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Crystallization and preliminary X-ray diffraction studies of the signal recognition particle receptor FtsY from *Mycoplasma mycoides*

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The prokaryotic signal recognition particle (SRP) pathway comprises two proteins, Ffh and FtsY, homologous to the SRP54 and SRα proteins in the more complex eukaryotic system. All four proteins are part of a unique subfamily of GTPases. Four truncated versions of the 412 amino-acid FtsY receptor protein from *Mycoplasma mycoides* have been cloned, expressed in *Escherichia coli* and purified. Purified proteins from all constructs and the full-length FtsY protein were subjected to crystallization trials. Crystals were obtained for the construct which comprised residues 98–412 corresponding to the conserved NG-domain (residues 194–497 in *E. coli*). A native data set at 1.9 Å resolution has been collected at 100 K using synchrotron radiation. The crystals belong to the space group $P2_12_12$, with unit-cell parameters $a = 68.7$, $b = 101.1$, $c = 42.5$ Å and one molecule in the asymmetric unit.

1. Introduction

The eukaryotic signal recognition particle (SRP) plays an important role in protein transport to and translocation across the endoplasmic reticulum (ER). The particle comprises six proteins of 9, 14, 19, 54, 68 and 72 kDa and one 7S (300 nucleotides) RNA (Walter & Blobel, 1980; reviewed in Lütcke, 1995). The 54 kDa subunit of SRP (SRP54) binds to a highly hydrophobic signal sequence present at the N-terminal end of proteins to be secreted as they emerge from the ribosome. Further translation of the nascent protein from the ribosome is arrested (Siegel & Walter, 1988) and SRP targets the new complex to the ER, where it interacts with the SRP receptor (or docking-protein complex) anchored in the ER. The SRP receptor is composed of two units, SRα and SRβ, which form a heterodimer. Regulation of this pathway is performed by the SRP54 and the SRP receptor. These three proteins are GTPases (Connolly & Gilmore, 1989; Miller *et al.*, 1995) and the switch from a GTP-active form to a GDP-inactive form releases the SRP from the complex. This initiates the co-translational translocation of the nascent chain in the ER pore (translocon) and a new round of targeting (Connolly & Gilmore, 1989).

Components of the SRP pathway have been found in several bacteria, in addition to the well studied Sec machinery constituents of the main translocation pathway. This SRP pathway targets specifically certain subsets of proteins to be secreted to the plasma membrane. First identified in *E. coli* (Bernstein *et al.*, 1989; Römisch *et al.*, 1989) but also present in many other organisms including *Mycoplasma* (Samuelsson, 1992), this bacterial SRP system is much simpler and consists of a 4.5S RNA, one SRP protein (Ffh) and one receptor protein (FtsY). *Mycoplasma* are prokaryotes distinct from bacteria and viruses. They have very small genomes of about 600 genes, which is approximately half that of *E. coli*. Therefore, mycoplasmas can be said to represent a 'minimal living system' comprising only the absolutely essential genes for survival. *Mycoplasma* lead a parasitic existence in close association with animal or plant cells. They completely lack cell walls, which gives them their characteristic jellyfish appearance. They are insensitive to penicillin and cause diseases including arthritis and pneumonia. The discovery of an SRP particle in this organism clearly emphasizes the importance of the SRP pathway for prokaryotic systems (Luirink & Dobberstein, 1994; Hutchison *et al.*, 1999).

The homologous proteins SRP54, Ffh, SRα and FtsY can be divided into three domains (Fig. 1). Common to all is the highly conserved GTPase domain (G-domain), which contains the GTP consensus elements G1–G4 unique to the SRP family. For example, an insertion in the effector-binding region (the so-called I-box) has been identified in the SRP GTPases (Montoya *et al.*, 1997*a*; Freymann *et al.*, 1997) and is suggested to act as a built-in nucleotide-exchange factor (Moser *et al.*, 1997). The short N-terminal domain (N-domain) upstream of the G-domain can be viewed as an extension of the G-domain and has no assigned function. The NG-domain can be obtained by mild

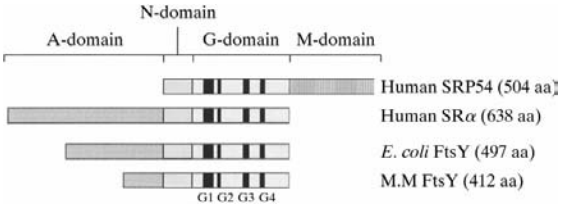

Figure 1
Linear representation of an SRP54 protein and SRα homologues (*M. mycoides* is shortened to M.M). In common is the GTPase G-domain containing the four GTP consensus (G1–G4, black bars). The positions of the N-, M- and A-domains are also indicated.

proteolysis (Kusters *et al.*, 1995). Unique to all SRP54 and Ffh homologues is the C-terminal methionine-rich domain (M-domain). This domain varies in length between different organisms and binds to SRP RNA and to signal sequences. The receptor homologues contain an extended N-terminal region (referred to as the A-domain) with a high content of charged residues. The A-domain can also vary significantly in length between species; its function remains unclear.

Recently, crystal structures of various domains of SRP54 and FtsY have become available. The NG-domains, as identified by limited proteolysis, of Ffh and FtsY from *Thermus aquaticus* and *E. coli*, respectively, were solved at high resolution and provided the first detailed description of these proteins at the molecular level (Montoya *et al.*, 1997*a*; Freymann *et al.*, 1997). As expected, the two proteins presented the same overall fold. Ffh from *T. aquaticus* has also been studied in complex with GDP (Freymann *et al.*, 1999) and the M-domain has been structurally characterized as part of the full-length Ffh protein (Keenan *et al.*, 1998), as a separate entity (Clemons *et al.*, 1999) and in complex with RNA (Batey *et al.*, 2000)

This paper describes the crystallization and preliminary diffraction data of the NG-domain of the FtsY protein from *M. mycoides*. The overall sequence identity of this protein to that from *E. coli* is 40.4% (25.5% for the N-domain and 47.0% for the G-domain). The two SRP systems have physiological differences: in *E. coli* the SRP RNA is compulsory for recognition and interaction between the Ffh protein and its receptor, but it is not in *M. mycoides* (Macao *et al.*, 1997). This difference implies that the *M. mycoides* SRP system represents the framework of all SRP systems. Structural studies on this system would allow a deeper insight into the understanding of the regulatory mechanisms between the SRP and its receptor.

2. Protein expression and purification

A plasmid pet9d-FtsY$_{wt}$ (a gift from Dr Samuelsson, Göteborg University) which includes the full-length *M. mycoides* FtsY protein was expressed in *E. coli*. The protein contains a C-terminal histidine tag and was purified as described (Samuelsson, 1992; Macao *et al.*, 1997) and used for crystallization trials. During this work, we noticed that storage of the protein at 277 K results in four degradation products (data not shown). N-terminal sequencing of the four degradation products, of which three could be assigned to residues 28 (A1), 49 (A2) and 69 (A3), identified all cleavage sites within the acidic A-domain. These sites are similar but not identical to the ones obtained by protease K cleavage (Macao *et al.*, 1997). A fourth construct (A4) was designed (residues 98–412) to correspond to the NG-domain of *E. coli* FtsY (residues 194–497), which had been reported to yield well diffracting crystals (Montoya *et al.*, 1997*b*). The four truncated proteins (A1–A4) and the full-length protein (AF) were subsequently cloned using PCR with the following primers at the 5′ end: CAT GCC ATG GGT TTT TGG GCT AAA TTA AAAG′, CAT GCCC ATG GAA CAA CAA GAT CAA CAA GAA C, CAT GCC ATG GTT AAT CAA GAT AAA CAA GTT G, CAT GCC ATC CCA AAA ACA AAA ACT AGT GAA AC and CAT GCC ATG GAA AAA GCA ATG CTT AAA TCA GC for the constructs AF, A1, A2, A3 and A4, respectively. The same primer, CGG GGT ACC TTA TTT TTC AAC TTC ATC ACC TTG, was used at the 3′ end for all the constructs. The oligonucleotides were purchased from Pharmacia Biotech and were tagged with *Nco*I and *Kpn*I sites at the N- and C-terminal ends, respectively. The PCR products were cleaved with *Nco*I and *Kpn*I and cloned in a pet24dN-His plasmid kindly provided by Gunther Stier (EMBL, Heidelberg). This plasmid expresses an N-terminal histidine tag. The absence of any nucleotide substitution in the cloned fragments was confirmed by DNA sequencing. Competent *E. coli* BL21 (DE3) (Novagen) cells were transformed with plasmid pet24dN-His and the transformants were grown at 310 K in Luria–Bertani (LB) broth supplemented with kanamycin (30 μg ml^{-1}) and induced (at A_{600} = 0.5) with 0.2 m*M* isopropyl thiogalactopyranoside (IPTG). After additional incubation for 3 h at 310 K, the cells were harvested by centrifugation and washed in 20 m*M* Tris–HCl pH 7.5 and 0.2 *M* NaCl before storage at 253 K.

All purification steps were carried out at 277 K. The thawed cells were resuspended in 50 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl and the suspension was passed twice through a French pressure cell at 30 MPa. The cell lysate was ultracentrifuged at 180 000*g* for 40 min. 2 ml of Ni–NTA Agarose (Qiagen) pre-equilibrated with buffer *A* (0.1 *M* potassium acetate pH 7.6, 20 m*M* Tris-acetate pH 7.6, 10% glycerol, 5 m*M* β-mercaptoethanol and 10 m*M* imidazole pH 7.6) was added to the supernatant and left on ice for 10 min. This suspension was packed into a Poly Prep chromatography column (BioRad). After extensive washing with buffer *A*, the salt concentration was increased to 0.6 *M* NaCl to reduce the amount of unspecifically bound protein. After one additional imidazole wash with buffer *A* plus 50 m*M* imidazole, the protein was finally eluted with buffer *A* plus 0.2 *M* imidazole. Fractions containing the protein were pooled and concentrated to a final volume of 2 ml and loaded onto a Superdex 75 gel-filtration column (Pharmacia Biotech) previously equilibrated with 20 m*M* Tris–HCl pH 8.0 and 0.3 *M* NaCl buffer. The peak fractions of FtsY or FtsY variants were pooled and concentrated using a Centriprep-10 (Millipore) to final concentrations exceeding 7 mg ml^{-1}.

3. Crystallization and preliminary X-ray diffraction studies

The hanging-drop vapour-diffusion method was used at 277 and 291 K (McPherson, 1982). All protein constructs were extensively subjected to crystallization trials, but only the construct A4, corresponding to the NG domain, yielded crystals suitable for X-ray diffraction. The first crystal form was obtained by equilibrating drops containing 2 µl of protein and 2 µl of reservoir solution against the reservoir solution (2 *M* ammonium sulfate, 0.2 *M* potassium tartrate, 10 m*M* $MgCl_2$, 0.1 *M* sodium citrate pH 5.6) at 291 K. The crystals grew to 0.2 × 0.2 × 0.3 mm in 2–3 weeks (Fig. 2). The second crystal form was obtained under similar experimental conditions except that the reservoir solution was 1.4 *M* sodium citrate pH 6.0 and the temperature was 277 K. These crystals were smaller (0.1 × 0.1 × 0.1 mm) and took about 4–5 weeks to grow. Preliminary X-ray diffraction experiments were performed using our in-house DIP2030 (MacScience) image-plate with an Enraf–

Table 1
Intensity data-collection statistics.

Values in parentheses are for the highest resolution shell.

	FtsY crystals grown at 291 K	FtsY crystals grown at 277 K
Space group	$P2_12_12$	$R32$
Resolution range (Å)	20–1.9 (1.97–1.9)	20–2.8 (2.9–2.8)
Number of observations	497368	419714
Number of unique reflections	22061	41523
Completeness (%)	95.7 (79.5)	91.2 (93.6)
$I/\sigma(I)$	17.45 (4.78)	16.92 (2.81)
R_{sym}† (%)	6.9 (32.1)	7.4 (58.4)
$I/\sigma(I) > 2$	83.2 (51.5)	73.0 (45.0)

† $R_{sym} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

Nonius FR-591 rotating-anode X-ray generator operating at 40 kV and 90 mA. Both crystal types were retrieved with a 0.15 mm nylon loop (Sauer & Ceska, 1997) and were flash-cooled in liquid nitrogen with an Oxford Cryosystems Cryostream operated at 100 K. The crystals grown at 291 K diffracted to about 2.7 Å in-house and belong to space group $P2_12_12$, with unit-cell parameters $a = 68.73$, $b = 101.13$, $c = 42.53$ Å, $\alpha = \beta = \gamma = 90°$. Calculation of the Matthews coefficient (Matthews, 1968) suggests that there is one molecule per asymmetric unit, with a solvent content of 35%. A native data set at 1.9 Å was collected at the synchrotron facility in DESY, Hamburg, on beamline X-11 (HASY Laboratory) at a wavelength of 0.91 Å. Additional experimental conditions were: crystal-to-detector distance, 170 mm; oscillation angle, 1°; exposure time, 3 min; total amount of data, 244 images. The data were processed using the *HKL* crystallographic data-reduction package *DENZO* (Otwinowski & Minor, 1997) and merged using the programs *TRUNCATE* and *SCALA* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The 277 K crystals displayed a much weaker diffraction, to 4 Å in-house. However, a data set to 2.8 Å resolution was collected at the ESRF synchrotron facility at beamline BM-14 at a wavelength of 1.00 Å. These crystals belong to the rhombohedral space group, with unit-cell parameters $a = b = 151.00$, $c = 223.34$ Å, $\alpha = 90$, $\gamma = 120°$. A summary of the data-collection statistics is given in Table 1. Structure determination using molecular-replacement methods is in progress.

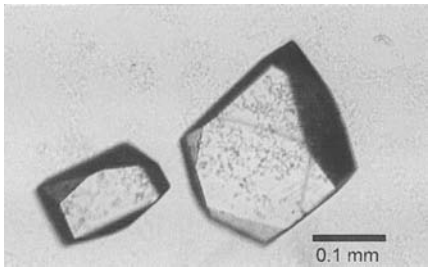

Figure 2
Crystals in space group $P2_12_12$ of the NG-domain of the FtsY protein (construct A4) from *M. mycoides*, with maximum dimensions of 0.2 × 0.2 × 0.3 mm.

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