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Purification, crystallization and preliminary X-ray diffraction analysis of the FeoB G domain from *Methanococcus jannaschii*

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The transmembrane protein FeoB plays a key role in ferrous iron acquisition in prokaryotes. The N-terminal domain of FeoB from *Methanococcus jannaschii* was overproduced, purified to homogeneity and crystallized in the presence of GTP and magnesium. The native protein crystallized in a tetragonal space group and the crystals diffracted to beyond 2.2 Å resolution, with unit-cell parameters $a = b = 84.77$, $c = 137.90$ Å. The Matthews coefficient and the solvent content were estimated to be 2.65 Å^3 Da^{-1} and 53.64%, respectively, which corresponds to the presence of two molecules per asymmetric unit. To obtain initial phases, selenomethionyl-substituted protein was overproduced, purified and crystallized.

1. Introduction

For most prokaryotes, the transition metal iron is an indispensable cofactor in biological processes such as DNA synthesis, oxygen reduction for ATP synthesis or haem formation (Neilands, 1995). In an aerobic environment, iron is present in the ferric (Fe^{3+}) form as insoluble iron hydroxide complexes. This form can be complexed in the extracellular space by siderophores and taken up mainly *via* ABC transporters (Braun *et al.*, 1998; Ratledge & Dover, 2000). Under anaerobic or reducing conditions, iron exists predominantly as ferrous iron (Fe^{2+}), which has higher solubility than Fe^{3+}. The major pathway for bacterial ferrous iron uptake is *via* the Feo system (Cartron *et al.*, 2006). The Feo system consists of up to three proteins encoded by the *feoABC* locus. The central unit of the Feo system is the transmembrane protein FeoB, while FeoA and FeoC are probably localized in the cytoplasm (Cartron *et al.*, 2006). FeoB-mediated ferrous iron uptake has been found to play an essential role in bacterial virulence (Boyer *et al.*, 2002), intracellular survival (Robey & Cianciotto, 2002) and/or gastrointestinal tract colonization of the host (Stojiljkovic *et al.*, 1993; Velayudhan *et al.*, 2000). FeoB is a putative permease with a hydrophilic region normally consisting of 250–300 amino acids in the N-terminus and a C-terminal transmembrane part (Cartron *et al.*, 2006) which is predicted to contain 8–12 membrane-spanning helices (Hantke, 2003). The N-terminal region contains a G domain as shown by biochemical experiments and sequence analysis showed the presence of four of the five motifs usually found in G domains (Marlovits *et al.*, 2002). These motifs are involved in the binding of magnesium and nucleotide and in the catalysis of GTP to GDP (Sprang, 1997). The G5 motif, which is not highly conserved among GTP-binding proteins, could not be identified in FeoB proteins. The N-terminal G domain is connected to the transmembrane domain *via* a linker region. The linker and part of the transmembrane domain have been shown to interact with the G domain and to increase the affinity of *Escherichia coli* FeoB for GDP and were therefore termed the GDP-dissociation inhibitor (GDI) domain (Eng *et al.*, 2008). As a full-length protein, FeoB is expected to exist mainly in its presumably inactive GDP-bound state and therefore it was concluded that FeoB follows a regulatory mechanism comparable to the regulatory G-protein cycle of eukaryotic cells (Eng *et al.*, 2008). This cycle demonstrates the existence of guanine

nucleotide-binding proteins (GNBPs) in a GTP-bound state and a GDP-bound state. Guanine-exchange factors (GEFs) activate GNBPs by exchanging GDP for GTP, while GAPs deactivate them by accelerating the intrinsic GTPase activity of the GNBPs (Vetter & Wittinghofer, 2001). Regulatory proteins equivalent to the GEFs and GAPs are so far unknown for FeoB.

Although many aspects pertaining to FeoB G-domain biochemistry are beginning to emerge, there are no structural data available to date. To fill this gap, we cloned, overproduced, purified and crystallized the G domain of FeoB from the archaeon *Methanococcus jannaschii* in the presence of GTP and Mg^{2+}. The structure will help to elucidate the detailed reaction mechanism of GTP hydrolysis in FeoB proteins.

2. Experimental procedures

2.1. Cloning

The gene segment encoding residues 1–184 of FeoB from *M. jannaschii* (MJ0566) was PCR-amplified with *Pfu* polymerase (Stratagene). The template was purchased from LGC Promochem (ATCC 625346). For protein overproduction with an N-terminal hexahistidine tag, the gene was integrated into the vector pSKB2LNB. This vector is a derivative of pET28a (Novagen) in which the thrombin recognition site was replaced by a PreScission recognition site. For insertion of the gene, *Eco*RI and *Xho*I sites were incorporated through PCR primer extensions at the 5′- and 3′-ends, respectively. After verifying the gene sequence, *E. coli* BL21(DE3) cells were transformed with the resulting plasmid.

2.2. Protein overproduction and purification

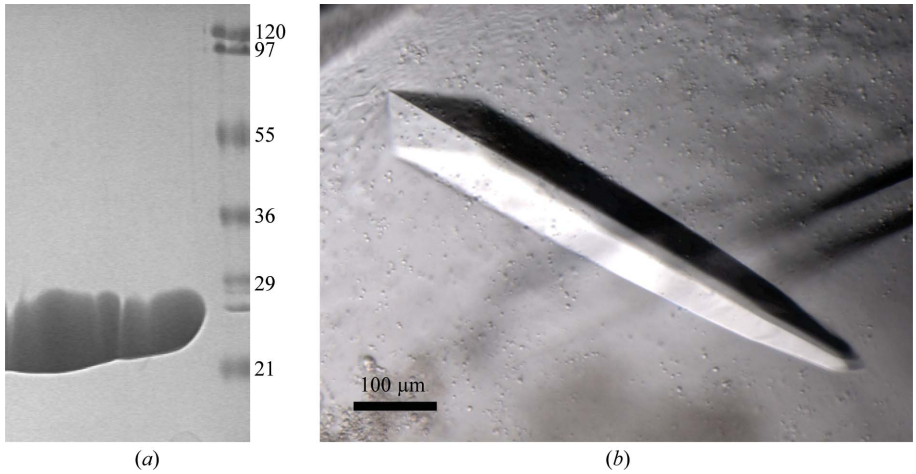
For heterologous overproduction of the recombinant protein, one bacterial colony was used to inoculate 100 ml Luria–Bertani broth containing 50 µg ml^{-1} kanamycin followed by overnight incubation at 310 K. The preculture was then transferred to 10 l Terrific broth (TB) with 50 µg ml^{-1} kanamycin and grown at 310 K to an OD_{600} of 1.5–2.0. After induction with 0.5 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) and cultivation for a further 5 h at 298 K, the cells were harvested by centrifugation (20 min, 4200*g*, 277 K), resuspended in lysis buffer (50 m*M* Tris–HCl pH 7.7, 100 m*M* NaCl) and passed four times through a microfluidizer (model M-110L; Microfluidics Corp., Newton, Massachusetts, USA) with a 100 µm interaction chamber and cooling coil both immersed in cold water (277 K).

Intact cells and cell debris were removed by centrifugation (1 h at 12 000*g*). The resulting cell-free extract was applied onto a 40 ml column containing His Select nickel-affinity gel (Sigma) at 277 K using an ÄKTA FPLC system (GE Healthcare). Contaminant proteins were removed by washing with washing buffer containing 50 m*M* Tris–HCl pH 7.7, 100 m*M* NaCl, 30 m*M* imidazole pH 8.0 (Tris–HCl and imidazole were prepared from 1 *M* stock solutions with the indicated pH). The target protein was eluted from the nickel column with elution buffer containing 50 m*M* Tris–HCl pH 7.7, 100 m*M* NaCl and 200 m*M* imidazole pH 8.0. The eluted protein was concentrated to 4 ml using a Vivaspin 10K filter (Sartorius). The concentrated protein was then loaded onto a Hiload 16/60 Superdex 200 (GE Healthcare) size-exclusion column pre-equilibrated with S200 buffer (25 m*M* Tris–HCl pH 7.7) with a flow rate of 1 ml min^{-1}. Fractions containing the pure protein were pooled, concentrated (Vivaspin 10K, Sartorius) to 20 mg ml^{-1}, flash-frozen in liquid nitrogen and stored at 193 K. The protein purity was estimated to be greater than 95% by SDS–PAGE analysis (Fig. 1*a*).

Selenomethionyl-substituted (SeMet) FeoB G domain was overproduced as for the native protein except that M9 minimal medium was used instead of TB, suppressing methionine biosynthesis by a fivefold increase in the concentrations of the amino acids Leu, Ile, Lys, Phe, Thr and Val. Pre-cultures were grown overnight in LB medium containing antibiotic and were spun down by gentle centrifugation prior to inoculation of the main culture. Protein overproduction and purification was performed as previously described for the native protein.

2.3. Crystallization

Before crystallization, $MgCl_2$ (1 *M* stock solution) and GTP (200 m*M* stock solution) were added to the protein solution to final concentrations of 10 and 5 m*M*, respectively. Initial screening was carried out using the hanging-drop vapour-diffusion technique using Index Screen and Crystal Screens 1 and 2 (Hampton Research). Equal volumes (400 nl) of protein and reservoir solution were pipetted and mixed by a Mosquito robot (Molecular Dimensions) and were incubated at 291 K against 100 µl reservoir solution. Initial crystallization setups yielded six hits. In five conditions only needles grew, while in one condition small rod-shaped crystals were found



(*a*) (*b*)

Figure 1
(*a*) SDS–PAGE of the purified FeoB G domain used for crystallization. The molecular masses of the markers are indicated in kDa. (*b*) Typical crystal of native FeoB(1–184) in the presence of $MgCl_2$ and GTP obtained by the hanging-drop vapour-diffusion technique, with an average length of 500 µm. Crystals were obtained using 425 m*M* potassium/sodium tartrate as a crystallizing agent.

Table 1
Crystal parameters and data-collection statistics for native crystals.

Values in parentheses are for the highest resolution shell.

Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 84.7$, $c = 137.9$
Matthews coefficient (Å^3 Da^{-1})	2.65
Solvent content (%)	53.64
No. of molecules per ASU	2
X-ray source	ID14.3, ESRF
Wavelength (Å)	0.931
Resolution (Å)	40–2.2 (2.3–2.2)
Total observations	343855 (35915)
Unique observations	24483 (2671)
Completeness (%)	93.3 (83.3)
$R_{\rm meas}$ (%)	7.0 (73.4)
$I/\sigma(I)$	29.2 (4.8)

Table 2
Crystal parameters for SeMet crystals.

Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 84.7$, $c = 137.9$
Matthews coefficient (Å^3 Da^{-1})	2.65
Solvent content (%)	53.64
No. of molecules per ASU	2
X-ray source	Cu $K\alpha$
Wavelength (Å)	$\lambda_{\rm peak} = 1.54$
Resolution (Å)	20–3

and were optimized in 24-well plates. For crystal optimization, 1 µl protein solution and 1 µl reservoir solution were mixed over a well containing 1 ml reservoir solution and equilibrated at 291 K.

2.4. X-ray data collection and processing

Native crystals were incubated for 2 min in a 1:1(*v*:*v*) mixture of reservoir solution and cryoprotectant solution (30% xylitol/5% inositol in water), which was followed by a short dip in cryoprotectant solution and flash-freezing in liquid nitrogen. Native data were collected under a stream of nitrogen gas (100 K) on synchrotron beamline ID14-3 at the European Synchrotron Research Facility (ESRF), Grenoble, France and were processed using *XDS* (Kabsch, 1993). SeMet crystals were processed as for the native crystals except that the cryoprotectant solution was 15% xylitol/5% inositol/20% ethylene glycol in water.

3. Results and discussion

The G domain containing amino acids 1–184 of FeoB from *M. jannaschii* was cloned, overproduced and purified to homogeneity (Fig. 1*a*). Initial crystallization screening resulted in several hits for the native protein. After optimization, the best quality crystals grew after 1 d in a 1:1 mixture of protein solution and 425 m*M* potassium/sodium tartrate. These crystals had a rod-like morphology with average dimensions of 150 × 150 × 500 µm (Fig. 1*b*). The crystals diffracted to 2.2 Å resolution (Fig. 2) and belonged to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 84.7$, $c = 137.9$ Å (Table 1). A full 180° data set was collected using a crystal-to-detector distance of 206 mm with 1° oscillation and 6 s exposure per image. The calculated Matthews coefficient $V_{\rm M}$ (Matthews, 1968) of 2.65 Å^3 Da^{-1} (solvent content 53.64%) suggested the presence of two molecules per asymmetric unit. Several attempts to solve the structure by molecular replacement using *Phaser* (McCoy, 2007) from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) were carried out with H-ras p21 in complex with GTP (Pai *et al.*, 1990) as a search model (PBD code 5p21) since other GNBPs have insertions of different length in their switch regions. However, these trials failed to give any satisfactory solution.

To obtain the phase information, we overproduced the FeoB G domain in the presence of SeMet and purified the (SeMet) FeoB G domain according to the protocol established for the native protein. This SeMet-substituted protein crystallized under the same conditions as the native protein. However, better diffracting SeMet crystals were obtained with a reservoir solution containing 100 m*M* sodium cacodylate pH 6.5 and 400 m*M* sodium acetate. The crystals obtained under these new conditions had the same morphology and dimensions as the native crystals. The SeMet crystals diffracted to ∼3 Å resolution using an in-house X-ray source (Cu $K\alpha$ radiation from a Rigaku rotating-anode generator) with an R-AXIS IV image-plate detector (Table 2). Although the crystallization conditions of the SeMet protein were different from those of the native protein, the

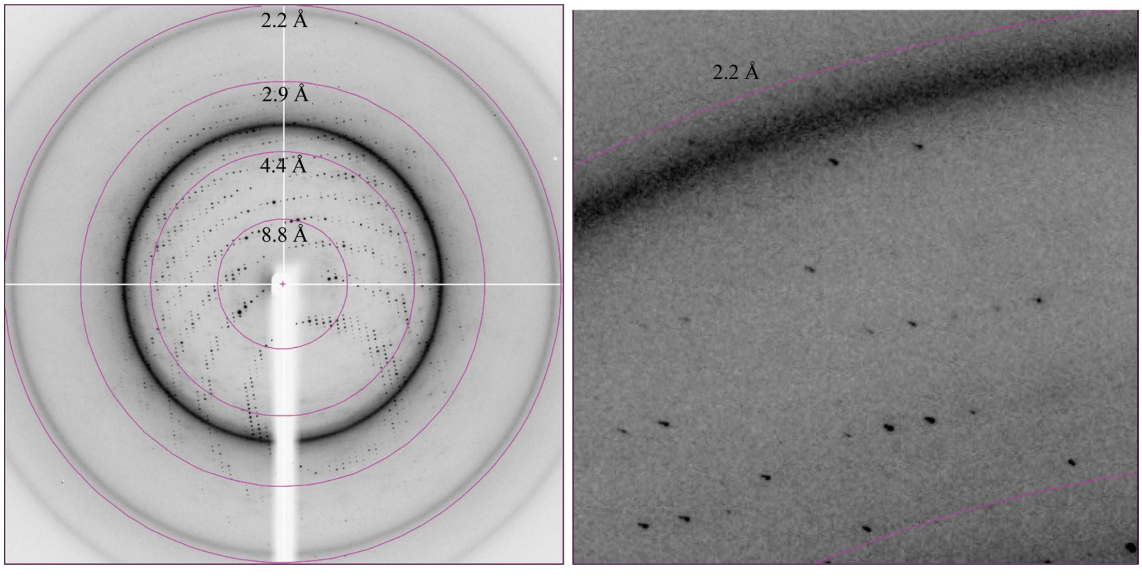


Figure 2
X-ray diffraction pattern collected from native FeoB(1–184) crystals with 1° oscillation per image displayed using *MOSFLM* (Powell, 1999). The purple circles and numbers correspond to the resolution shells. The pattern displays a maximum resolution of 2.2 Å.

space group and unit-cell parameters were the same. In order to solve the structure of the FeoB G domain, we intend to obtain experimental phases by the multiple-wavelength anomalous diffraction (MAD) approach using SeMet-incorporated crystals. We have collected a complete MAD data set and we are currently trying to obtain phase information for the FeoB G domain.

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References

Boyer, E., Bergevin, I., Malo, D., Gros, P. & Cellier, M. F. (2002). *Infect. Immun.* **70**, 6032–6042.
Braun, V., Hantke, K. & Koster, W. (1998). *Met. Ions Biol. Syst.* **35**, 67–145.
Cartron, M. L., Maddocks, S., Gillingham, P., Craven, C. J. & Andrews, S. C. (2006). *Biometals*, **19**, 143–157.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Eng, E. T., Jalilian, A. R., Spasov, K. A. & Unger, V. M. (2008). *J. Mol. Biol.* **375**, 1086–1097.
Hantke, K. (2003). *Trends Microbiol.* **11**, 192–195.
Kabsch, W. (1993). *J. Appl. Cryst.* **26**, 795–800.
Marlovits, T. C., Haase, W., Herrmann, C., Aller, S. G. & Unger, V. M. (2002). *Proc. Natl Acad. Sci. USA*, **99**, 16243–16248.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
McCoy, A. J. (2007). *Acta Cryst.* D**63**, 32–41.
Neilands, J. B. (1995). *J. Biol. Chem.* **270**, 26723–26726.
Pai, E. F., Krengel, U., Petsko, G. A., Goody, R. S., Kabsch, W. & Wittinghofer, A. (1990). *EMBO J.* **9**, 2351–2359.
Powell, H. R. (1999). *Acta Cryst.* D**55**, 1690–1695.
Ratledge, C. & Dover, L. G. (2000). *Annu. Rev. Microbiol.* **54**, 881–941.
Robey, M. & Cianciotto, N. P. (2002). *Infect. Immun.* **70**, 5659–5669.
Sprang, S. R. (1997). *Annu. Rev. Biochem.* **66**, 639–678.
Stojiljkovic, I., Cobeljic, M. & Hantke, K. (1993). *FEMS Microbiol. Lett.* **108**, 111–115.
Velayudhan, J., Hughes, N. J., McColm, A. A., Bagshaw, J., Clayton, C. L., Andrews, S. C. & Kelly, D. J. (2000). *Mol. Microbiol.* **37**, 274–286.
Vetter, I. R. & Wittinghofer, A. (2001). *Science*, **294**, 1299–1304.