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Crystallization and preliminary X-ray analysis of XC1015, a histidine triad-like protein from *Xanthomonas campestris*

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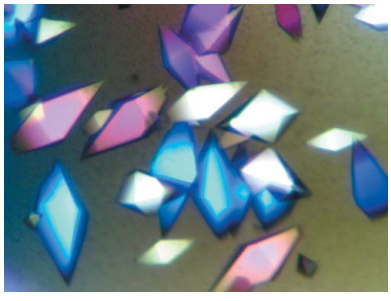

Histidine-triad (HIT) proteins are a superfamily of nucleotide hydrolases and transferases that contain a conserved HφHφH$\varphi\varphi$ motif (where φ is a hydrophobic amino acid) and are found in a variety of organisms. In addition to binding to a variety of nucleotides, other biological functions of the HIT superfamily proteins have been discovered and HIT malfunction has been implicated in several human diseases. Structural studies of HIT superfamily proteins are thus of particular interest. In this manuscript, the cloning, expression, crystallization and preliminary X-ray analysis of XC1015, a HIT protein present in the plant pathogen *Xanthomonas campestris* pathovar *campestris*, are reported. The XC1015 crystals diffracted to a resolution of 1.3 Å. They are tetragonal and belong to space group $P4_32_12$, with unit-cell parameters a = 40.52, b = 40.52, c = 126.89 Å.

1. Introduction

HIT-like homologues are almost ubiquitous and are present in almost all kingdoms of life. Currently, three branches of the HIT superfamily have been identified: the HINT branch, which comprises adenosine 5′-monophosphoramide hydrolases, the FHIT branch, which comprises diadenosine polyphosphate hydrolases, and the GalT branch, which comprises nucleoside monophosphate transferases (Brenner, 2002). A HIT-like protein has been found to interact with the Nit protein, possibly acting as a tumour suppressor and leading to cell apoptosis (Pace *et al.*, 2000), and malfunctions of certain HIT-like proteins have also been implicated in several human diseases, including ataxia-oculomotor apraxia syndrome and galactosaemia (Brenner, 2002). The structures of HIT superfamily proteins are therefore of interest.

Although several structures of mammalian HIT-like proteins are available (Lima, Klein *et al.*, 1997; Lima *et al.*, 1996; Brenner *et al.*, 1997; Lima, D'Amico *et al.*, 1997), only one structure is currently known for a prokaryotic HIT-like protein (PDB entry 1y23 from *Bacillus subtilis*). In this manuscript, we report the cloning, expression, crystallization and preliminary X-ray analysis of XC1015 (gi|21112027), a HIT-like protein from the plant pathogen *Xanthomonus campestris* strain 17 (Xcc). XC1015 is classified as belonging to the histidine triad-like (HIT-like) protein superfamily in the Pfam database (Bateman *et al.*, 2000). It consists of 116 amino acids and contains a conserved HIHLHLL motif characteristic of HIT proteins (Brenner, 2002; Brenner *et al.*, 1999). In the UniProt database, there are currently approximately 200 sequences that have sequence identities of greater than 34% with XC1015. Intriguingly, the XC1015 sequence is more similar to the sequences of the eukaryotic members of the HIT-like superfamily than the prokaryotic members.

2. Materials and methods

2.1. Cloning, expression and purification

The XC1015 gene fragment was PCR-amplified directly from genomic DNA of Xcc with forward 5′-TACTTCCAATGCTATGACCGACACCATTTTCGGC and reverse 5′-TTATCCACTTCCAATGTCACGGCGTACCGAAACGCC primers. A ligation-independent cloning (LIC) approach (Aslanidis & de Jong, 1990) was

carried out according to a previously published protocol (Wu *et al.*, 2005) in order to obtain the desired construct in the pTBSG1 expression vector (F. P. Gao, unpublished results). The final construct codes for an N-terminal His_6 tag, a 17-amino-acid linker [SSGVDL-GT**ENLYFQ**_SNA, with the tobacco etch virus (TEV) protease-recognition sequence in bold and the cutting site marked by an underscore] and the XC1015 target protein (116 amino acids) under the control of a T7 promoter. Overexpression of the His_6-tagged target protein was induced by the addition of 1 m*M* IPTG at 310 K for 5 h. The target protein was purified by immobilized metal-affinity chromatography (IMAC) on a nickel column (Sigma). The His_6 tag and linker were cleaved from XC1015 by TEV protease at 277 K for 24 h to obtain the final target protein with an extra tripeptide (SNA) at the N-terminal end (119 amino-acid residues in total). For crystallization, XC1015 was further purified on a DEAE anion-exchange column (AKTA; Pharmacia Inc.) The final target protein has greater than 90% purity (Fig. 1). The overexpression and purification of XC1015 were monitored by SDS–PAGE as shown in Fig. 1.

SeMet-labelled XC1015 was produced using a non-auxotrophic *Escherichia coli* strain BL21(DE3) as host in the absence of methionine but with ample amounts of SeMet (100 mg l^{-1}). The induction was conducted at 310 K for 4 h by the addition of 0.5 m*M* IPTG in M9 medium consisting of 1 g NH_4Cl, 3 g KH_2PO_4 and 6 g Na_2HPO_4 supplemented with 20%(*w*/*v*) glucose, 0.3%(*w*/*v*) $MgSO_4$ and 10 mg $FeSO_4$. Purification and crystallization of the SeMet-labelled XC1015 were performed using the protocols established for the native protein.

2.2. Crystallization

For crystallization, XC1015 was concentrated to 15 mg ml^{-1} in 20 m*M* Tris pH 8.0 and 250 m*M* NaCl using an Amicon Ultra-10 (Millipore). Screening for crystallization conditions was performed using sitting-drop vapour diffusion in 96-well plates (Hampton Research) at 295 K by mixing 0.5 µl protein solution with 0.5 µl reagent solution. Initial screens included the Hampton sparse-matrix Crystal Screens 1 and 2, a systematic PEG–pH screen and the PEG/Ion screen and were performed using the Gilson C240 crystallization workstation. Needle-like crystals appeared in 3 d from a reservoir solution comprising 0.2 *M* sodium acetate buffer pH 5.5, 0.1 *M* Tris buffer pH 8.5 and 25% PEG 4K MME (polyethylene glycol monomethyl ether). This initial condition was then optimized by varying the PEG concentration, with 22%(*v*/*w*) giving the best results. Diamond-shaped crystals suitable for diffraction experiments were grown by mixing 1.5 µl protein solution with 1.5 µl reagent solution at room temperature and reached maximum dimensions of 0.1 × 0.2 × 0.1 mm after 3 d (Fig. 2).

Table 1
Data-collection statistics for XC1015.

Values in parentheses are for the highest resolution shell.

		SeMet		
	Native	Remote	Inflection point	Peak
Space group	$P4_32_12$			
Unit-cell parameters (Å)	$a = b = 40.52$, $c = 126.89$			
Temperature (K)	100			
Wavelength (Å)	1.0	0.963945	0.979365	0.979173
Resolution range	30.0–1.3 (1.35–1.30)	50–1.5 (1.55–1.5)	50–1.52 (1.57–1.52)	50–1.52 (1.57–1.52)
Mosaicity (°)	0.3	0.32	0.33	0.33
Unique reflections	132916 (17876)	132812 (16579)	127982 (15320)	257001 (25740)
Redundancy	7.4 (7.0)	7.2 (7.0)	7.0 (6.8)	15 (14.5)
Completeness (%)	99.7 (100)	99.8 (100)	99.1 (94)	99.3 (96)
R_{merge} (%)	4.2 (25)	3.7 (22)	3.9 (23)	4.8 (22)
Mean $I/\sigma(I)$	44.0 (8.2)	38.7 (8.0)	39.2 (7.8)	40.2 (9.1)
Solvent content (%)	50.0			

2.3. Data collection

Crystals soaked in the mother liquor (22% PEG 4K MME) were mounted straight from the drop and then flash-cooled at 100 K in a stream of cold nitrogen. X-ray diffraction data were collected using beamline 13B1 at NSRRC, Taiwan. A 1.3 Å resolution native data set was obtained. The data were indexed and integrated using the *HKL* processing software (Otwinowski & Minor, 1997). A three-wavelength data set was also collected at the remote, peak and inflection-point wavelengths of Se absorption for the SeMet-labelled XC1015 using beamline 12B2 at the SPring-8 facility, Japan. All crystals belong to the tetragonal space group $P4_32_12$; the data-collection statistics are summarized in Table 1. An X-ray diffraction image collected at the Hsin-Chu synchrotron facility, Taiwan is shown in Fig. 3.

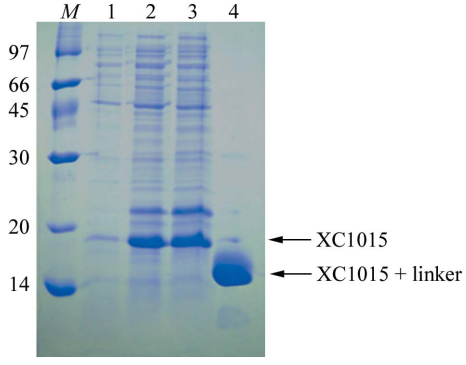

Figure 1
SDS–PAGE monitoring of the overexpression and purification of XC1015. Lane *M*, molecular-weight markers in kDa; lane 1, whole cell lysate before IPTG induction; lane 2, whole cell lysate after IPTG induction; lane 3, soluble fraction after IPTG induction; lane 4, purified XC1015 after TEV protease cleavage. The positions of linker target and free XC1015 were also marked.

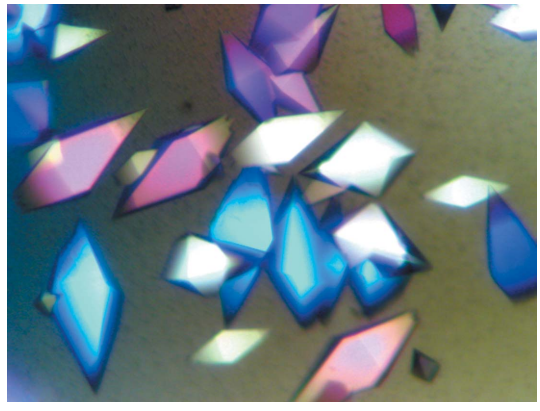

Figure 2
Diamond-shaped crystals of XC1015 from *X. campestris* grown by the hanging-drop vapour-diffusion method. The crystallization condition used was 0.2 *M* sodium acetate buffer pH 5.5, 0.1 *M* Tris buffer pH 8.5 and 22% PEG 4K MME. The average dimensions of these crystals were around 0.1 × 0.2 × 0.1 mm after 3 d.

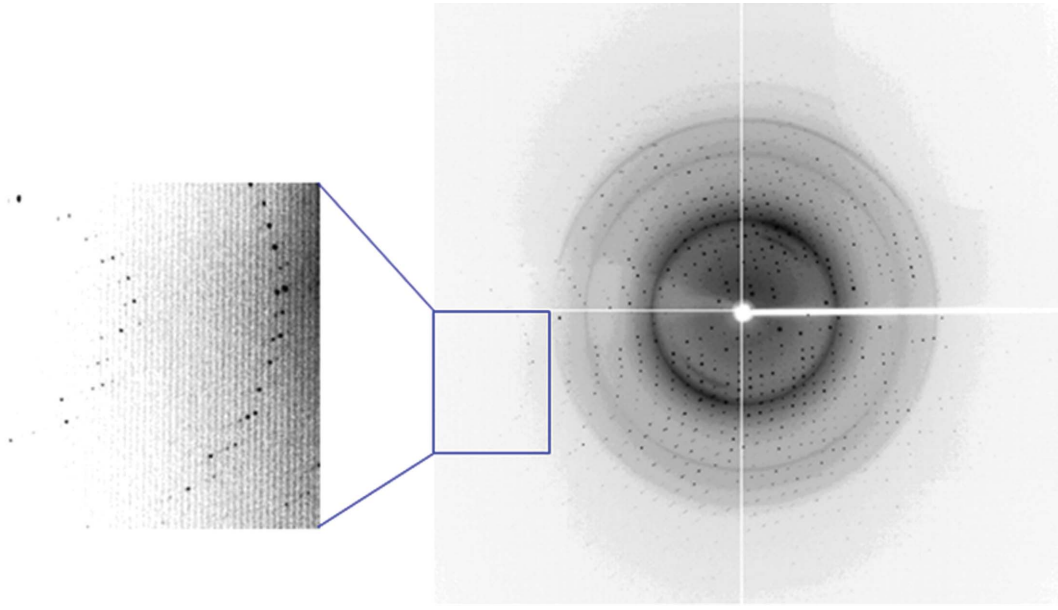

Figure 3
The diffraction pattern of XC1015 collected from a flash-frozen crystal in reservoir cryoprotectant using beamline 13B1, NSRRC, Taiwan. The exposure time was 10 s, with an oscillation range of 1° and a crystal-to-detector distance of 120 mm. The insert shows the enlarged view of diffraction points in the outmost shell at 1.3 Å.

3. Results and discussion

The final XC1015 target protein contained an extra tripeptide (SNA) at the N-terminal end and consisted of 119 amino-acid residues in total. The isoelectric point was calculated to be 5.80. The purified XC1015 showed a single band of approximate 15 kDa on SDS–PAGE (Fig. 1), slighter larger than the calculated value of 12 945.23 Da. This is not unusual, as some proteins migrate more slowly than expected on SDS–PAGE.

The high-resolution diffraction obtained from the native crystals established their suitability for X-ray structural analysis (Fig. 3). A good preliminary structure of XC1015 has been obtained by the MAD approach using the *SOLVE* and *RESOLVE* programs (Hendrickson & Ogata, 1997; Terwilliger & Berendzen, 1999). Detailed structural refinement of XC1015 is currently under way.

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