SHORT COMMUNICATIONS

Acta Cryst. (1997). D53, 195–196

Preliminary X-ray diffraction studies on asparaginyl-tRNA synthetase from *Thermus thermophilus*

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(Received 5 August 1996; accepted 11 November 1996)

Abstract

The recombinant asparginyl-tRNA synthetase from the thermophilic bacterium *Thermus thermophilus* expressed in *Escherichia coli* has been crystallized from PEG 6000 solutions. Depending on the PEG concentrations the crystals were in either tetragonal or hexagonal space groups. Although generally smaller, the latter (space group $P6_422$) diffracted better, to a resolution of 2.8 Å. Using the coordinates of the yeast aspartyl-tRNA synthetase structure molecular replacement methods were applied to both tetragonal and hexagonal crystals; a solution was found which gave excellent crystal packing in both space groups.

1. Introduction

Aminoacyl-tRNA synthetases are a set of enzymes which, by specifically catalysing the ligation of cognate amino acids to their cognate tRNA's, ensure the correct translation of genetic information into proteins. This catalysis proceeds *via* a two-step reaction. In the first the amino acid is activated as an aminoacyl-adenylate by reaction with ATP. This reaction takes place within the catalytic domains of the enzymes. The apparent diversity and lack of general primary structure homology was resolved by the observations that the 20 enzymes, for the 20 natural amino acids found in proteins, could be partitioned into two classes of ten enzymes based on primary structural motifs and folds of their catalytic domains (Eriani, Delarue, Poch, Gangloff & Moras, 1990; Cusack, Berthet-Colominas, Härtlein, Nassar & Leberman, 1990). In the class I synthetases this being a domain based on the Rossmann dinucleotide-binding fold and for the class II enzymes a domain consisting of seven antiparallel β-strands (Cusack *et al.*, 1990).

Of the ten class II aminoacyl-tRNA synthetases, three-dimensional structures of representative examples of five have been determined (Cusack, 1995). The class II synthetases can be further sub-classified according to the spatial relationship between the catalytic domains and domains responsible for tRNA recognition/interaction (Cusack, Härtlein & Leberman, 1991). Sub-class IIb enzymes possess in their primary structure an N-terminal domain proceeding the catalytic domain. This sub-class comprises the aspartyl, lysyl and asparaginyl-tRNA synthetases and for the first two members of this sub-class, high-resolution crystal structures have been determined both of the native enzyme (Delarue *et al.*, 1994; Onesti, Miller & Brick, 1995) and in complex with cognate tRNA (Ruff *et al.*, 1991; Yaremchuk, Kryklivyi, Cusack & Tukalo, 1995; Cusack, Yaremchuck & Tukalo, 1997). The tertiary structures of the lysyl and aspartyl enzymes are remarkably similar and it is to be expected that this similarity will apply to the asparaginyl enzyme. But it is of interest to determine the structural features that enable the cognate tRNA synthetases to distinguish between aspartic acid and asparagine.

Although the recombinant asparginyl-tRNA synthetase from *Escherichia coli* has been available in this laboratory for some years (Anselme & Härtlein, 1989), all attempts to crystallize this enzyme have proved unsuccessful. We have recently sequenced the asparginyl-tRNA synthetase from *Thermus thermophilus*, and purified and crystallized the recombinant enzyme expressed in *E. coli* (Seignovert, Härtlein & Leberman, 1996). We describe here the crystallization and preliminary X-ray diffraction data of the dimeric protein.

2. Experimental

2.1. *Materials*

Recombinant asparginyl-tRNA synthetase from *T. thermophilus* was isolated and purified as described (Seignovert *et al.*, 1996).

2.2. *Crystallization*

Preliminary crystallization trials were carried out by vapour diffusion using a simple 24-solution sparse matrix (R. Leberman, unpublished work). The sitting-drop method was used with initially 5 µl enzyme solution at 7 mg ml^{-1} mixed with 5 µl reservoir solution.

2.3. *Crystal analysis*

All data collections have been performed using a 30 cm Mar Research image-plate detector. Preliminary X-ray data were collected using a Siemens rotating-anode generator source ($\lambda = 1.5416$ Å) at room temperature. Higher resolution data were collected on frozen crystals at beamlines 3 (ID9, $\lambda = 0.733$ Å) and 19 (D14, $\lambda = 0.924$ Å) of the ESRF. In order to measure the crystals at low temperature, they were transferred to a mother liquor solution containing 30% glycerol and frozen at 100 K and maintained at this temperature in a nitrogen stream using an Oxford Instruments cryo-cooling system.

3. Results and discussion

3.1. *Crystallization*

Within a few days the preliminary trials produced small needles under five conditions; 50% saturated ammonium sulfate at pH 8.4 or pH 6.4 with addition of 0.2 *M* lithium sulfate or pH 7.5 with addition of 0.2 *M* potassium nitrate, 1 *M* triammonium citrate pH 7.5 with addition of 0.2 *M* lithium

sulfate or pH 8.4 with addition of 0.2 *M* potassium nitrate. Small plates were obtained from 20% PEG 6000 at pH 8.4 with the addition of 0.2 *M* lithium sulfate. This last condition was chosen for refinement and by a sequence of modifying the PEG concentration, pH, type and concentration of salt addition, single crystals suitable for diffraction analysis were obtained. These conditions were: protein 5 mg ml^{-1}, 12–15% PEG 6000, 0.3 *M* KCl at pH 8.4.

3.2. *Crystal data*

Data have been collected on two different crystals forms obtained from PEG 6000 solutions under very similar crytallization conditions; the variation being dependent on the concentration of the precipitant. At PEG concentrations of about 12% both forms can be found in the same drop, but the crystal habit is sufficient to distinguish between them.

At low PEG concentrations crystals were more commonly of a tetragonal form with unit-cell parameters $a = 83.9$, $b = 83.9$, $c = 167.9$ Å, $\alpha = \beta = \gamma = 90°$. Examination of systematic absences identified the space group as either $P4_12_12$ (number 95) or $P4_32_12$ (number 96). The crystals grew to a maximum size of $400 \times 400 \times 600$ µm and diffracted slightly anisotropically to a maximum of 3 Å resolution.

From 12 to 15% PEG 6000 crystals grew faster, were smaller and were of a hexagonal space group with unit-cell parameters $a = 124.7$, $b = 124.7$, $c = 122.6$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. Good-quality diffraction data have been collected from these crystals only at 100 K using synchrotron radiation. When the dimensions of the crystals were about 200 µm they diffracted to 2.8 Å resolution, but with larger crystals the resolution was reduced to 3.2 Å. Examination of systematic absences identified the space group as either $P6_222$ (number 180) or $P6_422$ (number 181).

It is interesting to recall that, on the basis of primary-sequence comparisons, prokaryotic asparaginyl-tRNA synthetases are more similar to eukaryotic aspartyl-tRNA synthetases than prokaryotic ones (Cusack *et al.*, 1991). The aminoacyl-tRNA synthetase of known structure most closely related to the *T. thermophilus* asparaginyl-tRNA synthetase is indeed yeast cytoplasmic aspartyl-tRNA synthetase, the identity being about 18%. *T. thermophilus* asparaginyl-tRNA synthetase and *T. thermophilus* aspartyl-tRNA synthetase are about 14.6% identical in sequence, but the aspartyl enzyme has the characteristically much larger insertion domain between motifs 2 and 3 of prokaryotic aspartyl-tRNA synthetases (Delarue *et al.*, 1994).

Using the program *AMoRe* in the *CCP*4 package (Collaborative Computational Project, Number 4, 1994), molecular replacement solutions were sought in both space groups using the monomer of yeast aspartyl-tRNA synthetase as search model. Promising solutions were found, in space groups $P4_32_12$ (number 96) and $P6_422$ (number 181) for the tetragonal and hexagonal forms, respectively. Using the resolution range 4–20 Å, the correlations for the best and next best solutions after rigid-body fitting were 0.196 (0.144) and 0.155 (0.145), respectively. Despite the relatively low correlations both solutions had excellent crystal packing, notably with the molecular twofold axis coinciding with a crystallographic twofold axis in each case. Improved solutions were obtained upon substitution of the asparaginyl-tRNA synthetase sequence and rigid-body refinement of the models with *X-PLOR* (Brünger, 1992) in which the N-terminal domain and C-terminal catalytic domain were treated separately. For instance in the case of space group 181, the correlation increased to 0.24 following a significant rotation of the N-terminal domain by local rotation angles of 3.98, 5.73 and −11.98°. Both molecular replacement solutions and the N-terminal domain rotation have subsequently been shown to be correct by independent determination of an experimental MIR map using uranium and samarium isomorphous derivatives. Model building and refinement of the structure, making use of averaging between the two space groups, is in progress.

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