Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

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

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Received 16 March 2005
Accepted 8 April 2005
Online 22 April 2005

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 *C*2, with unit-cell parameters a = 99.66, b = 73.93, c = 61.77 Å, β = 113.56°.

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 H*XTX* (where *X* is a hydrophobic amino-acid residue) motif (Fig. 1), which is a characteristic feature of the bacterial and archeal 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 archeal 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

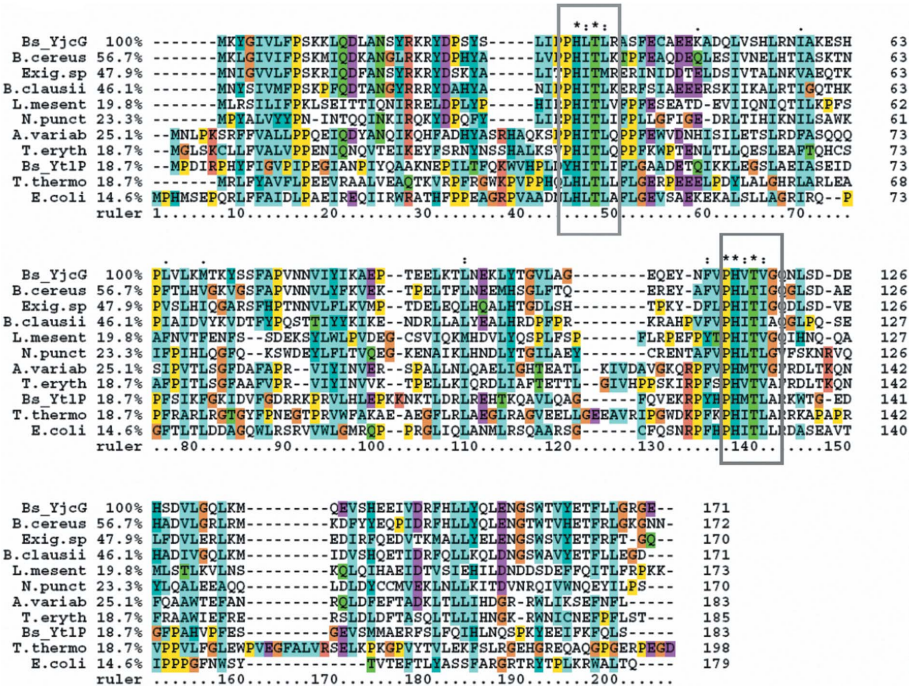

Figure 1
Multiple sequence alignment of YjcG homologues. The conserved H*XTX* motifs are marked with grey boxes and the percentage identities with *B. subtilis* YjcG are indicated after the organism names; asterisks indicate strictly conserved residues. The alignment was performed using the program *CLUSTALX* (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*.

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 H*X*T*X* 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 H*X*T*X* 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′-CACCATGAAATACGGAATCGTTTTAT-3′ and 5′-TTATTC-TCCTCTGCCTAGCAA-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 OD_{600} of 0.6–0.8 was reached. The cells were induced with 1 m*M* isopropyl-β-D-thiogalactopyranoside and grown for a further 5 h at 303 K. The cells were then harvested by centrifugation at 6700*g* for 10 min. The cell pellet was resuspended in buffer *A* containing 20 m*M* Tris–HCl, 500 m*M* NaCl pH 7.5 and then disrupted by sonication.

2.2. Protein purification

The disrupted cells were centrifuged at 34 700*g* 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 with buffer *A* and the low Ni-affinity proteins were eluted with buffer *B* (20 m*M* Tris–HCl, 500 m*M* NaCl, 500 m*M* 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 m*M* Tris–HCl, 150 m*M* NaCl pH 7.5. The purified proteins were examined by SDS–PAGE in each step.

Figure 2
Crystal of *B. subtilis* YjcG. The dimensions of the crystal are approximately 0.1 × 0.1 × 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_{sym}† (%)	7.6 (17.7)
Mean $I/\sigma(I)$	10.8 (4.7)
Space group	*C*2
Unit-cell parameters (Å, °)	a = 99.66, b = 73.93, c = 61.77, β = 113.56
No. of observed reflections	56855
No. of unique reflections	17981
Molecules per AU	2
V_M (Å^3 Da^{-1})	2.67

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

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 µl protein solution was mixed with 1 µl reservoir solution and equilibrated against 500 µl 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 *C*2, with unit-cell parameters a = 99.66, b = 73.93, c = 61.77 Å, β = 113.56°. Assuming the presence of two molecules per asymmetric unit gave a V_M value of 2.67 Å^3 Da^{-1} (Matthews, 1968), which corresponds to a solvent content of 53%. The data-collection statistics are listed in Table 1.

We thank the beamline staff (Drs Yu-Hui Dong and Peng Liu) at BSRF for their kind help with data collection. This work was supported by a grant from the National High Technology and

Development Program of China (863 program 2002BA711A13). Peking University's 985 and 211 grants are greatly acknowledged. YHL is the recipient of a Fok Ying Tong Education Foundation grant (94017).

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