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Crystallization and preliminary X-ray analysis of *Thermotoga maritima* ribosome recycling factor

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Thermotoga maritima ribosome recycling factor (RRF) is one of the proteins catalyzing the fourth step in prokaryotic protein synthesis, ribosome recycling. The RRF protein was crystallized with ammonium sulfate. Native diffraction data to 2.55 Å resolution were obtained at the MAX II synchrotron from a flash-frozen crystal at 100 K. The crystals belong to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 47$, $c = 298$ Å, and probably contain one monomer per asymmetric unit.

1. Introduction

Protein synthesis is a fundamental biological process and biochemistry textbooks usually describe three steps of translation: initiation, elongation and termination (for example, Stryer, 1995). What happens after the termination step, the recycling of the components of the termination complex, is less well known but equally essential (for reviews, see Janosi, Hara *et al.*, 1996; Janosi, Ricker *et al.*, 1996; Kaji *et al.*, 1998). During the termination step, the translating 70S ribosome reaches a termination codon on the mRNA. The nascent polypeptide is removed from the peptidyl tRNA in the P-site by the action of RF-1 or RF-2 and RF-3 under GTP hydrolysis (Freistroffer *et al.*, 1997; Grentzmann *et al.*, 1998). The resulting post-termination complex is the substrate of RRF. At this stage, the ribosome presumably has an empty A site and a deacylated tRNA in the P site (Pavlov *et al.*, 1997). RRF together with EF-G (Hirashima & Kaji, 1973) or RF3 (Grentzmann *et al.*, 1998) catalyzes disassembly of this complex into 70S ribosomes, mRNA and tRNA, as measured by sedimentation analysis after the RRF reaction on naturally occurring mRNA (Hirashima & Kaji, 1972). The 70S ribosomes can subsequentially be dissociated into subunits by the action of IF-3 (Subramanian & Davis, 1970). On the other hand, kinetic analysis of a coupled *in vitro* oligopeptide synthesis system programmed by a relatively short synthetic mRNA containing a Shine–Dalgarno sequence shows that only the 50S subunits are released by the action of RRF and EF-G, and that IF-3 subsequentially removes the deacylated tRNA (Karimi *et al.*, 1999). In any case, after the disassembly reaction the ribosome is ready to enter a new round of protein synthesis.

In the absence of RRF, the ribosome not only stays on the mRNA but also initiates unscheduled translation downstream from the termination codon. This reinitiation takes place randomly in all frames downstream from the termination codon *in vivo* (Janosi *et al.*, 1998).

Ribosome-recycling factor is an essential protein in *Escherichia coli* (Janosi *et al.*, 1994). It is present in all sequenced eubacterial genomes, but not in those of archaeons (Bult *et al.*, 1996). Sequence homologues are also found in yeast mitochondria (Kanai *et al.*, 1998; Ouzounis *et al.*, 1995), plant chloroplast (Rolland *et al.*, 1999) and in human mitochondria (Zhang & Spremulli, 1998). Some bacterial RRFs, such as *Pseudomonas aeruginosa* RRF, function in *E. coli* (Ohnishi *et al.*, 1999), while others, such as *T. maritima* RRF, function as a specific inhibitor of the *E. coli* RRF activity (Atarashi & Kaji, 1999). *P. aeruginosa* RRF has 64.5% sequence identity (Ohnishi *et al.*, 1999) to *E. coli* RRF, while *T. maritima* RRF has 40% sequence identity to *E. coli* RRF.

The molecular mechanism of action of RRF remains to be elucidated. As in many other systems, the understanding of this mechanism will be greatly aided by structural information. For this reason, we have undertaken the task of determining the crystal structure of RRF. Here, we report the successful crystallization and preliminary X-ray analysis of *T. maritima* RRF.

2. Materials and methods

T. maritima RRF was expressed and purified as described (Atarashi & Kaji, 1999). Crystals of *T. maritima* RRF grew at 298 K in hanging-drop vapour-diffusion experiments. 3 µl of protein solution (5 mg ml^{-1} in 10 m*M* Tris–HCl pH 7.6, 50 m*M* NH_4Cl, 0.5 m*M* dithiothreitol) was mixed with an equal volume of reservoir solution (0.1 *M* sodium acetate pH 5.5, 2.0 *M* ammonium sulfate) and equilibrated against 600 µl of reservoir solution. Crystals

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell, 2.65–2.55 Å.

Resolution (Å)	30–2.55
Total observations	79947 (7119)
Unique reflections	11927 (1261)
Average redundancy	6.7 (5.6)
R_{sym}† (%)	4.9 (15.6)
Data completeness (%)	99.8 (99.1)
$I/\sigma(I)$	26.4 (8.9)

† $R_{sym} = \sum |I - \langle I \rangle| / \sum I$, where I is the observed intensity and $\langle I \rangle$ is the average intensity of symmetry-related reflections.

grew in 3 d as bipyramids of dimensions 0.3 × 0.3 × 0.5 mm (Fig. 1).

To allow rapid and reproducible freezing of the crystals, the crystallization conditions were modified to include glycerol. For cryo data collection, crystals were grown using the sitting-drop vapour-diffusion technique, in which 5–10 µl protein solution (as above) was mixed with an equal volume of reservoir solution (0.1 *M* sodium acetate pH 5.5, 2.0 *M* ammonium sulfate, 10% glycerol) and equilibrated against 600 µl of reservoir solution. After equilibration for 24 h, streak seeding was performed by gently touching a suitable crystal with a cat whisker and immediately streaking the whisker through the pre-equilibrated drop (Stura, 1999). Crystals appeared overnight.

Native data were collected at beamline BL711 (λ = 0.947 Å) at the MAX II synchrotron using a MAR345 image-plate detector. The crystal was mounted in a nylon loop (Hampton Research) and flash-frozen at 100 K in a stream of boiled-off nitrogen (Oxford Cryosystems Cryostream). The *c* axis of the crystal was approximately aligned to the spindle axis to allow a rotation of 1° per image. This was readily achievable since the crystals were normally longer in the direction of the *c* axis and thus oriented themselves in this direction in the loop. If not, the crystals could be returned to the mother liquor and remounted without loss of diffraction power as judged by visual inspection. The programs *XDS* and *XSCALE* (November 1998 version; Kabsch, 1993) were used for indexing, integration, scaling and merging of data.

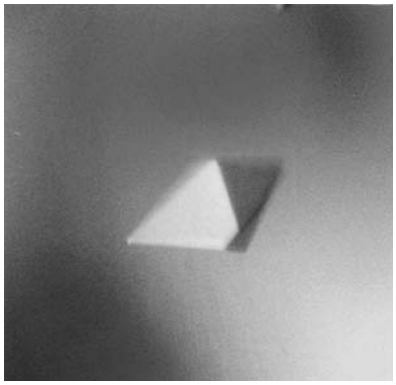

Figure 1
Native crystal of *T. maritima* ribosome recycling factor. The bipyramidal crystal has approximate dimensions 0.3 × 0.3 × 0.5 mm.

3. Results and discussion

The crystals belong to space group $P4_12_12$ or $P4_32_12$. The unit-cell dimensions are normally $a = b = 47$, $c = 298$ Å, but the length of the *c* axis varies between 294 and 302 Å between individual crystals. Assuming one RRF molecule of 21.5 kDa per asymmetric unit, the solvent content for this crystal form is 68% (Andersson & Hovmöller, 1998). Because of the non-isomorphism between different crystals and limited resolution of the crystals, this seems more reasonable than having two molecules per asymmetric unit.

T. maritima RRF crystals diffract to about 2.3 Å under cryo conditions. A single crystal was used to collect a complete native data set to 2.55 Å resolution on a 345 mm MAR image plate (Table 1).

Molecular-replacement phasing was not an option, as *T. maritima* RRF only showed significant sequence homology (above 25% identity) to RRF from other species in a SWISS-PROT (Bairoch & Apweiler, 1999) database search using the program *FASTA* (Pearson, 1990). The sequence does not contain any recognizable sequence motifs. The secondary-structure prediction program *PREDATOR* (Frishman & Argos, 1997) predicts that RRF contains mainly α-helix in the regions of residues 1–30 and 105–185, and both α-helix and β-strand elements in the intermediate region.

A search for suitable heavy-atom derivatives for MIR phasing is in progress but seems problematic because of non-isomorphism between the individual crystals. Therefore, as *T. maritima* RRF contains four methionines, the system seems suitable for selenomethionine MAD phasing and selenomethionine-substituted RRF is currently in preparation.

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