecule in the asymmetric unit gave a Matthews coefficient of 4.69 Å^3 Da^{-1}, corresponding to a solvent content of 73.7%.

1. Introduction

Human ASC-1 is a transcriptional regulator that is widely conserved in eukaryotes and is a component of a potential RNA-interacting protein complex (Kim *et al.*, 1999; Jung *et al.*, 2002). Nonetheless, it contains a novel uncharacterized superfamily region, the ASCH domain, at its C-terminus, which is a small polypeptide of ∼110 amino acids. Careful sequence analysis of ASCH domain-containing proteins and their elucidated and predicted structures have suggested that they might belong to the nucleic acid-binding proteins, with a β-barrel fold structure similar to that of the PUA domain (Ishitani *et al.*, 2003; Pan *et al.*, 2003; Li & Ye, 2006), and may function as RNA-binding proteins in contexts related to coactivation, RNA processing and possibly prokaryotic translation regulation (Iyer *et al.*, 2006). However, an inspection of recently elucidated structures and computational data suggested that PUA, ASCH and EVE domains have related but distinct structures that may differentiate their functions (Bertonati *et al.*, 2009) and leaves the exact function of this superfamily of proteins ambiguous.

ASCH proteins are widely dispersed in all three superkingdoms of life and their sequence identity is relatively low, sometimes less than 15% (Iyer *et al.*, 2006). The ZMO0922 gene of *Zymomonas mobilis* ZM4 codes for a polypeptide of 148 amino acids which forms an ASCH domain-containing protein in *Z. mobilis* (zmASCH). It is a representative of ASCH family 2, members of which are predominantly found in bacteria and archaea (Iyer *et al.*, 2006). Like other ASCH proteins (Shen *et al.*, 2005; Iyer *et al.*, 2006; Bertonati *et al.*, 2009), zmASCH contains a highly conserved G*x*K*xxxx*R octapeptide sequence motif that is predicted to form an RNA-binding cleft. However, zmASCH also has an additional region of ∼40 amino acids at the C-terminus, the function of which has not yet been characterized.

Proteins may display similarity at the three-dimensional structural level even if their sequences appear to be nonhomologous (Kim, 1998; Sali, 1998; Teichmann *et al.*, 1999; Bertonati *et al.*, 2009) and structure determination can provide new insights into protein function. Moreover, a crystal structure of the functionally uncharacterized zmASCH may enrich the structural information on ASCH domain-containing proteins and provide a better understanding of their probable functional roles. As an initial step towards elucidating its

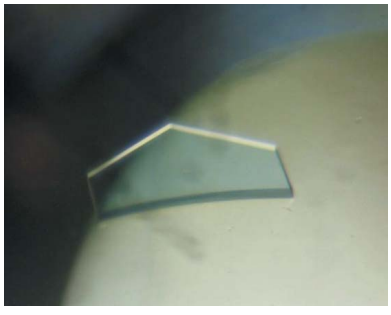

Table 1
Data-collection statistics for zmASCH.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.00
Space group	$P3_121$ or $P3_221$
Unit-cell parameters (Å, °)	$a = b = 51.51$, $c = 206.60$, $\alpha = \beta = 90$, $\gamma = 120$
Resolution range (Å)	41.0–2.10 (2.21–2.10)
Measured reflections	124149 (18029)
Unique reflections	18068 (2488)
Multiplicity	6.9 (7.2)
Temperature (K)	100
Completeness (%)	92.9 (90.2)
Mean $I/\sigma(I)$	7.2 (3.0)
$R_{\rm merge}$† (%)	7.6 (15.7)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of an individual reflection and $\langle I(hkl)\rangle$ is the mean intensity of that reflection.

function based on its structure, we cloned, expressed, purified and crystallized zmASCH. Diffraction data were collected and preliminarily analyzed.

2. Methods

2.1. Cloning, expression and purification of zmASCH

The *Z. mobilis* ZM4 gene coding for zmASCH (ZMO0922; Met1–Glu148) was amplified from *Z. mobilis* ZM4 chromosomal DNA by polymerase chain reaction (PCR). The PCR product was then cloned into a pET21a derivative which expresses 18 extra N-terminal amino acids encoding a cleavable hexahistidine tag followed by the tobacco etch virus (TEV) protease cleavage site. The expression construct was transformed into *Escherichia coli* BL21 (DE3) Star and was grown in LB medium containing 100 µg ml^{-1} ampicillin at 310 K. After induction with 1.0 m*M* isopropyl β-D-1-thiogalactopyranoside for a further 8 h at 310 K, the cells were harvested by centrifugation at 5000*g* at 277 K. The cell pellet was resuspended in ice-cold buffer *A* (20 m*M* Tris–HCl pH 7.5 and 500 m*M* NaCl) and disrupted by ultrasonication. The cell debris was removed by centrifugation at 11 000*g* for 1 h. The zmASCH fusion protein was purified using a 5 ml HisTrap HP chelating column (GE Healthcare, Uppsala, Sweden). After treatment with recombinant TEV protease to cleave the hexahistidine tag and the removal of salts by dialysis, the protein containing five additional amino acids (GGGGG) at the N-terminus was purified using a 5 ml HiTrap SP HP cation-exchange column (GE Healthcare, Uppsala, Sweden). The purified protein was >95% pure as judged by Coomassie Blue-stained SDS–PAGE (data not shown).

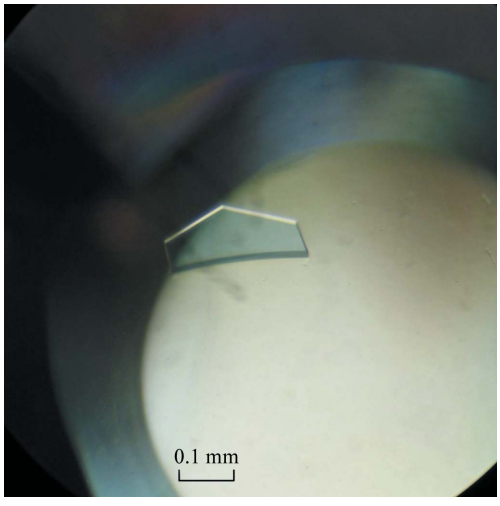

Figure 1
A representative crystal of zmASCH. The crystal grew at 291 K within 1 d to maximum dimensions of approximately 0.2 × 0.1 × 0.01 mm.

2.2. Crystallization

For crystallization, the purified zmASCH protein was concentrated to 6.0 mg ml^{-1} in a buffer consisting of 20 m*M* Tris–HCl pH 7.5 and 200 m*M* NaCl. The protein concentration was determined using an extinction coefficient of 1.712 mg ml^{-1} cm^{-1} at 280 nm, which was calculated from its amino-acid sequence. Crystallization of zmASCH was attempted at 295 K by the sitting-drop vapour-diffusion method. The initial trials used Crystal Screen, Crystal Screen 2, Index 1, Index 2 (Hampton Research, Riverside, California, USA) and Wizard I and II (Emerald BioStructures, Bainbridge Island, Washington, USA). For each crystallization trial, a 2 µl drop was prepared by mixing 1 µl purified protein solution with an equal volume of reservoir solution. The reservoir contained 80 µl of the precipitating solution. Small crystals were obtained in 1 d from five of the drops (Crystal Screen solution No. 9, Index 1 solution No. 19, Index 2 solution No. 76 and Wizard II solution Nos. 12 and 35). The most promising results were obtained using Wizard II solution No. 12 [30%(*w*/*v*) polyethylene glycol 400, 0.1 *M* cacodylic acid pH 6.5 and 0.2 *M* lithium sulfate] and this condition was optimized by changing the precipitant and protein concentration, the buffer pH, the temperature and the vapour-diffusion method to obtain crystals that were suitable for X-ray diffraction.

2.3. X-ray diffraction data collection

For diffraction experiments, crystals were briefly immersed into precipitant solution containing 20%(*v*/*v*) glycerol as a cryoprotectant and were immediately placed in a 100 K nitrogen-gas stream. Native X-ray diffraction data were collected on the MAX4A beamline at

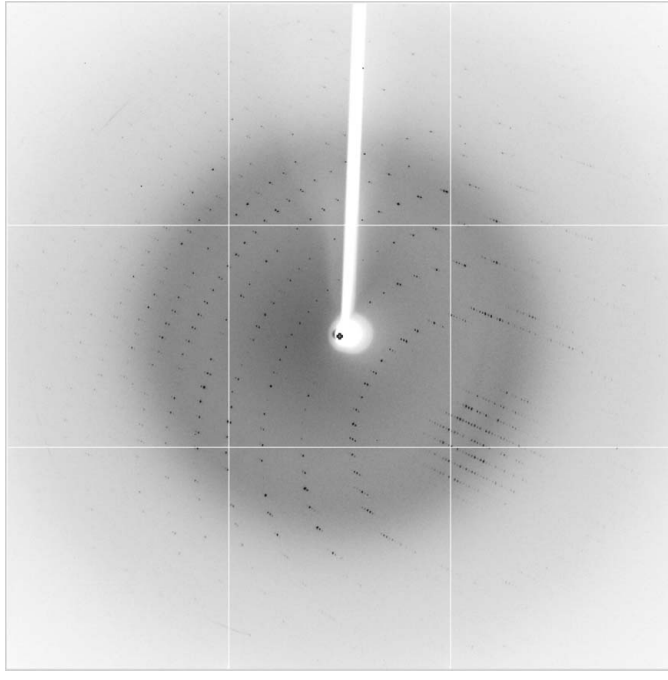

Figure 2
A representative X-ray diffraction image from a zmASCH crystal. The crystal was exposed for 20 s over a 1° oscillation range. The edge of the detector corresponds to a resolution of 2.10 Å.

Pohang Accelerator Laboratory (PAL, Republic of Korea) using a 1° oscillation per image and a crystal-to-detector distance of 300 mm. The crystal was exposed for 20 s per image at a wavelength of 1.00 Å. A data set of 270 images was collected to 2.10 Å resolution from a single crystal. The data were processed using *MOSFLM* (Leslie, 2006) and *SCALA* (Evans, 2006). The data-collection statistics are summarized in Table 1.

3. Results and discussion

Recombinant zmASCH protein was successfully expressed in *E. coli* and purified. The purified protein was concentrated to 6.0 mg ml^{-1} in 20 m*M* Tris–HCl pH 7.5, 200 m*M* NaCl. Crystals that were suitable for diffraction experiments were obtained within 1 d using the hanging-drop vapour-diffusion method at 291 K by mixing 1 µl protein solution and 1 µl reservoir solution and equilibrating against 200 µl reservoir solution, which consisted of 30%(*w*/*v*) polyethylene glycol 400, 0.1 *M* cacodylic acid pH 6.5 and 0.2 *M* lithium sulfate. The dimensions of the crystal used for data collection were approximately 0.2 × 0.1 × 0.01 mm (Fig. 1) and the crystal diffracted to 2.1 Å resolution (Fig. 2). The crystal belonged to the primitive trigonal space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 51.67$, $c = 207.30$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. Assuming the presence of one zmASCH molecule of 17.2 kDa in the asymmetric unit, the asymmetric unit volume per dalton was calculated as 4.69 Å^3 Da^{-1}, corresponding to a solvent content of 73.7% (Matthews, 1968). There is sufficient space for a second monomer in the asymmetric unit, giving a more typical solvent content of 47.4%, but self-rotation and native Patterson maps do not show evidence of a second monomer. We have attempted molecular replacement for phase determination with the programs *Phaser* v.1.3 (McCoy, 2007) and *MOLREP* from the *CCP*4 program suite (Vagin & Teplyakov, 2010; Collaborative Computational Project, Number 4, 1994) using the structures of reported ASCH-superfamily proteins such as PDB entries 1s04 (G. Liu, R. Xiao, D. K. Sukumaran, T. Acton, G. T. Montelione & T. Szyperski, unpublished work), 1kw8 (Sato *et al.*, 2002), 2eve (Bertonati *et al.*, 2009), 1j2b (Ishitani *et al.*, 2003), 1r3e (Pan *et al.*, 2003) and 2hvy (Li & Ye, 2006) as search models, but have not yet been successful. As mentioned above, their sequence identity is relatively low at less than 15%. Therefore, the crystal structure of zmASCH is now being solved by the MAD or SAD method with selenium as the anomalous scatterer using synchrotron radiation.

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