crystallization communications

Crystallization and preliminary X-ray crystallographic analysis of the *Sulfolobus solfataricus* nucleotide-exchange factor 1*β*

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The nucleotide-exchange factor isolated from the hyperthermophilic archaeon *Sulfolobus solfataricus* (*Ss*EF-1*β*) consists of 90 residues and differs from eukaryal EF-1*β*s. The protein has been successfully crystallized using either microbatch-under-oil or vapour-diffusion methods. Crystals of native *Ss*EF-1*β* diffract to 1.97 Å resolution and belong to space group $P2_12_12$, with unit-cell parameters $a = 106.46$, $b = 54.87$, $c = 44.03$ Å. Diffraction data have also been collected from a selenomethionine derivative of *Ss*EF-1*β* at 1.83 Å resolution. Model building using the phases derived from the MAD experiment is in progress.

1. Introduction

Protein biosynthesis is a key event in all living organisms. Most of the actions linked to this biological process are performed by the ribosome (Ramakrishnan, 2002). However, ribosome function relies on several ancillary proteins such as initiation, elongation and termination factors. A particularly important role is played by the elongation factors EF-Tu/EF-1*α* that carry the aminoacyl-tRNA to the ribosome in the elongation cycle of the process (Andersen *et al.*, 2003). These elongation factors are GTPases characterized by two important specific properties: (i) an extremely low intrinsic GTPase activity and (ii) a high affinity for the reaction product (GDP). The affinity for GDP is generally higher than that observed for the substrate (GTP). These features allow the transport of the aminoacyl-tRNA, bound to the GTP complex of EF-Tu/EF-1*α*, to the ribosome, as well as efficient modulation of the elongation factor function. The GDP is so tightly bound to these GTPases that other accessory proteins, denoted nucleotide-exchange factors, are necessary for GDP release and for regeneration of the active GTP-bound form of these enzymes. In contrast to elongation factors, which display some common basic properties in all forms of life, exchange factors isolated from the three domains of life display larger differences (Cherfils & Chardin, 1999). Indeed, no sequence similarity is detected between exchange factors isolated from bacteria (EF-Ts) and those extracted from archaea/eukarya (EF-1*β*s). Moreover, differences are also observed between archaeal and eukaryal EF-1*β*s. The molecular mass of eukaryal EF-1*β*s (23 kDa) is twice that exhibited by archaeal counterparts. A limited sequence identity (approximately 20%) is observed between the C-terminal domain of eukaryal exchange factors and archaeal EF-1*β*s.

Structural studies have been carried out by NMR on human (C-terminal fragment; Perez *et al.*, 1999) and *Methanobacterium thermoautotrophicum* EF-1*β* (*Mt*EF-1*β*; Kozlov *et al.*, 2000). The crystallographic structure of the C-terminal fragment of *Saccharomyces cerevisiae* EF-1*β* in complex with the corresponding elongation factor (*Sc*EF-1*α*–EF-1*β*) has also been reported (Andersen *et al.*, 2000, 2001).

The exchange factor EF-1*β* isolated from the hyperthermophilic archaeon *Sulfolobus solfataricus* (*Ss*EF-1*β*) has some distinctive properties (Arcari *et al.*, 1995; Raimo *et al.*, 1996). This protein is endowed with a remarkable thermostability. Indeed, no transition is observed in thermal denaturation curves up to 378 K monitored by far-UV circular dichroism (unpublished results) and 10 h of heating
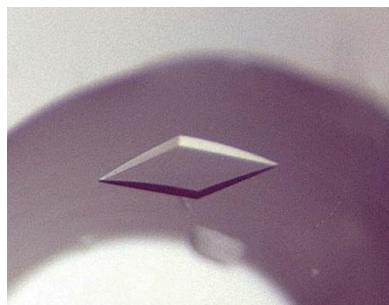
at 373 K is required to achieve 90% inactivation of the protein (Raimo *et al.*, 1996). Additionally, non-denaturing gel filtration of *Ss*EF-1β indicates an apparent molecular mass of 20 kDa, suggesting a dimeric form of this exchange factor (Raimo *et al.*, 1996), since the protein consists of 90 residues with a corresponding molecular mass of 10 kDa. Furthermore, solution studies of the complex between *Ss*EF-1β and its elongation-factor counterpart (*Ss*EF-1α) have also shown an unexpected stoichiometry that indicates that two monomers of the exchange factor bind to a single elongation-factor molecule (Raimo *et al.*, 1999). Therefore, structural studies on *Ss*EF-1β are important for elucidation of its structure–stability and structure–function relationships. Here, we report the crystallization and preliminary crystallographic investigation of this protein.

2. Experimental methods

2.1. Purification and crystallization

Recombinant *Ss*EF-1β was expressed in *Escherichia coli* BL21(DE3) strain as previously reported (Ianniciello *et al.*, 1998). *E. coli* cells were mechanically disrupted and the cell debris was removed by centrifugation. After heating the supernatant at 353 K, the protein was purified using anion-exchange chromatography. The purity and homogeneity were tested by SDS–PAGE. The protein was concentrated to 15 mg ml^{-1} in a Centricon YM-3 concentrator and stored at 277 K in a solution containing 20 mM Tris–HCl pH 7.8.

The selenomethionine (SeMet) derivative of *Ss*EF-1β was prepared by growing the *E. coli* strain expressing the enzyme in minimal media containing 1 mg l^{-1} vitamins (riboflavin, niacinamide, pyridoxine monohydrochloride and thiamine), 0.4% glucose, 2 mM MgSO$_4$, 0.1 mM CaCl$_2$, 50 mg l^{-1} of the amino acids Phe, Thr, Lys, Ile, Leu and Val and 60 mg l^{-1} seleno-L-methionine. 5 mM DTT was added to all solutions used for purification of the SeMet derivative.

Crystallization experiments were performed at 293 K using either the microbatch-under-oil or hanging-drop vapour-diffusion methods. Preliminary crystallization trials were carried out using commercially available sparse-matrix screens (Crystal Screen and Wizard kits I and II from Hampton Research and deCode Genetics, respectively). Crystals suitable for X-ray diffraction were obtained by tuning the protein and precipitant concentrations.

A similar approach was used to grow crystals of the SeMet derivative. In this case, to prevent oxidation of the SeMet derivative, 5 mM DTT was added to the crystallization medium.
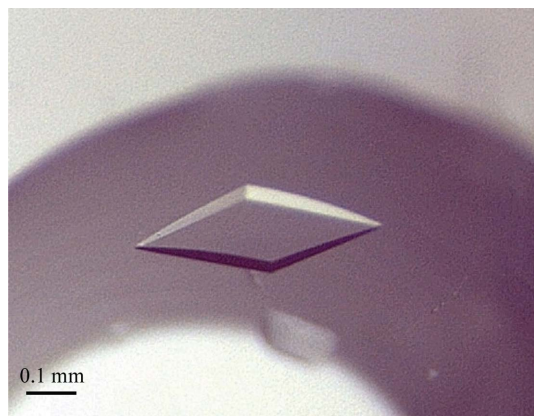

Figure 1
Image of a typical *Ss*EF-1β crystal grown using PEG 3000 as a precipitating agent (see text for details).

2.2. Data collection and processing

Preliminary diffraction data were collected in-house at 298 K using a MacScience DIP 2030b imaging plate equipped with a Nonius FR591 generator producing Cu $K\alpha$ radiation of wavelength 1.5418 Å. Higher resolution data were collected at beamline BW7B of the DESY synchrotron (Hamburg, Germany) at 100 K. Crystals were flash-cooled after the addition of 20%(v/v) ethylene glycol to the crystallization buffer. Data processing was performed using the program *DENZO* (Otwinowski & Minor, 1997). The data sets were scaled and merged using the program *SCALEPACK* (Otwinowski & Minor, 1997).

Multiwavelength anomalous diffraction (MAD) data were collected at beamline ID29 of the ESRF synchrotron (Grenoble, France). Three different data sets were collected from a single crystal using wavelengths determined from the selenium-absorption spectrum.

2.3. Structure determination

Solution of the *Ss*EF-1β structure using MAD methods is in progress. The program *SOLVE* (Terwilliger & Berendzen, 1999) was used to identify and localize the selenium sites present in the asymmetric unit and to derive the experimental phases. Phases were improved by density modification using the program *DM* (Cowtan & Main, 1998). Model building using both automatic and manual approaches is in progress.

3. Results and discussion

The initial screenings using commercially available solutions revealed several promising conditions for crystallization of *Ss*EF-1β. All favourable conditions were characterized by the presence of polyethylene glycol as precipitating agent. The quality of the crystals was improved by fine-tuning the concentration of the protein and of the precipitants. *Ss*EF-1β crystals (Fig. 1) suitable for X-ray diffraction
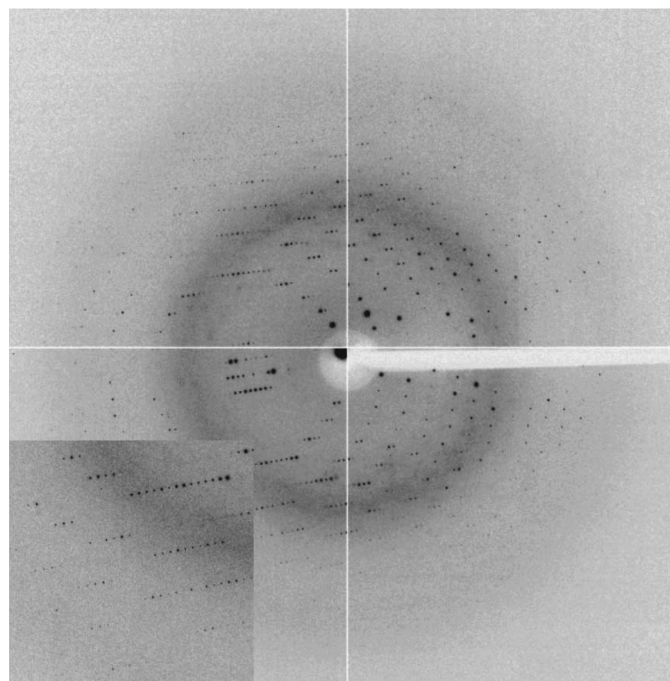

Figure 2
Diffraction pattern of a *Ss*EF-1β crystal (SeMet derivative). Diffraction data are detectable to 1.8 Å resolution.

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| | Native | SeMet derivative | | |
		Peak	Edge	Remote
Crystal				
Beamline	BW7B, DESY	ID29, ESRF		
Space group	$P2_12_12$	$P2_12_12$		
Unit-cell parameters (Å)				
a	106.46	105.72		
b	54.87	54.69		
c	44.03	43.42		
Resolution (Å)	30–1.97	30–1.83	30–1.83	30–1.83
	(2.04–1.97)	(1.86–1.83)	(1.86–1.83)	(1.86–1.83)
Wavelength (Å)	0.850	0.9792	0.9794	0.9756
Mean redundancy	3.6	3.8	3.8	3.8
Completeness (%)	90.4 (79.5)	99.4 (97.1)	99.4 (96.5)	99.4 (97.1)
Unique reflections	17141	42642	42600	42703
R_{merge}† (%)	8.9 (37.3)	5.7 (29.8)	5.9 (35.4)	5.9 (35.9)
Mean $I/\sigma(I)$	12.9 (2.3)	19.4 (3.0)	18.9 (2.4)	18.9 (2.3)

† $R_{merge} = \sum_h \sum_i |I(h,i) - \langle I(h) \rangle| / \sum_h \sum_i I(h,i)$, where $I(h,i)$ is the intensity of the ith measurement of reflection h and $\langle I(h) \rangle$ is the mean value of the intensity of reflection h. For the SeMet derivative the R_{merge} value has been calculated considering the Bijovet pairs individually.

data collection (0.3 × 0.2 × 0.1 mm) were obtained using 5–10 mg ml^{-1} protein solution and 9–10%(w/v) PEG 3000 as precipitant in 50 mM sodium acetate pH 4.5 buffer. Notably, crystals with a similar morphology were obtained using other rather different conditions. In particular, they were obtained in the presence of (i) 15%(w/v) PEG 8000 and 0.1 M (NH$_4$)$_2$SO$_4$, (ii) 10–15%(w/v) PEG 8000, 100 mM lithium sulfate and 50 mM sodium acetate pH 4.5 and (iii) 15–20%(w/v) PEG 1000, 100 mM lithium sulfate and 50 mM phosphate–citrate buffer pH 4.2.

All these crystals proved to be isomorphous, with unit-cell parameters $a = 106.46$, $b = 54.87$, $c = 44.03$ Å (space group $P2_12_12$). Using DESY synchrotron radiation, crystals of SsEF-1β diffracted to 1.97 Å (Fig. 2 and Table 1). Matthews coefficient calculations (Matthews, 1968) suggested the presence of either two ($V_M = 3.2$ Å^3 Da^{-1}, with 62% solvent content) or three ($V_M = 2.1$ Å^3 Da^{-1}, with 41% solvent content) SsEF-1β molecules per asymmetric unit. However, the self-rotation function did not exhibit any significant peak apart from that located at the origin.

Several attempts were made to solve the structure by molecular replacement (MR) using *S. cerevisiae* and *M. thermoautotrophicum* EF-1β structures as starting models. However, all these attempts were unsuccessful. The failure of MR trials using the structure of ScEF-1β extracted from the ScEF-1α–EF-1β complex (PDB code 1f60; Andersen *et al.*, 2000) can be ascribed to the very low sequence identity (<20%) and to the fact that the ScEF-1β structure may be affected by the formation of the complex with ScEF-1α. Moreover, taking into account that MR often fails when NMR models are used (Chen, 2001), it is not surprising that the use of MtEF-1β (PDB code 1gh8; Kozlov *et al.*, 2000) did not yield correct solutions, despite the sequence identity shown by SsEF-1β and MtEF-1β (36%). In both cases the presence of two SsEF-1β molecules in the asymmetric unit of the crystal was a further difficulty for the MR approach.

Since MR proved to be unsuccessful, MAD experiments were carried out to obtain experimental phases. Therefore, an SeMet derivative of the protein was prepared. It is worth mentioning that, excluding the N-terminal methionine, a sole methionine is present in SsEF-1β sequence, which consists of 90 residues. Crystals of SeMet SsEF-1β were obtained from solutions containing 10–12%(w/v) PEG 8000, 50 mM lithium sulfate and 50 mM sodium acetate pH 4.5. In order to determine the peak and the inflection wavelengths, a fluorescence scan was recorded on a single SeMet-labelled SsEF-1β crystal. Using data sets collected at wavelengths optimized for selenomethionine, the program *SOLVE* (Terwilliger & Berendzen, 1999) identified two selenium sites in the asymmetric unit of the protein. This finding supports the hypothesis that two SsEF-1β molecules are present in the asymmetric unit. The program *SOLVE* also provided a set of initial phases. These phases were improved using the methods implemented in the program *DM* (Cowtan & Main, 1998). Using these improved phases, the program *ARP/wARP* (Perrakis *et al.*, 1999) was able to automatically trace nearly 60% of the residues present in the asymmetric unit. Manual model-building sessions aimed at defining the complete SsEF-1β structure are in progress.

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