Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction analysis of *Thermus thermophilus* prolyl-tRNA synthetase

Anna Yaremchuk,[a,b]* Stephen Cusack[a] and Mikhail Tukalo[a,b]

[a]EMBL Grenoble Outstation, 156X, 38042 Grenoble CEDEX 9, France, and [b]Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 252627 Kiev 143, Ukraine

Correspondence e-mail: yarem@embl-grenoble.fr

© 2000 International Union of Crystallography
Printed in Denmark – all rights reserved

Received 16 September 1999
Accepted 29 November 1999

Prolyl-tRNA synthetase from *Thermus thermophilus* (ProRSTT) was purified to homogeneity using a five-step purification procedure and was crystallized using ethylene glycol as a precipitant. Crystals of ProRSTT belong to the space group $P2_12_12$, with unit-cell parameters $a = 132$, $b = 191$, $c = 125$ Å, have two homodimers per asymmetric unit and diffract to 2.4 Å resolution. A complete native data set to 2.43 Å resolution has been collected and a data set from ProRSTT in complex with proline has been collected to 2.9 Å resolution.

1. Introduction

Aminoacyl-tRNA synthetases constitute a family of enzymes that carry out the specific esterification of an amino acid to the 3′ end of its cognate tRNA. This is achieved through a two-step reaction: the enzyme first activates the amino acid using ATP to form the aminoacyl-adenylate intermediate and then the aminoacyl moiety is transferred to either the 2′ or 3′ position of the 3′-terminal ribose of the cognate tRNA. These enzymes are divided into two quite distinct classes on the basis of primary structure and the fold of the catalytic domain as found by structure determination (Eriani *et al.*, 1990; Cusack *et al.*, 1990). Crystal structures of the majority of aminoacyl-tRNA synthetases have now been determined together with a variety of substrate complexes, which have yielded much detailed information on substrate specificity, enzyme mechanism and the evolution of the two classes (Cusack, 1995; Arnez & Moras, 1997; Martinis *et al.*, 1999).

Prolyl-tRNA synthetase (ProRS) is a class II aminoacyl-tRNA synthetase having the closest structural similarities to threonyl-, histidyl- and glycyl-tRNA synthetases which are all members of sub-class IIa (Cusack, 1995). The sequence of *T. thermophilus* ProRS (ProRSTT) has been determined recently (Yaremchuk *et al.*, unpublished results). Comparison of the primary structure with other known ProRSs shows that it is significantly more similar to eukaryote cytoplasmic ProRSs than to other eubacterial prolyl-tRNA synthetases. Such an analysis reveals that there are two distinct structural forms of ProRS: (i) 'eukaryote/archae-like', but including ProRSTT, characterized by the absence of an insertion domain between motif 2 and motif 3 and by the presence of an extra C-terminal domain beyond the normal class IIa anticodon-binding domain ending in an absolutely conserved tyrosine, and (ii) 'prokaryote-like' including ProRS from *Escherichia coli, Chlamydia trachomatis* and mitochondria of eukaryotes, which have a very large insertion between motifs 2 and 3 and no extra C-terminal domain (Cusack *et al.*, 1998). Here, we report the purification and crystallization of ProRSTT and preliminary X-ray studies of the crystals. Crystallization of the ProRSTT–$tRNA^{Pro}$ complex is reported in the accompanying paper (Yaremchuk *et al.*, 2000).

2. Methods and results

ProRSTT was purified to homogeneity using a five-step procedure. The *T. thermophilus* (strain HB8) cells were harvested by centrifugation, washed and disrupted using a French press in 100 m*M* Tris–HCl buffer containing 2 m*M* dithiothreitol, 0.1 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride, Complete (a protease inhibitor cocktail, 1 tablet per 25 ml extraction buffer), 5 m*M* $MgCl_2$, 1 m*M* NaN_3. The extract was centrifuged for 2 h at 105 000*g* and the supernatant was subjected to 30–65% ammonium sulfate fractionation. The precipitate containing ProRSTT activity was dissolved in 20 m*M* Tris–HCl buffer pH 7.5 containing 5 m*M* $MgCl_2$, 0.1 m*M* phenylmethylsulfonyl fluoride, 2 m*M* DTT, 1 m*M* NaN_3 (buffer *A*), dialyzed against buffer *A* and absorbed on a DEAE–Sepharose column (5 × 55 cm) equilibrated with buffer *A*. ProRSTT was eluted with a 2 × 2.5 l linear gradient of 0.03–0.3 *M* sodium chloride in buffer *A*. The fractions containing ProRSTT activity were salted out by ammonium sulfate (50% saturation) and applied to a Toyopearl HW-65 column (3 × 80 cm) equilibrated with 40% ammonium sulfate in buffer *A*. The enzyme was eluted with a 2 × 2 l gradient of 40–10% saturated ammonium sulfate in buffer *A*. Fractions containing ProRSTT activity were pooled,

dialyzed and applied to a hydroxylapatite column (3 × 40 cm) with a 2 × 2 l potassium phosphate buffer linear gradient from 0.01–0.25 *M* (pH 7.9). After dialysis of the active fractions in buffer *A*, the enzyme was loaded onto a heparin Sepharose CL-6B column (1 × 40 cm). A 1.0 l linear 0–0.25 *M* KCl gradient in buffer *A* was used to elute the ProRSTT. All enzyme purification steps were carried out at 277 K. The final yield was approximately 15 mg of pure enzyme from 520 g of cells, with a specific activity of 869 U mg^{-1} (1 U of enzyme catalyzes the formation of 1 nmol prolyl-$tRNA^{Pro}$ per minute at 328 K). The enzyme is an α_2 homodimer with an apparent molecular mass of 108 kDa.

A rapid initial screening of crystallization conditions was conducted using a sparse matrix, without any success. Conventional precipitants alone, such as ammonium sulfate, PEG, sodium formate, sodium citrate, sodium chloride and MPD, at different concentrations over a broad range of pH and protein concentration did not yield any crystals. Many different additives were tried in combination with these precipitants. After a long search, only a mixture of ammonium sulfate and ethylene glycol was successful. A single crystal of ProRSTT was first obtained at 285 K by equilibrating 10 µl drops containing 6 mg ml^{-1} protein in 20 m*M* Tris–HCl pH 7.9, 2 m*M* DTT, 5 m*M* $MgCl_2$, 1 m*M* NaN_3, 0.5% ammonium sulfate, 10% ethylene glycol against 800 µl reservoir solution containing 28% ethylene glycol, 100 m*M* Tris–HCl pH 7.9, 1 m*M* NaN_3. Microcrystals of ProRSTT have also been obtained at 293 K under the same crystallization conditions. These microcrystals were enlarged by macroseeding. Before the seed crystals were added, drops of protein solution (3–4 mg ml^{-1}) were equilibrated against 25% ethylene glycol for 5–6 d. After seed crystals were added, the concentration of ethylene glycol in the reservoir was increased to 32%. Under these conditions, single crystals grew within approximately 2–3 weeks, with the largest crystals having dimensions 0.4 × 0.2 × 0.15 mm. Protein from solubilized crystals as well as the protein used for crystallization was verified to be full-length ProRSTT by SDS–PAGE. Under the crystallization conditions at room temperature the enzyme retains its enzymatic activity to aminoacylate tRNA.

The crystals of ProRSTT belong to space group $P2_12_12$, with unit-cell dimensions a = 132, b = 191, c = 125 Å at 100 K. A complete native data set to 2.43 Å resolution has been collected from a flash-frozen crystal on beamline BM14 at the ESRF, Grenoble (98.6% complete, R_{merge} = 0.053). Artificial mother liquor in 35% ethylene glycol was used as cryoprotectant. The crystal structure of ProRSTT has now been determined by SAD (single-wavelength anomalous diffraction) using only the optimized anomalous scattering from a mercury aniline derivative (S. Cusack, A. Yaremchuk & M. Tukalo, submitted). The results show that there are two dimers per asymmetric unit (V_m = 3.6 $Å^3$ Da, 65% solvent); fourfold non-crystallographic symmetry averaging was crucial in improving the initial map. A crystal of ProRSTT was soaked with L-proline and a complete data set has been collected to 2.9 Å resolution on the Swiss–Norwegian beamline at the ESRF (99% complete, R_{merge} = 0.068). Comparison of the ligand-free and proline-bound structures shows significant conformational changes in the enzyme associated with proline binding, which will be described elsewhere (S. Cusack, A. Yaremchuk, M. Grøtli & M. Tukalo, submitted). Crystallization of the complex between ProRSTT and cognate $tRNA^{Pro}$ is described in the accompanying article (Yaremchuk *et al.*, 2000); the corresponding structure is described elsewhere (Cusack *et al.*, 1998).

We thank A. Thompson, S. Monaco and S. McSweeney for their help with the measurements on BM14. The research of MT was supported in part by an International Research Scholar's award from the Howard Hughes Medical Institute.

References

Arnez, J. G. & Moras, D. (1997). *Trends Biochem. Sci.* **22**, 211–216.
Cusack, S. (1995). *Nature Struct. Biol.* **2**, 824–831.
Cusack, S., Berthet-Colominas, C., Härtlein, M., Nassar, N. & Leberman, R. (1990). *Nature (London)*, **347**, 249–255.
Cusack, S., Yaremchuk, A., Kriklivyi, I. & Tukalo, M. (1998). *Structure*, **6**, 101–108.
Eriani, G., Delarue, M., Poch, O., Gangloff, J. & Moras, D. (1990). *Nature (London)*, **347**, 203–206.
Martinis, S. A., Plateau, P., Caverelli, J. & Florentz, C. (1999). *Biochemie*, **81**, 683–700.
Yaremchuk, A., Kriklivyi, I., Cusack, S. & Tukalo, M. (2000). *Acta Cryst.* D**56**, 197–199.