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Preparation, crystallization and preliminary X-ray analysis of YjcG protein from *Bacillus subtilis*

Bacillus subtilis YjcG is a functionally uncharacterized protein with 171 residues that has no structural homologue in the Protein Data Bank. However, it shows sequence homology to bacterial and archaeal 2'-5' RNA ligases. In order to identify its exact function *via* structural studies, the *yjcG* gene was amplified from *B. subtilis* genomic DNA and cloned into the expression vector pET21-DEST. The protein was expressed in a soluble form in *Escherichia coli* and was purified to homogeneity. Crystals suitable for X-ray analysis were obtained that diffracted to 2.3 Å and belonged to space group C2, with unit-cell parameters $a = 99.66$, $b = 73.93$, $c = 61.77$ Å, $\beta = 113.56^\circ$.

1. Introduction

The *Bacillus subtilis* *yjcG* gene encodes a putative protein of 171 amino-acid residues with a predicted molecular weight of 19.5 kDa. The YjcG protein shows a sequence identity of 57% to a 2'-5' RNA ligase from *B. cereus* and contains two copies of the highly conserved HXTX (where X is a hydrophobic amino-acid residue) motif (Fig. 1), which is a characteristic feature of the bacterial and archaeal 2'-5' RNA ligase family (Kato *et al.*, 2003). 2'-5' RNA ligase activity was first found in *Escherichia coli* and then detected in many other bacterial and archaeal species and *in vitro* the RNA ligases can ligate 5' and 3' half-tRNA molecules into a mature tRNA molecule through a 2'-5' phosphodiester linkage (Greer *et al.*, 1983; Arn & Abelson, 1996). Using sensitive sequence profile-analysis methods, it was

Figure 1

Multiple sequence alignment of YjcG homologues. The conserved HXTX motifs are marked with grey boxes and the percentage identities with *B. subtilis* YjcG are indicated after the organism names; asterisks indicate strictly conserved residues. The alignment was performed using the program CLUSTALX (Thompson *et al.*, 1997). Bs_YjcG, *B. subtilis* YjcG; Exig.sp, *Exiguobacterium* sp.; L.mesent, *Leuconostoc mesenteroides*; N.punct, *Nostoc punctiforme*; A.variab, *Anabaena variabilis*; T.eryth, *Trichodesmium erythraeum*; Bs_YtlP, *B. subtilis* YtlP; T.thermo, *Thermus thermophilus*.

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recently revealed that the bacterial and archeal 2'-5' RNA ligase family belongs to the 2H phosphoesterase protein superfamily, named after the two highly conserved histidines in the highly conserved HXTX motifs. The *B. subtilis* YjcG protein represents a YjcG-like subgroup belonging to this superfamily. The proteins from this group were suggested to react on some unknown molecules with potential 2'-3' cyclic phosphoester linkages (Mazumder *et al.*, 2002). So far, the crystal structure of the 2'-5' RNA ligase from *Thermus thermophilus* HB8 has been determined and showed a high structural similarity to *Arabidopsis thaliana* cyclic phosphodiesterase (Kato *et al.*, 2003). Except for the two HXTX motifs, the sequence identity between the *T. thermophilus* 2'-5' RNA ligase and the *B. subtilis* YjcG protein is below 20% (Fig. 1) and no homologues were found in the Protein Data Bank for the *B. subtilis* YjcG protein. Determination of the *B. subtilis* YjcG structure will help us to understand the function of YjcG-like proteins in bacteria.

2. Materials and methods

2.1. Cloning and expression

The genomic DNA of *B. subtilis* strain 168 was used as the template for the polymerase chain reaction (PCR). The *yjcG* gene was cloned into the pET21-DEST destination vector using Gateway cloning technology (Ren *et al.*, 2004). The primers used in cloning were 5'-CACCATGAAATACGGAATCGTTTAT-3' and 5'-TTATTC-TCCCTGCCTAGCAA-3'. *E. coli* strain BL21(DE3) cells harbouring the *yjcG* expression plasmid were grown aerobically in Luria-Bertani (LB) medium containing 50 mg ml⁻¹ ampicillin at 310 K until an OD₆₀₀ of 0.6–0.8 was reached. The cells were induced with 1 mM isopropyl- β -D-thiogalactopyranoside and grown for a further 5 h at 303 K. The cells were then harvested by centrifugation at 6700g for 10 min. The cell pellet was resuspended in buffer A containing 20 mM Tris-HCl, 500 mM NaCl pH 7.5 and then disrupted by sonication.

2.2. Protein purification

The disrupted cells were centrifuged at 34 700g at 277 K for 30 min. The supernatant was loaded onto a 5 ml HiTrap Ni column (Amersham) equilibrated with buffer A. The unbound proteins were flushed

Figure 2
Crystal of *B. subtilis* YjcG. The dimensions of the crystal are approximately 0.1 $\times$ 0.1 $\times$ 0.8 mm.

Table 1

Data-collection statistics of YjcG.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	0.981
Resolution (Å)	50–2.3 (2.38–2.3)
Completeness (%)	98.8 (95.0)
$R_{\text{sym}}^{\dagger}$ (%)	7.6 (17.7)
Mean $I/\sigma(I)$	10.8 (4.7)
Space group	C2
Unit-cell parameters (Å, °)	$a = 99.66$, $b = 73.93$, $c = 61.77$, $\beta = 113.56$
No. of observed reflections	56855
No. of unique reflections	17981
Molecules per AU	2
V_M (Å ³ Da ⁻¹)	2.67

$\dagger R_{\text{sym}} = \sum |I_{\text{obs}} - I_{\text{avg}}| / \sum I_{\text{obs}}$, where the summation is over all reflections.

with buffer A and the low Ni-affinity proteins were eluted with buffer B (20 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole pH 7.5; 20% imidazole concentration). The bound proteins were successively eluted with buffer B. Further purification to homogeneity was carried out using a HiLoad Superdex 75 column (Amersham) with an elution buffer consisting of 20 mM Tris-HCl, 150 mM NaCl pH 7.5. The purified proteins were examined by SDS-PAGE in each step.

2.3. Crystallization

The purified protein was concentrated to 10 mg ml⁻¹ by ultrafiltration (Millipore Amincon). Crystallization was carried out using the hanging-drop vapour-diffusion method at 293 K using Crystal Screen I, Crystal Screen II and Index kits (Hampton Research) as initial screening conditions. 1 μ l protein solution was mixed with 1 μ l reservoir solution and equilibrated against 500 μ l reservoir solution.

2.4. Data collection

X-ray diffraction data were collected on a MAR 165 CCD detector at beamline 3W1A, Beijing Synchrotron Radiation Facility (BSRF), People's Republic of China. The crystal was flash-frozen and maintained at 100 K using nitrogen gas (Oxford) during data collection; 5% (v/v) glycerol solution was used for cryoprotection. The data were processed with DENZO and SCALEPACK (Otwinowski & Minor, 1997).

3. Results

The YjcG protein was expressed in *E. coli* BL21(DE3) in a soluble form and purified to homogeneity in two steps. SDS-PAGE shows the purified protein to have a molecular weight about 25 kDa, which is in agreement with the predicted molecular weight of 19.7 kDa plus an added 4 kDa fusion part.

Microcrystals appeared in Index condition No. 47 (Hampton Research), consisting of 28% PEG MME 2000, 0.1 M bis-Tris pH 6.5. After further optimization, crystals suitable for X-ray diffraction were obtained in an optimized condition containing 0.1 M bis-Tris pH 7.3 and 24% PEG MME 2000 (Fig. 2). The YjcG crystal diffracted to a resolution of 2.3 Å and belonged to space group C2, with unit-cell parameters $a = 99.66$, $b = 73.93$, $c = 61.77$ Å, $\beta = 113.56^\circ$. Assuming the presence of two molecules per asymmetric unit gave a V_M value of 2.67 Å³ Da⁻¹ (Matthews, 1968), which corresponds to a solvent content of 53%. The data-collection statistics are listed in Table 1.

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