CRYSTALLISATION OF TRYPSIN-MODIFIED METHIONYL-tRNA SYNTHETASE FROM *ESCHERICHIA COLI*

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1. Introduction

The elucidation of the molecular basis for the specific interaction between aminoacyl-tRNA synthetases and their corresponding tRNAs requires prior knowledge of both the primary structure and the conformation of these molecules. Considerable progress towards this goal has been achieved in the case of tRNAs: the primary structure of close to twenty of these have been determined, and X-ray diffraction analysis of crystalline tRNAs is now beginning to provide information on their internal geometry [1]. Similar progress has not been achieved in the case of aminoacyl-tRNA synthetases: there have been no reported attempts at determining the primary structure of any of these enzymes, and progress towards the elucidation of their tertiary structure has been hampered by lack of suitable, stable crystals amenable to high resolution X-ray diffraction analysis.

In this paper we report on the crystallization of a modified, fully active form of methionyl-tRNA synthetase. The best crystals obtained have properties suitable for high resolution crystallographic analysis: they provide strong reflections at 2.5 Å, are stable in the X-ray beam, contain only one molecule per assymmetric unit, and retain full enzymatic activity.

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2. Materials and methods

Native methionyl-tRNA synthetase (EC 6.1.1.10) was purified from *E. coli* K12 carrying the F32 episome, as described earlier [2]. Homogenous trypsin-modified methionyl-tRNA synthetase was obtained by incubation of the native enzyme with trypsin, followed by purification of the active modified form by chromatography on hydroxylapatite, as described in detail elsewhere [3].

The diffraction studies were carried out with precession cameras (Supper and Enraf-Nonius) having a crystal-to-film distance of 60 mm. All experiments, including crystallization and diffraction studies, were performed in the cold room.

3. Results

Trypsin-modified methionyl-tRNA synthetase could be readily isolated in crystalline form under a variety of conditions. As will be described, salting-out by concentrated ammonium sulfate gave small crystals, whereas the use of concentrated ammonium citrate yielded good crystals suitable for a detailed X-ray analysis.

The first crystals were obtained by the method of Jacoby [4] from 49% saturated ammonium sulfate buffered at pH 7.2 by 0.1 M potassium phosphate. These crystals had the shape of parallelepipedic plates,

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Table 1
Unit cell parameters of methionyl-tRNA synthetase crystals, grown in 1.5 M ammonium citrate at pH 6.8 or 7.4.

Unit cell parameters [a] Å	Unit cell volume Å 3	Space-group	Number of dimeric molecules per unit cell	Calculated [b] water content of crystals by volume %
a = 78.5 b = 46.4 c = 87.9 α = 90° β = 109°05 γ = 90°	302,500	$P2_1$	2	48

[a] Estimated precision: $\sigma < 0.1$.
[b] The water content was calculated assuming 2 molecules per unit cell and a partial specific volume of 0.74 cm^3/g for each dimer of 64,000.

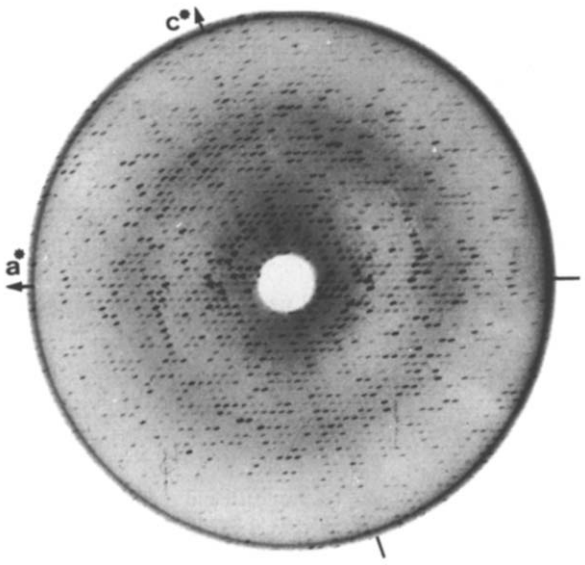

Fig. 1. A 18° precession photograph of the hol zone of methionyl-tRNA synthetase.

the largest having dimensions of about 0.1 X 0.1 X 0.01 mm. The smallest of these microcrystals were subsequently used as seeds in experiments where the protein solution (0.1% protein by weight) was dialyzed in capillaries according to Weber and Goodkim [5] against 48% ammonium sulfate in phosphate buffer, pH 7.2. The crystals thus obtained had the same shape as the seeds, with maximal dimensions of 0.4 X 0.2 X 0.02 mm. Their extreme thinness made them very fragile, and the diffracting volume was too small to enable a routine study with a precession camera.

Crystals suitable for X-ray work were obtained by dialyzing in the cold room the protein solution (5 mg/ml in 0.1 M potassium phosphate buffer, pH 7.2) in conventional dialysis tubing against 1.5 M ammonium citrate buffered by 0.1 M phosphate at pH's varying between 6.4 and 8.0. Crystals appeared in a matter of days, the largest being observed at pH 6.8 and 7.4–7.6. The smallest of these crystals had a well-defined shape of parallelepipedic plates, while the biggest (about 1 X 1 X 0.2 mm) were often cracked. The enzyme recovered from these crystals retained full activity in the ATP–PP_i exchange assay as well as for aminoacylation of tRNA.

The study of the unit cell was undertaken with two samples obtained at pH 6.8 and pH 7.4. In both cases the crystals were found to be monoclinic, space-group $P2_1$ (table 1 and fig. 1), with one dimeric molecule per assymmetric unit. These crystals suffered no detectable alteration after 100 hr of exposure to the X-ray beam, in contrast to crystals of lysyl tRNA synthetase from yeast, which were reported as very sensitive to exposure to X-rays [6]. As shown in fig. 1, the reflections are still strong in the outer part of the film, corresponding to Bragg spacings of 2.5 Å, thus making these crystals suitable for high resolution studies.

4. Discussion

Native methionyl-tRNA synthetase from *E. coli* K12, composed of four, probably identical subunits having a molecular weight of 43,000 each [7, 8], has proven highly susceptible to proteolytic cleavage [3]. Incubation with trypsin is accompanied by release of close to 25% of the original protein, with consequent dissociation of the original tetramer into modified dimers, each now composed of two, apparently identical subunits of molecular weight 32,000. In addition to its drastically altered molecular structure, the trypsin-modified enzyme is characterized by its unimpaired specificity and affinity towards the normal substrates, a 20% lower catalytic efficiency, a significantly lower magnesium ion requirement for the aminoacylation of both methionine-tRNAs, and a considerably enhanced thermal stability, compared to the native enzyme [3]. The present study indicates that the trypsin-modified methionyl-tRNA synthetase also shows a pronounced tendency to crystallize, in contrast to the native enzyme which has repeatedly failed to do so.

A detailed study of the properties of the trypsin-modified enzyme has led to the formulation of a structural model for native methionyl-tRNA synthetase [3] in which the subunit polypeptide chains, assumed to be structurally identical, are each composed of two functionally distinct parts (designated A and B for convenience). These are folded into separate tertiary structures, one of molecular weight 32,000 (globule A) comprising the catalytic and tRNA recognition sites as well as the monomer-monomer interaction sites, and the other of 11,000 molecular weight (globule B), in some way implicated in promoting association of the dimers into tetramer. Proteolytic attack of the enzyme would utimately eliminate that portion of the subunit molecules corresponding to globule B and the connecting region of the chain, leaving enzymatically active intact A-A dimers which are resistant to further proteolysis.

The enhanced thermal stability of modified methionyl-tRNA synthetase, and its propensity to crystallize, which both reflect a highly ordered tertiary structure, thus appear to be directly related to loss of globule B from the native subunits through proteolytic cleavage.

If the subunits of trypsin-modified methionyl-tRNA synthetase are, indeed, identical, then the polypeptide chain of molecular weight 32,000 represents, to date, the shortest amino acid sequence endowed with the information required for full expression of the catalytic properties characteristic of aminoacyl-tRNA synthetases. The isolation of this enzyme in crystalline form suitable for high resolution X-ray crystallography, the availability of a method for the isolation of homogenous enzyme in high yields [2, 3] from an episomal strain derived from *E. coli* K12 which contains four times the amount of methionyl-tRNA synthetase normally found in strain K12 [9], as well as the accumulation of a wealth of information on the properties of the homologous methionine-specific tRNAs, thus designate this modified methionyl-tRNA synthetase as a most favorable candidate for further studies aimed at defining the structural basis of the specific interaction between aminoacyl-tRNA synthetases and tRNA. Work on the high resolution crystallographic analysis of the enzyme is in progress.

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References

[1] S.H.Kim, G.Quigley, F.L.Suddath and A.Rich, Proc. Natl. Acad. Sci. U.S. 68 (1971) 841.
[2] D.Cassio and J.P.Waller, FEBS Letters 12 (1971) 309.
[3] D.Cassio and J.P.Waller, European J. Biochem. 20 (1971) 283.
[4] W.B.Jacoby, Anal. Biochem. 26 (1968) 295.
[5] B.H.Weber and P.E.Goodkim, Arch. Biochem. Biophys. 141 (1970) 489.
[6] L.Rymo, V.Lagerkvist and A.Wonacott, J. Biol. Chem. 245 (1970) 4308.
[7] F.Lemoine, J.P.Waller and R.van Rapenbusch, European J. Biochem. 4 (1968) 213.
[8] C.J.Bruton and B.J.Hartley, J. Mol. Biol. 52 (1970) 165.
[9] D.Cassio, F.Lawrence and D.Lawrence, European J. Biochem. 15 (1970) 331.