Linda Rasubala,^{a‡} Dominique
Fourmy,^{b‡} Toyoyuki Ose,^{a‡}
Daisuke Kohda,^a Katsumi
Maenaka^{a*} and Satoko
Yoshizawa^{b*}

^aDivision of Structural Biology, Medical Institute
of Bioregulation, Kyushu University,
3-1-1 Maidashi, Higashi-ku, Fukuoka,
Fukuoka 812-8582, Japan, and ^bLaboratoire de
RMN, ICSN-CNRS, 1 Avenue de la Terrasse,
91190 Gif-sur-Yvette, France

‡ These authors contributed equally to this
work.

Correspondence e-mail:
kmaenaka@bioreg.kyushu-u.ac.jp,
yoshizawa@icsn.cnrs-gif.fr

Received 17 December 2004

Accepted 2 February 2005

Online 12 February 2005

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

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 over-expressed 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 consecutive 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 OD₆₀₀ 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

© 2005 International Union of Crystallography
All rights reserved

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 mM NaCl, 16 mM Na₂HPO₄, 4 mM NaH₂PO₄ pH 7.3) and lysed by sonication. The lysate was centrifuged at 10 000g 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 mM reduced glutathione (Sigma) in 50 mM 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 mM 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 mM Tris-HCl pH 7.0, 100 mM NaCl, 0.1 mM EDTA. The complex solution was stored at 277 K until use.

2.2. Crystallization 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, Crystal 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. 2a).

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 mM 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. 2b) at 100 K from the single largest crystal at beamline BL41XU of SPring-8 (Harima, Japan; $\lambda = 0.984$ Å). The diffraction data were autoindexed, integrated and scaled with the *HKL2000* program package (Otwinowski & Minor, 1997) and indicated space group *P*2₁2₁2, with unit-cell parameters $a = 81.69$, $b = 169.58$, $c = 71.69$ Å ($R_{\text{merge}} = 5.2\%$ 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).

(a)

(b)

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.

We thank M. Kawamoto, H. Sakai and K. Miura for assistance in data collection at SPring-8. This work was supported by the joint program JSPS-CNRS to SY and KM, and the French national program GEOMEX to SY and DF. KM and DK were supported in part by Ministry of Education, Science, Sports, Culture and Technology of Japan and the Protein 3000 project. LR was supported by JSPS postdoctoral fellowship for foreign researchers.

References

- Forchhammer, K., Leinfelder, W. & Böck, A. (1989). *Nature (London)*, **342**, 453–456.
- Fourmy, D., Guittet, E. & Yoshizawa, S. (2002). *J. Mol. Biol.* **324**, 137–150.
- Gajiwala, K. S. & Burley, S. K. (2000). *Curr. Opin. Struct. Biol.* **10**, 110–116.
- Kromayer, M., Wilting, R., Tormay, P. & Böck, A. (1996). *J. Mol. Biol.* **262**, 413–420.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Selmer, M. & Su, X.-D. (2002). *EMBO J.* **21**, 4145–4153.
- Smith, D. B. & Johnson, K. S. (1988). *Gene*, **67**, 31–40.
- Yoshizawa, S., Rasubala, L., Ose, T., Kohda, D., Fourmy, D. & Maenaka, K. (2005). *Nature Struct. Mol. Biol.* **12**, 198–203.