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Crystallization and preliminary X-ray analysis of the mRNA-binding domain of elongation factor SelB in complex with RNA

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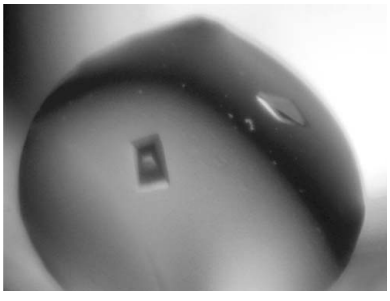

In bacteria, the selenocysteine-specific elongation factor SelB is necessary for incorporation of selenocysteine, the 21st amino acid, into proteins by the ribosome. SelB binds to an mRNA hairpin formed by the selenocysteine-insertion sequence (SECIS) and delivers selenocysteyl-tRNA (Sec-tRNA^{Sec}) at the ribosomal A site. The minimum fragment (residues 512–634) of *Moorella thermoacetica* SelB (SelB-M) required for mRNA binding has been overexpressed and purified. The complex of SelB-M with 23 nucleotides of the SECIS mRNA hairpin was crystallized at 293 K using the hanging-drop vapour-diffusion or oil-batch methods. The crystals diffract to 2.3 Å resolution using SPring-8 BL41XU and belong to the space group $P2_12_12$, with unit-cell parameters a = 81.69, b = 169.58, c = 71.69 Å.

1. Introduction

In bacteria, the incorporation of selenocysteine (Sec), an unusual genetically encoded amino acid, into proteins requires elongation factor SelB (Forchhammer *et al.*, 1989). SelB has the unusual property of binding to both tRNA and mRNA. The protein recognizes an mRNA hairpin formed by the selenocysteine-insertion sequence (SECIS), which serves as a recognition signal to deliver Sec-tRNA^{Sec} to a UGA codon at the ribosomal A site. Hence, a UGA codon with a downstream SECIS is recoded as a Sec codon instead of being used as a termination signal. The N-terminal domain of SelB, which has sequence homology to the three domains of elongation factor Tu (EF-Tu), binds guanine nucleotides and Sec-tRNA^{Sec}, while an extra C-terminal domain (SelB-C) recognizes the SECIS hairpin (Kromayer *et al.*, 1996). Although the crystal structure of SelB-C from *Moorella thermoacetica* (Selmer & Su, 2002) and the solution structure of the *Escherichia coli* SECIS hairpin (Fourmy *et al.*, 2002) have been reported, the molecular basis of mRNA recognition by SelB-C is unknown. Also, the SelB-C is formed of four consecutives winged-helix motifs, a protein module found in many DNA-binding proteins (Gajiwala & Burley, 2000) and recently discovered in RNA-binding proteins. To date, there is no structural data available on a winged-helix motif complexed to RNA. In this paper, we report the production and crystallization of the minimum fragment of the mRNA-binding domain (SelB-M, residues 512–634) in complex with SECIS mRNA hairpin.

2. Results and discussion

2.1. Cloning, expression and purification of SelB-M and formation of its SECIS RNA complex

The DNA encoding SelB-M (residues 512–634) was amplified by PCR using appropriate primers (5′-CGCGGATCCGAGACGCAA-AAGAAGCTTCTA-3′ and 5′-CCGGAATTCTCAATTACCAAC-AACTACCCG-3′) and *M. thermoacetica* genomic DNA and cloned into the plasmid vector pGEX-2T (Amersham Biosciences) in between *Bam*HI and *Eco*RI sites for overexpression as a GST-fusion protein. SelB-M was overexpressed in *E. coli* BL21 grown at 310 K to an OD_{600} of 0.4 in LB medium containing 100 µg ml^{-1} ampicillin. IPTG was added to a final concentration of 1 mM to induce GST-SelB-M expression and the cells were grown for a further 3 h at

310 K. The fusion protein was purified essentially as described by Smith & Johnson (1988) with slight modifications. The harvested cells were suspended in MTPBS (150 m*M* NaCl, 16 m*M* Na_2HPO_4, 4 m*M* NaH_2PO_4 pH 7.3) and lysed by sonication. The lysate was centrifuged at 10 000*g* for 10 min at 277 K and the supernatant was loaded onto a glutathione agarose column (Sigma). The GST-fusion protein was eluted with 5 m*M* reduced glutathione (Sigma) in 50 m*M* Tris–HCl pH 8.0. The GST was removed from SelB-M by cleavage with thrombin (Sigma) and purified by glutathione-agarose column chromatography (Fig. 1). The SelB-M fragment was further purified by CM-Sepharose column chromatography (Amersham Biosciences). The absence of ribonuclease activity in the purified protein was confirmed by incubation with the RNA at 310 K for 48 h. The 23 nucleotides of the SECIS RNA hairpin (5′-GGCGUUGCCGGU-CUGGCAACGCC-3′) derived from the *M. thermoacetica fdhA* gene were chemically synthesized and purified by denaturing polyacrylamide gel electrophoresis (PAGE). Equal volumes of the SelB-M protein and SECIS RNA were mixed at a low concentration of 0.1 m*M* and the SelB-M–SECIS RNA complex was concentrated to 8 mg ml^{-1} by ultrafiltration on a Centricon YM-3 device (Millipore) and dialyzed against 10 m*M* Tris–HCl pH 7.0, 100 m*M* NaCl, 0.1 m*M* EDTA. The complex solution was stored at 277 K until use.

2.2. Crystalization of the SelB-M–SECIS RNA complex

Crystals of the complex were obtained by the hanging-drop vapour-diffusion or sitting-drop method at 293 K using 24-well Linbro plates (Hampton Research). Typically, a hanging drop was prepared by mixing equal volumes (1 µl each) of protein–RNA complex solution (at 8 mg ml^{-1}) and reservoir solution. Each hanging drop was placed over 0.7 ml reservoir solution. Preliminary crystallization trials were conducted at 293 K using Crystal Screens I and II and Natrix (Hampton Research). Crystals were obtained with Crystal Screen I solutions 9, 10, 28, 30, 38, 43 and 46 and Natrix solutions 25, 27 and 48. The crystals obtained with one of the solutions, Cystal Screen I solution 38 (0.1 *M* Na HEPES pH 7.5, 1.4 *M* trisodium citrate dihydrate), were suitable for structure determination, as shown in the following section. To obtain larger crystals, an oil-batch crystallization was performed in a similar hanging-drop setup strategy as described above. Crystals appeared within 7 d and reached maximum dimensions (0.3 × 0.3 × 0.4 mm) after two weeks (Fig. 2*a*).

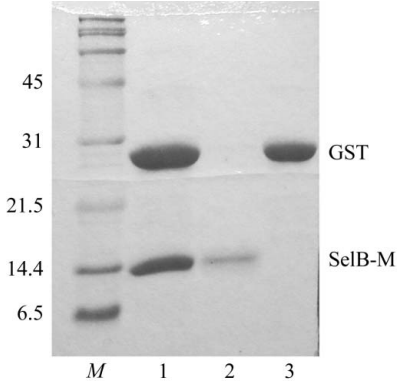

Figure 1
SDS–PAGE showing purification of SelB-M protein. Lane 1, fraction treated by thrombin cleavage before being applied to the glutathione-agarose column. Lane 2, flowthrough of the glutathione column (SelB-M protein). Lane 3, fraction eluted with 5 m*M* glutathione (GST domain). Lane *M*, molecular-weight standards in kDa.

2.3. Preliminary X-ray diffraction analysis

Crystals of the SelB-M–SECIS RNA complex were made suitable for cryo-experiments by increasing the concentration of ethylene glycol to 15%. A diffraction data set was collected to 2.3 Å resolution (Fig. 2*b*) at 100 K from the single largest crystal at beamline BL41XU of SPring-8 (Harima, Japan; λ = 0.984 Å). The diffraction data were autoindexed, integrated and scaled with the *HKL*2000 program package (Otwinowski & Minor, 1997) and indicated space group $P2_12_12$, with unit-cell parameters a = 81.69, b = 169.58, c = 71.69 Å (R_{merge} = 5.2% on data to 2.3 Å resolution, data completeness = 93.0%) and contained three to four complexes per asymmetric unit. The structure was finally solved by molecular replacement with two search probes: the NMR structure of the 23-base fragment of *E. coli* SECIS RNA (Fourmy *et al.*, 2002) and the SelB-M protein of the previously determined free SelB-C structure (Selmer & Su, 2002).

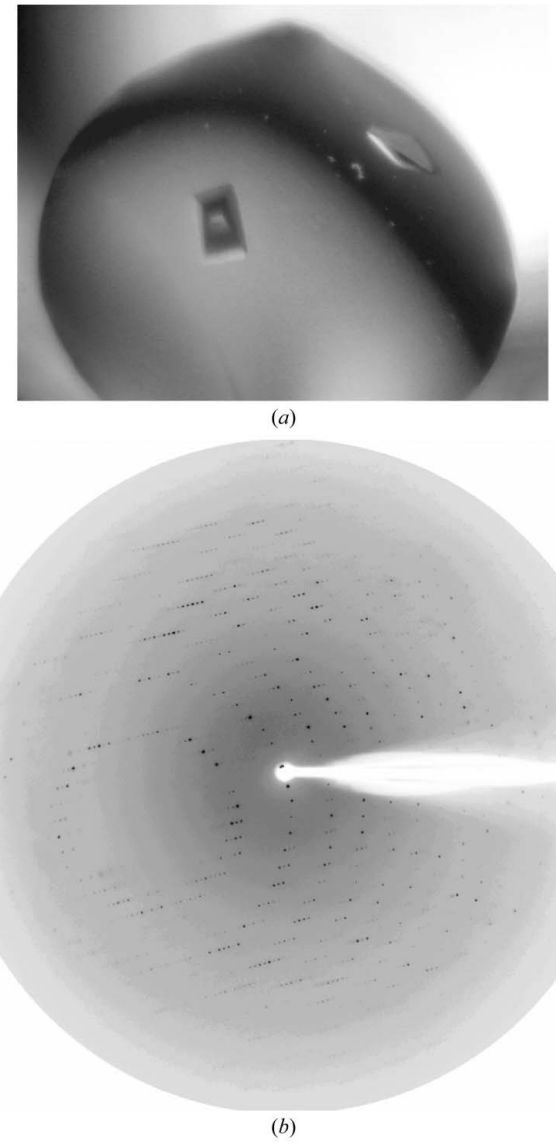

Figure 2
(*a*) Crystals of the SelB-M–SECIS RNA complex in an oil-batch drop. Crystal dimensions are 0.3 × 0.3 × 0.4 mm. (*b*) A typical diffraction pattern of SelB-M–SECIS RNA complex crystals. The detector edge corresponds to 2.3 Å resolution. The exposure time was 9 s, the detector distance was 200 mm and the oscillation range per frame was 1°.

The detailed structural description will be published elsewhere (Yoshizawa *et al.*, 2005); the PDB code is 1wsu.

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