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Crystals of *Thermus thermophilus* tRNA^{Asp} Complexed with its Cognate Aspartyl-tRNA Synthetase Have a Solvent Content of 75%. Comparison with Other Aminoacylation Systems

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Abstract

Thermus thermophilus tRNA^{Asp}, purified from a non-recombinant source, has been crystallized in a complex with its cognate dimeric ($\alpha 2$) aspartyl-tRNA synthetase. Crystals diffract to 2.9 Å resolution and belong to space group $P6_3$ with cell parameters $a = b = 258$, $c = 90.9$ Å. The crystals contain one aspartyl-tRNA synthetase dimer and two tRNA molecules in the asymmetric unit, corresponding to a V_m of 4.85 Å^3 Da^{-1} and 75% solvent content. When compared with those obtained for globular proteins these values are high, but fall within the range observed for other aminoacyl-tRNA synthetases, either free or complexed with their tRNAs. A comparative survey is presented here.

1. Introduction

Faithful translation of genetic information relies on the correct attachment of a given amino acid to its cognate transfer RNA (tRNA). This attachment is catalysed by a set of 20 enzymes (one for each amino acid) known as aminoacyl-tRNA synthetases (aaRS). The reaction proceeds *via* a two-step process. In the first step, the amino acid is activated by attacking a molecule of ATP at the α-phosphate and giving rise to an aminoacyl adenylate. In the second step, the activated amino acid is linked to the 3′-terminal ribose of a cognate tRNA (see Arnez & Moras, 1997; Cusack, 1995, for recent reviews).

Aspartyl-tRNA synthetases (AspRS) are modular enzymes which can be described in terms of four distinct structural domains: the N-terminal domain is responsible for the tRNA anticodon recognition, and the hinge and the catalytic C-terminal domains are involved both in catalysis and tRNA recognition. Finally an extra domain, also involved in tRNA recognition, allows a clear structural partition between eukaryotic (small extra domain) and prokaryotic (large extra domain) AspRS (Delarue *et al.*, 1994). The structure of *T. thermophilus* AspRS in its free state, as well as complexed with its cognate aminoacyl adenylate, has enabled the analysis of the first step of the reaction, the adenylation (Poterszman *et al.*, 1994). In the AspRS system, the transfer reaction has been extensively studied in the eukaryotic *Saccharomyces cerevisiae* enzyme (Cavarelli *et al.*, 1994). To analyse the transfer reaction catalysed by the *T. thermophilus* AspRS and, therefore, obtain a complete description of the aminoacylation reaction for this enzyme, structural data on *T. thermophilus* AspRS complexed with its cognate tRNA are required.

We report here the crystallization and preliminary crystal structure analysis of the homologous RNA–protein complex, tRNA^{Asp} and AspRS from *T. thermophilus*. Crystals belong to space group $P6_3$ and contain one biological dimer in the asymmetric unit (one protein dimer of 130 kDa and two tRNA molecules).

2. Experimental

2.1. *Purification*

AspRS from *T. thermophilus* was expressed in *Escherichia coli* and purified as described by Poterszman *et al.* (1993). After the usual flocculation step and the anion exchange column, an additional hydrophobic interaction chromatographic step was carried out. The protein was loaded on a Merk Fractogel TSK-butyl 650s column (diameter = 26 mm; flow rate = 1.5 ml min^{-1}) equilibrated in 1.4 *M* ammonium sulfate, 10 m*M* Tris–HCl pH 7.5 and subsequently eluted by decreasing the ammonium sulfate concentration. The protein, which eluted at approximately 1 *M* ammonium sulfate, was dialysed against a buffer containing 5 m*M* Tris–HCl pH 7.5 and 100 m*M* ammonium sulfate. The protein was then concentrated to 70 mg ml^{-1} (assuming an extinction coefficient of 1 ml mg^{-1} cm^{-1} for the protein at 280 nm) with a Centricon-10 microconcentrator (Amicon) and stored at 277 K in the dialysis buffer.

tRNA^{Asp} was purified from *T. thermophilus* HB8 cells as described by Keith *et al.* (1993). Starting with 500 g of wet cells, a 1 mg yield of pure tRNA^{Asp} could be obtained. The tRNA was dialysed against 5 m*M* sodium cacodylate pH 6.5 and 10 m*M* $MgCl_2$, and concentrated with a Centricon-10 device to 22 mg ml^{-1} (assuming an

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extinction coefficient of 2.5 ml mg^{-1} cm^{-1} for the tRNA at 260 nm) and stored at 253 K in the dialysis buffer.

2.2. *Crystallization*

Crystallization was conducted using the hanging-drop technique. In all experiments, a 4 µl drop (2 µl complex and 2 µl reservoir solution) was equilibrated against 500 µl reservoir solution. Initial screening was carried out using Crystal Screen (Hampton Research) with 15 mg ml^{-1} AspRS and 7.9 mg ml^{-1} tRNA, which corresponds to a ratio of three tRNA molecules per dimer of AspRS. Whenever a screening solution led to immediate precipitation of the contents of a drop, the well was diluted twofold with distilled water. A single condition yielded crystals: condition 38 from the Hampton Research Crystal Screen, which had been diluted twofold. It was optimized by varying the pH (6.5–8.0), the tRNA:dimer ratio (2.0–3.0) and the precipitant concentration (0.5–0.9 *M* sodium citrate). The influence of substrates (ATP and amino acid) was also tested.

The best crystals of the homologous complex between AspRS from *T. thermophilus* and its cognate tRNA were grown by mixing 2 µl of a reservoir solution containing 0.6 *M* sodium citrate, 10 m*M* $MgCl_2$ and 50 m*M* Na HEPES pH 7.3 with 2 µl of a solution containing 15 mg ml^{-1} protein and 7.35 mg ml^{-1} tRNA (*i.e.* 2.8 tRNA per AspRS dimer). The crystals exhibit hexagonal morphology and can reach a size of 0.7 × 0.5 × 0.5 mm in 14 d at 290 K.

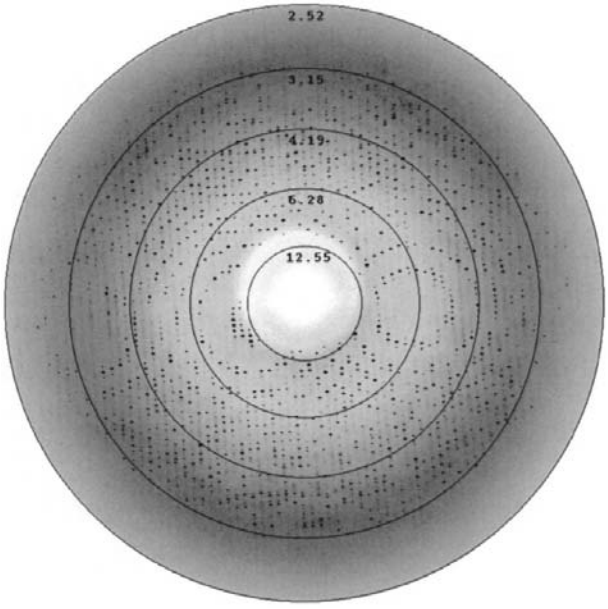

Fig. 1. A typical diffraction pattern of *T. thermophilus* AspRS complexed with its cognate tRNA. The image was recorded on a MAR Research imaging-plate detector using X-rays from the W32 beamline of LURE. The oscillation range was 0.5° and the exposure time 300 s. The crystal-to-film distance was set to 400 mm, giving a resolution limit of 2.52 Å at the edge of the photograph. Circles corresponding to resolutions of 2.52, 3.15, 4.19, 6.28 and 12.55 Å are drawn.

2.3. *Space-group determination and data collection*

Crystals mounted in a thin-wall capillary diffract anisotropically (2.6 Å resolution along *c* and better than 3 Å resolution along *a*; see Fig. 1). For subsequent diffraction experiments, crystals were stabilized by adjusting the citrate concentration in the well to 0.9 *M*. Under these conditions, reservoir solution can be added to the drop without alteration of the crystals in terms of morphology or diffraction pattern. However, a rapid decay after two or three X-ray exposures at 277 K limits the usable data set to 3.5 Å resolution.

Preliminary data were collected at 277 K using synchrotron radiation (beamline W32 at LURE, $\lambda = 0.901$ Å) on a 30 cm MAR Research imaging-plate detector by the oscillation method. The crystal-to-detector distance was set to 450 mm and the oscillation range was 0.6°. Using the *MARXDS* data-processing software package (Kabsch, 1988), the cell dimensions were determined to be $a = b = 258$, $c = 90.9$ Å in the hexagonal space group $P6_3$.

A preliminary dataset with an *R* factor of 6.7% on intensities was collected from four crystals; 80.54% of the reflections were measured with an average multiplicity of 3.3 between 15 and 3.5 Å. In the highest resolution shell (3.6–3.5 Å), the *R* factor is 35.74%, $I/\sigma(I)$ is 3.03, the multiplicity is 1.6, the completeness is 58.9% and 22.8% of the reflections were measured with $I > 3\sigma(I)$.

3. Results and discussion

Using the preliminary 3.5 Å data set, the structure was solved by molecular replacement. Calculations were performed with the *AMoRe* package (Navaza, 1994) using 8096 reflections (80% of the data) between 6 and 15 Å. Using the structure of the apo-enzyme from *T. thermophilus* as a search model (Delarue *et al.*, 1994), two highly contrasting solutions related by a twofold axis, which corresponds to the dimer axis derived from the unique peak of the self-rotation function, were found (for the two solutions, correlation coefficients were 53.3 and 53.7% and *R* factors were 38.4 and 38.8%). The model was then subjected to successive steps of rigid-body refinement in the ranges 15–6, 15–4.5 and 15–3.5 Å, followed by torsion-angle molecular dynamics at a resolution of 3.5 Å. The corresponding $3F_o - 2F_c$ and $F_o - F_c$ maps display interpretable electron density for the protein dimer, and electron density is visible for most of the sugar–phosphate backbone atoms of the tRNAs.

Table 1. V_m values for various aaRS crystals

Compilation of the V_m values from crystals of free or complexed aaRSs. V_m values were taken from the references, except where noted otherwise. Data sets are complete to the indicated resolution and were collected at room temperature or at 277 K, except where noted otherwise. The average V_m values are $\langle V_m \rangle$ = 3.3 Å^3 Da^{-1} for free aaRS and $\langle V_m \rangle$ = 4.2 Å^3 Da^{-1} for the complexes with tRNAS. These values were calculated from the solved crystal structure only (11 out of 13 for 13 of the complexes and 10 out of 12 for the aaRSs).

Synthetase	V_m (Å^3 Da^{-1})	Solvent content (%)†	Resolution (Å)	References
Seryl-tRNA synthetase–$tRNA^{Ser}$ complex from *T. thermophilus*, form Ia‡	6.10	79.9	6§	Yaremchuk *et al.* (1992)
Aspartyl-tRNA synthetase–$tRNA^{Asp}$ complex from *Saccharomyces cerevisiae*, cubic form	5.30	76.8	7	Lorber *et al.* (1983)
Lysyl-tRNA synthetase–$tRNA^{Lys}$ complex from *T. thermophilus*	4.90¶	74.9	2.9††	Cusack *et al.* (1996); Yaremchuk *et al.* (1995)
Aspartyl-tRNA synthetase–$tRNA^{Asp}$complex from *T. thermophilus*	4.85	74.6	3.0	This work
Seryl-tRNA synthetase–$tRNA^{Ser}$ complex from *E. coli*	4.30	71.5	4	Price *et al.* (1993)
Aspartyl-tRNA synthetase–$tRNA^{Asp}$ complex from *E. coli*	4.21	70.8	3.2	Eiler *et al.* (1992)
Phenyl-tRNA synthethase–$tRNA^{Phe}$ complex from *T. thermophilus*	4.20¶	70.7	3.28	Reshetnikova *et al.* (1993)
Seryl-tRNA synthetase–$tRNA^{Ser}$ complex from *T. thermophilus*, form II‡	3.90	68.5	5.5§	Yaremchuk *et al.* (1992)
Seryl-tRNA synthetase–$tRNA^{Ser}$ complex from *T. thermophilus*, form VI	3.90	68.5	2.5††	Belrhali *et al.* (1994); Yaremchuk *et al.* (1992)
Aspartyl-tRNA synthetase–$tRNA^{Asp}$ complex from *S. cerevisiae*	3.79	67.6	2.9	Ruff *et al.* (1988)
Glutaminyl-tRNA synthetase–$tRNA^{Gln}$ complex from *E. coli*	3.67	66.5	2.8	Perona *et al.* (1988)
E. coli aspartyl-tRNA synthetase in a complex with yeast $tRNA^{Asp}$	3.66	66.5	2.7††	Boeglin *et al.* (1996)
Seryl-tRNA synthetase–$tRNA^{Ser}$ complex from *T. thermophilus* form III	3.20	61.6	3.5	Yaremchuk *et al.* (1992)
Lysyl-tRNA synthetase from *E. coli*	4.95¶	75.2	2.8	Onesti *et al.* (1994, 1995)
Phenyl-tRNA synthetase from *T. thermophilus*	4.80	74.4	2.9	Mosyak *et al.* (1995); Reshetnikova *et al.* (1992)
Threonyl-tRNA synthetase from *T. thermophilus*‡	3.70	66.8	2.7§	Cura *et al.* (1995)
Glycyl-tRNA synthetase from *T. thermophilus*	3.60	65.9	2.75	Logan *et al.* (1994, 1995)
Histidyl-tRNA synthetase from *E. coli*	3.40	63.9	2.6	Arnez *et al.* (1995); Francklyn *et al.* (1994)
Amino-terminal fragment of the alanyl-tRNA synthetase from *E. coli*‡	3.30	62.8	3§	Frederick *et al.* (1988)
Aspartyl-tRNA synthetase from *T. thermophilus*	3.22	61.9	2.5	Poterszman *et al.* (1993)
Tyrosyl-tRNA synthetase from *Bacillus sterarothermophilus*	3.17	61.2	2.3	Brick *et al.* (1989); Reid *et al.* (1973)
Glutamyl-tRNA synthetase from *T. thermophilus*	3.01	59.2	2.5	Nureki *et al.* (1992, 1995)
Tryptophanyl-tRNA synthetase from *B. stearothermophilus*	2.66	53.8	2.9	Carter & Carter (1979); Doublie *et al.* (1995)
Trypsin-modified methionyl-tRNA synthetase from *E. coli*	2.36¶	47.9	2.5	Brunie *et al.* (1990); Waller *et al.* (1971)
Histidyl-tRNA synthetase from *T. thermophilus*	2.26	45.6	2.7††	Aberg *et al.* (1997)

† Calculated with the Matthews relation $\{T_{sol} = [1-(1.66 \times 0.74)]/V_m\}$ (Matthews, 1968). ‡ Unsolved crystal structure. § Incomplete data set. ¶ Calculated from cell parameters and number of molecules in the asymmetric unit. †† Data from flash-frozen crystal.

The crystals contain one dimeric complex in the asymmetric unit. This corresponds, assuming a molecular weight of 180 kDa for the complex, to a calculated volume per unit mass of V_m = 4.85 Å^3 Da^{-1} and to a solvent content of 75% computed with the formula $T_{sol} = [1-(1.66 \times 0.74)]/V_m$. The V_m value is high when compared to those given by Matthews (1968) for a set of 116 globular proteins [$\langle V_m \rangle$ = 2.61, V_m(min) = 1.68, V_m(max) = 3.53 Å^3 Da^{-1}; $\langle V_m \rangle$, average value; V_m(min), minimum value; V_m(max), maximum value]. However, this value falls within the range observed for other aaRSs crystallized in their free state [$\langle V_m \rangle$ = 3.3, V_m(min) = 2.26, V_m(max) = 4.95 Å^3 Da^{-1}] or complexed with their cognate tRNAs [$\langle V_m \rangle$ = 4.2, V_m(min) = 3.20, V_m(max) = 6.10 Å^3 Da^{-1}]. These high values (Table 1) should be correlated to the size and to the modular

organization of the aaRS proteins. They have an irregular shape and are more difficult to pack than small globular proteins. A second observation can be made: on average, crystals of aaRSs have lower V_m values and diffract better than those obtained for aaRS–tRNA complexes (See Fig. 2). The poor diffraction observed for aaRS/tRNA complexes could be related to the fact that the tRNA molecules are often, if not always, involved in packing contacts. These polyelectrolytes have a tendency to favour non-specific contact and to create local disorder.

4. Conclusions

Crystals of a complex formed by native $tRNA^{Asp}$ from *T. thermophilus* and its cognate AspRS were obtained in the absence of the two other substrates, ATP and amino acid. By comparison with the known structures of complexes obtained in the presence of aminoacyl adenylate (Cavarelli *et al.*, 1994; Ruff *et al.*, 1991), this structure will identify the specific interactions occurring upon recognition of the tRNA. The high correlation value obtained using the apo-enzyme model suggests that only small conformational changes occur. Preliminary tests show that data can be recorded at low temperature to a resolution better than 3.0 Å using flash-freezing techniques. Crystals for flash-freezing were soaked between 30 and 40 s in a cryo-protecting buffer consisting of reservoir solution containing 20% glycerol.

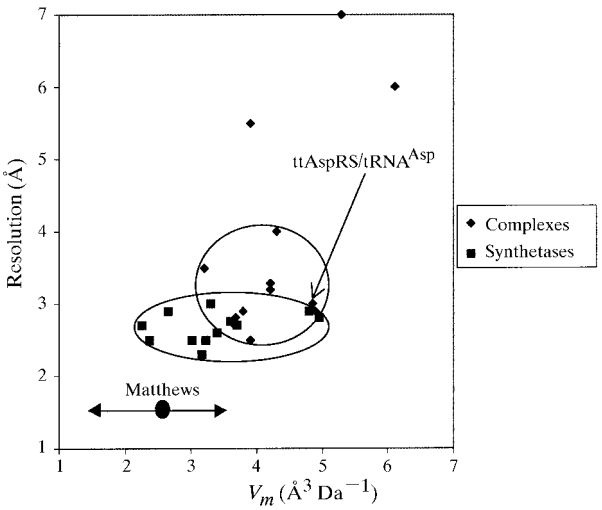

Fig. 2. Resolution *versus* V_m for some aaRSs. aaRSs crystallized in the absence of tRNA are represented by squares and aaRS–tRNA complexes by diamonds. Ellipsoids were drawn to surround most of the points for free and complexed aaRSs without taking into consideration the three crystal forms which diffract only to a low resolution. The V_m values (Matthews, 1968) for 116 small globular proteins are indicated.

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