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Crystallization and preliminary X-ray diffraction studies on the conserved GTPase domain of the signal recognition particle from *Acidianus ambivalens*

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The signal recognition particle (SRP) of bacteria consists of only one protein, known as Ffh or the SRP54 homologue, which forms a complex with 4.5S RNA. It also binds to signal peptides and contains a GTPase which displays interesting differences to Ras GTPases. The conserved NG-domain of Ffh from the archaebacterium *Acidianus ambivalens* was cloned and overexpressed with a C-terminal His tag in *Escherichia coli*. Crystallization experiments of the native protein as well as of the Thr112Ala mutant, which is deficient in GTP hydrolysis, resulted in crystals suitable for X-ray diffraction. The crystals belong to the orthorhombic space group $C222_1$, with unit-cell parameters $a = 64.5$, $b = 128.3$, $c = 72.0$ Å. At cryogenic temperatures, the crystals diffracted to a resolution limit of 2.8 Å using a rotating-anode generator and contain one molecule per asymmetric unit. A native data set has been collected using synchrotron radiation to around 2.0 Å resolution. Selenomethionine protein was produced; its crystals diffract in-house to about 2.8 Å resolution.

1. Introduction

The transport and translocation of proteins to and across membranes is mediated by the specific recognition of a hydrophobic N-terminal signal sequence by a specialized targeting machinery. The high conservation of this system between eukaryotes and prokaryotes suggests that the same basic mechanism of membrane targeting and translocation is used in both kingdoms (Luirink & Dobberstein, 1994). In mammalian cells, targeting of most proteins to the endoplasmic reticulum (ER) is mediated by the signal recognition particle (SRP), which consists of one RNA molecule (SRP7S RNA) and six polypeptides of 9, 14, 19, 54, 68 and 72 kDa (Walter & Blobel, 1980; for a review, see Lütcke, 1995). The SRP binds to the signal sequence of the nascent polypeptide chain *via* its 54 kDa subunit (SRP54; Siegel & Walter, 1988). The complex composed of the ribosome, the nascent polypeptide chain and the SRP is targeted to the ER by interaction with a docking-protein complex at the membrane. The SRP is released from the complex after GTP hydrolysis and translation proceeds (Connolly & Gilmore, 1989). The ribosome–nascent chain complex remains bound to the membrane, interacting with the translocon – a membrane–protein complex which catalyzes membrane insertion and translocation of the nascent chain. In bacteria, the SRP consists only of one protein, the SRP54 homologue (Ffh) and 4.5S RNA. The SRP may support co-translational translocation in a secretory pathway similar to its eukaryotic counterpart. The SRP54 homologue was recently also described for archaea (Moll *et al.*, 1999), although the precise composition of the SRP in archaea remains to be determined. In all systems, however, Ffh consists of three domains: an N-terminal domain (the N-domain), a G-domain, which contains the consensus elements for GTP

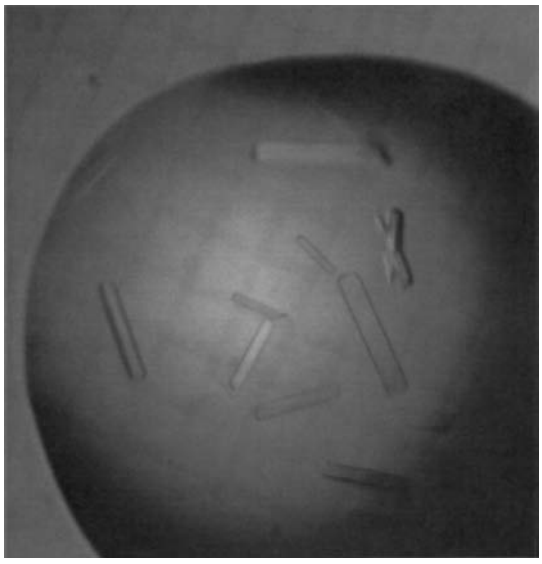

Figure 1
Orthorhombic crystals of Ffh-NG from *A. ambivalens*. Approximate dimensions are 0.2 × 0.2 × 0.5 mm (see text for details).

Table 1
Data-collection statistics.

(*a*) Native Ffh-NG in-house.

Resolution limits (Å)	Intensity	σ	R_{sym}†	Completeness (%)
30.0–7.56	9033.1	197.3	0.029	99.3
7.56–6.02	3247.1	88.9	0.047	100
6.02–5.26	3562.5	100.8	0.049	100
5.26–4.78	5073.1	128.1	0.045	100
4.78–4.44	7076.4	164.7	0.042	100
4.44–4.18	6132.3	154.8	0.048	100
4.18–3.97	5916.1	155.1	0.048	100
3.97–3.80	4540.7	138.9	0.057	100
3.80–3.65	4695.9	148.1	0.058	100
3.65–3.53	3936.8	141.3	0.069	100
3.53–3.42	3310.4	134.2	0.080	100
3.42–3.32	3186.5	135.0	0.078	100
3.32–3.23	2412.6	124.0	0.095	100
3.23–3.15	1897.0	123.1	0.119	100
3.15–3.08	1790.2	122.0	0.127	100
3.08–3.02	1700.1	120.2	0.135	100
3.02–2.96	1518.3	120.5	0.149	100
2.96–2.90	1299.6	121.9	0.170	100
2.90–2.85	1270.4	121.8	0.181	100
2.85–2.80	1223.1	121.5	0.192	100
Average values	3695.2	133.5	0.066	100

(*b*) Native Ffh-NG at beamline BM-14 (ESRF).

Resolution limits (Å)	Intensity	σ	R_{sym}†	Completeness (%)
35.0–4.93	12075.1	249.7	0.061	99.1
4.93–3.91	13215.4	250.9	0.062	100
3.91–3.42	8733.4	168.5	0.070	100
3.42–3.11	5138.4	108.3	0.080	100
3.11–2.88	3081.3	73.6	0.097	100
2.88–2.71	2085.2	57.0	0.118	100
2.71–2.58	1511.8	46.7	0.142	100
2.58–2.47	1292.3	44.4	0.164	100
2.47–2.37	1015.8	43.0	0.161	100
2.37–2.29	959.8	44.9	0.154	100
2.29–2.22	802.5	43.7	0.175	100
2.22–2.15	673.6	43.5	0.209	100
2.15–2.10	549.0	43.5	0.240	100
2.10–2.05	466.1	43.2	0.265	100
2.05–2.00	387.5	44.0	0.304	100
Average values	3545.2	88.4	0.084	99.9

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

binding, and a C-terminal domain rich in methionine residues (the M-domain), which interacts with the RNA and the signal peptide. The NG-domain can be obtained as a stable fragment by proteolysis. SRP GTPases form a distinct subfamily of GTPases which display a number of interesting differences to other small GTPases, *e.g.* Ras. In particular, an insertion in the effector-binding region (the I-box) has been identified (Montoya *et al.*, 1997*a*; Freymann *et al.*, 1997). The off-rates of the nucleotides are about five orders of magnitude higher than in Ras-type GTPases (Moser *et al.*, 1997), which suggests a different mechanism for regulation of GTP hydrolysis. Based on nucleotide-binding kinetics and structural data of the NG-domain from the *Escherichia coli* receptor FtsY, the I-box was proposed to act as a built-in nucleotide-exchange factor (Moser *et al.*, 1997).

Here, we describe the first crystals of an SRP component from archaebacteria. We report the expression, purification, crystallization and preliminary X-ray studies of an engineered fragment of Ffh (Ffh-NG) from *Acidianus ambivalens*, the selenomethionine-substituted Ffh-NG and the Thr112Ala mutant, which is inactive in GTP hydrolysis.

2. Materials and methods

2.1. Expression and purification

Methods used to express and purify recombinant Ffh have been described (Moll *et al.*, 1999). Some modifications have been implemented in order to improve the expression and purification of the protein; for instance, the use of a gel-filtration column after the ion-exchange chromatography step. Ffh-NG comprising amino-acid residues 1–293 plus six histidines at the C-terminus was expressed in *E. coli* strain BL21(DE3)pLysS (Novagen) using a pET16b plasmid. The procedures followed for purification of both proteins, wild type and the Thr112Ala mutant, were identical.

Cells were transformed by electroporation and colonies were selected by double resistance against ampicillin (30 µg ml^{-1}) and chloramphenicol (34 µg ml^{-1}). Cells were grown in LB medium plus ampicillin and chloramphenicol. When the culture reached an OD of 0.4 at 600 nm, the cells were induced with 0.4 m*M* IPTG for 2 h. Cells were collected by centrifugation and stored at 193 K until use. The cells were disrupted using a French press in buffer *A* (50 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl and 10 m*M* $MgCl_2$). After two passes through the French press, the homogenized cells were centrifuged for 30 min at 20000*g*. The supernatant was collected and loaded onto a nickel affinity column (Chelating Sepharose Fast Flow, Pharmacia) equilibrated with buffer *A*. The column was initially washed with a gradient of 0–100 m*M* imidazole. A second gradient of 100–800 m*M* imidazole was then applied. The protein eluted as a clear single peak around 300 m*M* imidazole. The peak fractions were pooled and concentrated to 8 mg ml^{-1}. After this step, the sample was subjected to gel-filtration chromatography using a Superose 12 column (Pharmacia) and a mobile phase composed of 10 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl and 10 m*M* $MgCl_2$. The protein eluted as a single peak, followed by the excess of imidazole.

Selenomethionine protein was produced using the *E. coli* methionine auxotroph strain B834(DE3). Cells were transformed by electroporation and colonies were selected using carbenicillin and chloramphenicol (for a detailed description, see Montoya *et al.*, 1997*b*). The purification and crystallization procedure for the selenomethionine protein was the same as that used for the native protein. The purities of the Thr112Ala mutant, native Ffh-NG and the selenomethionine protein were checked by SDS–PAGE and mass spectrometry. The purity was higher than 98% in each case. Mass spectrometry showed 100% incorporation of selenomethionine.

2.2. Crystallization

Ffh-NG was concentrated to 8 mg ml^{-1} and crystallization trials were performed using the vapour-diffusion method in hanging-drop mode at 293 and 277 K. Orthorhombic crystals were obtained at 277 K (see Fig. 1). The crystals grew in 50 m*M* acetate buffer pH 5.00, 100 m*M* calcium acetate and 18% PEG 8000 to maximum dimensions of about 0.3 × 0.4 × 0.3 mm within one to two weeks. These crystals were suitable for X-ray diffraction studies (Fig. 1). The Thr112Ala mutant crystallized under the same conditions.

2.3. Data collection and reduction

Initial inspection of the crystals was performed at room temperature on a 180 mm MAR Research image plate using Cu $K\alpha$ radiation produced by an Enraf–Nonius generator operating at 40 kV and 70 mA. The crystals diffracted to around 3 Å and suffered from radiation damage. Cryocooling effectively eliminated radiation damage and also increased the in-house resolution to about 2.8 Å. The crystals were soaked for 1 min in a cryoprotectant solution composed of the mother liquor containing 20% MPD and were then directly flash-frozen at the diffractometer, which was equipped with an Oxford Cryosystems Cryostream set to 100 K. Native data were collected using synchrotron radiation at the European Synchrotron Radiation Facility (ESRF) in Grenoble, beamline BM-14. Synchrotron radiation increased the average resolution of cryocooled crystals from 2.8 to 2.0 Å (statistics are given in Table 1). Data were collected with a 300 mm MAR Research MAR345 image plate with a crystal-to-detector distance of 250 mm, $\Delta\varphi = 0.75^\circ$, $\lambda = 0.932$ Å and 120 s exposures. Data were collected through a 130° rotation in φ. Crystals were mounted in loops and flash-frozen as above. Selenomethionine protein crystals were studied in-house and

Table 2
Comparison of Ffh-NG data.

Ffh-NG	d_{min}† (Å)	Total observations	Unique reflections	Completeness (%)	R_{sym}† (%)
Native in-house	2.8	111572	7679	100	6.6
T112A mutant BM-14 (ESRF)	1.95	450721	21100	99.6	6.9
Native BM14 (ESRF)	2.0	451808	20842	100	8.1
Se-Met protein in-house	2.8	100599	6867	98.3	8.0

† Minimum Bragg spacing with $I/\sigma = 2.5$. † $R_{sym}(I) = \sum_{hkl}\sum_i |I_i(hkl) - \overline{I(hkl)}|/\sum_{hkl}\sum_i I_i(hkl)$.

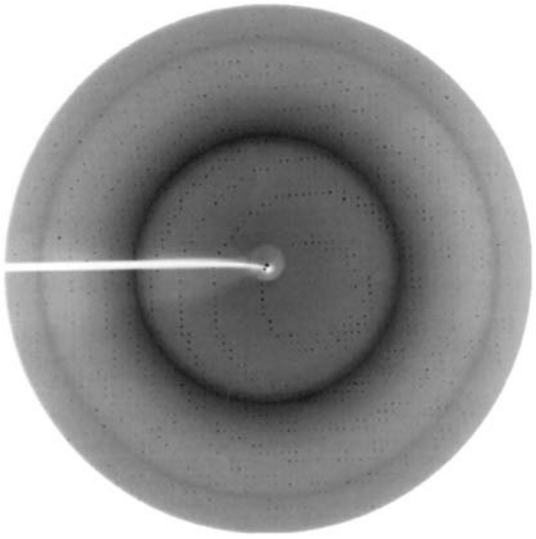

Figure 2
Diffraction pattern from the native crystals using synchrotron radiation on beamline BM-14 at the ESRF (see text for details). The resolution at the edge of the plate is 1.98 Å.

show similar radiation damage to the native crystals. However, selenomethionine crystals were smaller in size and diffracted to somewhat lower resolution than the native crystals. Crystals of the Thr112Ala mutant were isomorphous with the wild-type crystals. Data were integrated with either *DENZO* (Otwinowski & Minor, 1997) or *MOSFLM* and were reduced using *SCALEPACK* or *SCALA* (*CCP*4 suite; Collaborative Computational Project, Number 4, 1994), depending on the integration package.

3. Results and discussion

The conserved core domain of Ffh (Ffh-NG) from *A. ambivalens* can be expressed routinely with yields of around 10 mg per litre of *E. coli* culture. The protein has been purified using a His_6 tag at the C-terminus. Crystals have been obtained which belong to the orthorhombic space group $C222_1$, with unit-cell parameters $a = 64.5$, $b = 128.3$, $c = 72.0$ Å. A Matthews coefficient of 2.35 $Å^3\ Da^{-1}$ suggests one protein molecule per asymmetric unit and a solvent content of 37%.

The crystals diffract beyond 3 Å at room temperature, but radiation damage decreased the diffracton limit to 4 Å after 7–9 h. Cryocooling effectively eliminated radiation damage and also increased the resolution to about 2.8 Å. A native data set at 100 K was collected on beamline BM-14 at the European Synchrotron Radiation Facility (ESRF) in Grenoble. Synchrotron radiation increased the average resolution to 1.95 Å. Statistics of the different native data sets are given in Table 2.

Heavy-metal soaks are in progress. Since Ffh-NG contains six methionine residues, it seemed feasible to use the MAD approach for solving the structure. Crystals of the Se-Met-containing Ffh-NG have been obtained following the same protocol as for the native protein. The Se-Met crystals are isomorphous with the native crystals and diffract to about 2.8 Å on a rotating-anode generator (Table 2).

So far, only one structure of an Ffh NG-domain from the thermophilic bacterium *Thermus aquaticus* and one of the NG-domain of the SRP receptor protein FtsY from *E. coli* have been determined. The structure of the Thr112Ala mutant should provide details about the role of Thr112 in GTP hydrolysis in *A. ambivalens* Ffh. Thr112 is part of the P-loop, which interacts with the α- and β-phosphates of GTP and is homologous to Ser17 in Ras, which stabilizes the nucleotide-binding site by forming a hydrogen bond with the aspartate of the G3 region (Pai *et al.*, 1990). The structure of Ffh-NG from *A. ambivalens* will be the first structure of an SRP component from an archaebacterium to be determined and should provide detailed information on the similarities and differences between the conserved NG-domains of SRP GTPases as well as insights into special features of thermostable proteins.

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