Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of *Escherichia coli* methionyl-tRNA$_f^{Met}$ formyltransferase complexed with formyl-methionyl-tRNA$_f^{Met}$

Emmanuelle Schmitt,* Sylvain Blanquet and Yves Mechulam

Laboratoire de Biochimie, Unité Mixte de Recherche No. 7654 du Centre National de la Recherche Scientifique, Ecole Polytechnique, F-91128 Palaiseau CEDEX, France

Correspondence e-mail: emma@botrytis.polytechnique.fr

Received 10 July 1998
Accepted 15 September 1998

The structure of methionyl-tRNA$_f^{Met}$ formyltransferase from *E. coli*, a monomeric protein of 34 kDa, has previously been determined at 2.0 Å resolution. In the present work, this enzyme was crystallized as a complex with its macromolecular product, the initiator formyl-methionyl-tRNA$_f^{Met}$ (25 kDa). Polyethylene glycol 5000 monomethyl-ether was used as a precipitating agent. The crystals are orthorhombic and have unit-cell parameters a = 201.7, b = 68.1, c = 86.4 Å. They belong to space group $P2_12_12$ and diffract to 2.8 Å resolution. The structure is being solved with the help of a mercury derivative.

1. Introduction

All living organisms use a specialized tRNA^{Met} species for the initiation of protein translation. In eubacteria, the amino group of the methionine carried by this particular tRNA (tRNA$_f^{Met}$) undergoes additional formylation. This modification, which is crucial for prokaryotic cell growth (Guillon, Mechulam *et al.*, 1992), directs the initiator tRNA$_f^{Met}$ towards the translation-initiation apparatus and prevents it from entering the elongation pathway (Guillon *et al.*, 1993, 1996; Varshney *et al.*, 1993). In *Escherichia coli*, methionyl-tRNA$_f^{Met}$ formyltransferase (E.C. 2.1.2.9; formylase) sustains the addition of the formyl group to the free amino group of the methionine esterified on the 3′ acceptor end of tRNA$_f^{Met}$ (Blanquet *et al.*, 1984; Kahn *et al.*, 1980). The formyl donor is 10-formyl-tetrahydrofolate (FTHF). The elongator Met-tRNAm^{Met} is not processed by the formylase. The identity elements of tRNA governing its recognition as a substrate by the formylase are located inside the acceptor-stem sequence (Guillon, Meinnel *et al.*, 1992; Lee *et al.*, 1991). The lack of strong base-pairing at position 1–72 in initiator tRNA^{Met} is the major determinant allowing its formylation. The aminoacyl moiety attached to tRNA can be varied without a large loss of enzyme activity (Giegé *et al.*, 1973). However, a methionine increases the efficiency of the formylation reaction (Varshney & Rajbhandary, 1992). The mechanism used by formylase to select its substrate appears, therefore, to be mainly based on a specific feature of the secondary structure of tRNA, rather than on the occurrence of given nucleotides at given locations. This situation raises an intriguing question in relation to the general problem of specificity in RNA–protein recognition. The crystalline structure of native formylase, a monomer of 34 kDa, was recently determined at 2.0 Å resolution (Schmitt, Blanquet *et al.*, 1996). To investigate the interaction of the enzyme with its polynucleotidic ligand, we have chosen formyl-methionyl-tRNA$_f^{Met}$ (fMet-tRNA$_f^{Met}$), the enzyme product, rather than the highly unstable methionyl-tRNA$_f^{Met}$ (Met-tRNA$_f^{Met}$) substrate. The present work describes the preparation of large amounts of this product and its use to obtain crystals of the formylase–formyl-methionyl-tRNA$_f^{Met}$ complex. A crystal form suitable for high-resolution X-ray crystallographic studies is described.

2. Materials and methods

Maturated tRNA$_f^{Met}$ was produced in *E. coli* cells using the pBStRNA$_Y^{fMet}$ overproducing plasmid (Meinnel & Blanquet, 1995). tRNA$_{f2}^{Met}$ accepting 1700 ± 100 pmol of methionine per A_{260} unit was purified from these cells as described (Guillon, Meinnel *et al.*, 1992). Purified tRNA$_f^{Met}$ (20 µ*M*) was aminoacylated at 298 K in the presence of 2 m*M* ATP, 80 µ*M* L-methionine and 1 µ*M* M547 methionyl-tRNA synthetase (Mellot *et al.*, 1989) in 20 m*M* Tris–HCl (pH 7.6) containing 7 m*M* $MgCl_2$ and 150 m*M* KCl. After a 10 min incubation, formylation was triggered by the addition of 125 µ*M* FTHF and 1 µ*M* *E. coli* formylase purified as described (Schmitt, Mechulam *et al.*, 1996). After a further 10 min incubation at 298 K, the reaction was quenched by ethanol precipitation. The obtained material, corresponding to 70 A_{260} units of tRNA$_f^{Met}$, was redissolved in 10 m*M* ammonium acetate (pH 6.3) plus 1.7 *M* ammonium sulfate and purified

by hydrophobic interaction chromatography on a TSK phenyl 5-PW column (8 mm × 7.5 cm, Bio-Rad) equilibrated in the same buffer. A reverse ammonium sulfate gradient was applied (1.7–0 *M*, 0.7 ml min^{-1}, 0.2 *M* h^{-1}). fMet-tRNA$_f^{Met}$ eluted as a single peak at 1.5 *M* ammonium sulfate. The fractions were pooled, dialysed against 10 m*M* sodium acetate (pH 5) and loaded onto a Mono Q column (Pharmacia, 10 mm × 10 cm) equilibrated in the same buffer. The column was then washed with 30 ml of 10 m*M* sodium acetate plus 0.6 *M* NaCl, and fMet-tRNA$_f^{Met}$ was eluted by the application of a linear NaCl gradient (0.6–1 *M*, 1 ml min^{-1}, 0.2 *M* h^{-1}). The recovered nucleic acid was then precipitated with 2-propanol, redissolved in water and stored at 253 K as an ethanol precipitate. The final yield of the purification procedure was usually 50–60%. fMet-tRNA$_f^{Met}$ was dissolved in water prior to use in crystallization assays.

Methionyl-tRNA$_f^{Met}$ formyltransferase was purified as described (Schmitt, Mechulam *et al.*, 1996), except that the phosphate buffer was systematically replaced by Tris–HCl (pH 7.6). An additional hydrophobic interaction chromatographic step on a BakerBond HiPropyl column (10 mm × 10 cm, Baker) was introduced at the end of the purification. Formylase was eluted from this column with a reverse ammonium sulfate gradient (1.7–0 *M*, 0.7 ml min^{-1}, 0.6 *M* h^{-1}) in the presence of 10 m*M* ammonium acetate (pH 6.7). The pooled fractions were concentrated by precipitation with ammonium sulfate, redissolved in 10 m*M* MOPS–NaOH (pH 6.7), 10 m*M* 2-mercaptoethanol, 100 m*M* potassium chloride and dialysed against the same buffer. Protein concentration was deduced from the absorbance at 280 nm using a specific extinction coefficient of 1.39 cm^2 mg^{-1} (Kahn *et al.*, 1980).

3. Results

Crystallization trials were conducted using the hanging-drop technique at 279 K (McPherson, 1982). An initial search for crystallization conditions was undertaken using sparse-matrix sampling (Jancarik & Kim, 1991; Crystal Screen solutions, Hampton Research). For this purpose, stoichiometric mixtures of formylase (a monomer of 34 kDa) and fMet-tRNA$_f^{Met}$ (25 kDa) containing 10 m*M* magnesium chloride were used. Twinned crystals could be observed in the presence of 30% polyethylene glycol 5000 monomethylether, 0.2 *M* ammonium sulfate and 0.1 *M* morpholinoethanesulfonate buffer (pH 6.5). The quality of these crystals could be improved by performing a second screen in which the concentration of PEG, the pH and the complex concentration were varied. Further improvement was obtained by using seeding techniques. Finally optimal growth conditions were defined as 23–26% of polyethylene glycol 5000 monomethylether, 0.2 *M* ammonium sulfate and 50 m*M* morpholinoethanesulfonate buffer (pH 6.6–6.8), in the presence of 60 µ*M* fMet-tRNA$_f^{Met}$, 66 µ*M* formylase, 10 m*M* magnesium chloride and 50 m*M* potassium chloride. Under these conditions, crystals reached sizes of 1 × 0.3 × 0.15 mm within 3 weeks of growth.

Crystals were assayed for diffraction using a synchrotron source (λ = 1 Å) at the wiggler station DW32 of the Laboratoire pour l'Utilization du Rayonnement Electromagnetique, Orsay, France and an MAR Research image-plate detector. The crystals diffracted beyond 3 Å resolution, but the quality of the diffraction decreased very rapidly to reach 3.5 Å resolution after a few recorded frames. This prompted us to search for conditions allowing cryocooling of the crystals. Ethylene glycol (8%) behaved as an effective cryoprotectant when added to the stabilizing solution. To avoid the shock resulting from soaking the crystals in the cryoprotective solution, crystals were directly grown in the presence of 8% ethylene glycol. Resulting crystals could be directly flash-cooled in liquid ethane after washing with a solution identical to the crystallization solution but containing 30% of polyethylene glycol 5000 monomethylether.

The material from washed and dissolved crystals was analyzed by SDS polyacrylamide gel electrophoresis. Silver staining of the gel suggested a 1:1 stoichiometry for the enzyme and the nucleic acid. Analysis of the same crystals by urea polyacrylamide gel electrophoresis under acidic conditions (Varshney *et al.*, 1991) indicated that after one month of crystal growth, more than 80% of the tRNA molecules had retained the formyl-methionyl moiety.

Data corresponding to 90° of oscillation were collected to 2.8 Å resolution from a single crystal at 100 K using the same synchrotron source. For this purpose, a modified goniometric head with an extended arc was used (Litt *et al.*, 1998). Crystals were orthorhombic, with unit-cell parameters *a* = 201.7, *b* = 68.1, *c* = 86.4 Å. The space group was identified as $P2_12_12$. This leads to a calculated V_m value of 5.3 Å^3 Da^{-1} and a solvent content of the order of 75%, assuming of one molecule of complex per asymmetric unit (Matthews, 1968). With two molecules per asymmetric unit, V_m would be 2.6 Å^3 Da^{-1} and the solvent content near 50%. The calculations therefore favour two molecules per asymmetric unit. Images were integrated using *MOSFLM* (A. G. W. Leslie, Laboratory of Molecular Biology, Cambridge) and data reduced using the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The data set was found to be 99.4% complete to 2.8 Å resolution. $R_{sym}(I)$ was 5.1% with an average of 3.3 observations per reflection and a mean ratio of intensity to standard deviation of 10.9. $R_{sym}(I)$ in the resolution shell between 2.95 and 2.80 Å was 35% with a completeness of 99.6%.

Finally, data were also collected from a sodium parachloromercuriphenylsulfonate derivative. Initial phases were obtained by using the *Sharp* program (de la Fortelle & Bricogne, 1997) combined with solvent flattening using *Solomon* (Collaborative Computational Project, Number 4, 1994). Examination of the corresponding map clearly showed the presence of two complex molecules per asymmetric unit. Model building and phase refinement are in progress.

The authors are much indebted to François Cluzeau whose skill has allowed the modification of a goniometric head for data collection at cryogenic temperatures, and to Christian Vasseur, Didier Lenoir and Marc Godfrein (Ecole Polytechnique) for their technical help. We gratefully acknowledge Alain Litt and Andre Mitschler (UPR 9004, Illkirch France) who kindly provided the drawings of the goniometric head, as well as Marc Ruff for helpful advice. We also thank the members of the Laboratoire pour l'Utilization du Rayonnement Electromagnetique for technical assistance with station W32.

References

Blanquet, S., Dessen, P. & Kahn, D. (1984). *Methods Enzymol.* **106**, 141–152.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Fortelle, E. de la & Bricogne, G. (1997). *Methods Enzymol.* **276**, 472–494.
Giegé, R., Ebel, J. P. & Clark, B. F. C. (1973). *FEBS Lett.* **30**, 291–295.
Guillon, J. M., Heiss, S., Suturina, J., Mechulam, Y., Laalami, S., Grunberg-Manago, M. & Blanquet, S. (1996). *J. Biol. Chem.* **271**, 22321–22325.

Guillon, J. M., Mechulam, Y., Blanquet, S. & Fayat, G. (1993). *J. Bacteriol.* **175**, 4507–4514.
Guillon, J. M., Mechulam, Y., Schmitter, J. M., Blanquet, S. & Fayat, G. (1992). *J. Bacteriol.* **174**, 4294–4301.
Guillon, J. M., Meinnel, T., Mechulam, Y., Lazennec, C., Blanquet, S. & Fayat, G. (1992). *J. Mol. Biol.* **224**, 359–367.
Jancarik, J. & Kim, S. H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Kahn, D., Fromant, M., Fayat, G., Dessen, P. & Blanquet, S. (1980). *Eur. J. Biochem.* **105**, 489–497.
Lee, C. P., Seong, B. L. & RalBhandary, U. L. (1991). *J. Biol. Chem.* **266**, 18012–18017.
Litt, A., Arnez, J. G., Klaholz, B., Mitschler, A. & Moras, D. (1998). *J. Appl. Cryst.* **31**, 638–640.
McPherson, A. (1982). *The Preparation and Analysis of Protein Crystals.* New York: John Wiley and Sons.
Matthews, B. (1968). *J. Mol. Biol.* **33**, 491–497.
Meinnel, T. & Blanquet, S. (1995). *J. Biol. Chem.* **270**, 15006–15014.
Mellot, P., Mechulam, Y., LeCorre, D., Blanquet, S. & Fayat, G. (1989). *J. Mol. Biol.* **208**, 429–443.
Schmitt, E., Blanquet, S. & Mechulam, Y. (1996). *EMBO J.* **15**, 4749–4758.
Schmitt, E., Mechulam, Y., Ruff, M., Mitschler, A., Moras, D. & Blanquet, S. (1996). *Proteins*, **25**, 139–141.
Varshney, U., Lee, C. P. & RajBhandary, U. L. (1991). *J. Biol. Chem.* **266**, 24712–24718.
Varshney, U., Lee, C. P. & RajBhandary, U. L. (1993). *Proc. Natl Acad. Sci. USA*, **90**, 2305–2309.
Varshney, U. & RajBhandary, U. L. (1992). *J. Bacteriol.* **174**, 7819–7826.