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Crystallization and preliminary X-ray diffraction data of the second and archaebacterial-type aspartyl-tRNA synthetase from *Thermus thermophilus*

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The archaebacterial-type aspartyl-tRNA synthetase (AspRS2) from the thermophilic eubacterium *Thermus thermophilus* was crystallized using the hanging-drop vapour-diffusion method. Crystals grew at pH 9.5 in the presence of PEG 8000 and NaCl. A native diffraction data set has been collected at 2.5 Å resolution using synchrotron radiation and cryocooling. Crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 57.3$, $b = 121.9$, $c = 166.9$ Å and $V_M = 3.03$ Å^3 Da^{-1}. There is one dimer of M_r 96 000 per asymmetric unit. A molecular-replacement analysis gave solutions for the rotation and translation functions.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) constitute a family of enzymes of structural and functional diversity (Martinis *et al.*, 1999). They have a pivotal role in protein synthesis, where they ensure translation of the genetic code into the protein language. The correctness of translation relies on the specific charging of the cognate isoaccepting tRNAs with the homologous amino acid by each of the 20 aaRSs (reviewed by Kisselev & Wolfson, 1994; Ibba & Söll, 2000). However, exceptions to the rule of unicity of tRNA aminoacylation systems, according to which one aaRS corresponds to each of the 20 amino acids, exist. Such exceptions appeared with the discovery of duplicated or missing aaRSs in certain organisms (reviewed by Becker & Kern, 1998). The thermophilic eubacterium *T. thermophilus* contains two genetically distinct aspartyl-tRNA synthetases (AspRSs) which show only 29% sequence identity (Becker *et al.*, 1997). The distinction is also functional, since AspRS1 charges solely $tRNA^{Asp}$, in strong contrast to AspRS2, which aspartylates both $tRNA^{Asp}$ and $tRNA^{Asn}$ with similar efficiencies. However *T. thermophilus* also contains a fully functional asparaginyl-tRNA synthetase (AsnRS) that directly forms asparaginyl-$tRNA^{Asn}$. In fact, AspRS2 is involved in an indirect pathway of asparaginyl-$tRNA^{Asn}$ formation, since aspartate bound on $tRNA^{Asn}$ is converted into asparagine by a tRNA-dependent aspartate amidotransferase. While AspRS1 shares functional and structural features with other eubacterial AspRSs, AspRS2 strikingly resembles the AspRSs of dual specificity present in archaea deprived of AsnRS (Becker *et al.*, 2000).

We are aiming towards understanding the structural basis of the relaxed specificity of the archaebacterial-type AspRS2 from *T. thermophilus* and at finding what distinguishes its three-dimensional structure from that of AspRS1 (Delarue *et al.*, 1994) and more generally from the known structures of AspRSs from other organisms, namely the eubacterium *Escherichia coli* (Eiler *et al.*, 1999), the yeast *Saccharomyces cerevisiae* (Sauter *et al.*, 2000) and the archaeon *Pyrococcus kodakaraensis* (Schmitt *et al.*, 1998). We describe here the crystallization of the thermophilic AspRS2 and the preliminary analysis of the X-ray diffraction data showing that the crystals are suitable for structure determination.

2. Materials and methods

2.1. Preparation of pure aspartyl-tRNA synthetase 2 from *T. thermophilus*

AspRS2 from *T. thermophilus* was overexpressed in *E. coli* BL21 strain transformed by the pET-3b vector (Becker *et al.*, 2000). Overproducing cells were harvested 10 h after induction of AspRS biosynthesis at 310 K. The original purification procedure of AspRS2 (Becker *et al.*, 2000) was slightly modified to obtain enzyme of crystallographic grade. Briefly, 15 g biomass was suspended in 45 ml of 100 m*M* Tris–HCl buffer pH 8.0 containing 5 m*M* β-mercaptoethanol, 0.5 m*M* EDTA and 1 m*M* AEBSF (a serine protease inhibitor provided by Uptima). Aliquots of 15 ml were sonicated and lysates were centrifugated for 2 h at 105 000*g*. The supernatant was then heated for 30 min at 343 K and the floculated proteins were sedimented by 15 min centrifu-

gation at 5000*g*. After dialysis against 20 m*M* potassium phosphate buffer pH 7.2, the proteins were adsorbed on a DEAE-cellulose column and eluted with a linear gradient of 20 m*M* at pH 7.2 to 250 m*M* at pH 6.8 potassium phosphate. The active fractions eluting at 140 m*M* salt were dialyzed, adsorbed on a HPLC hydroxyapatite column (CHT20, Bio-Rad) equilibrated with 10 m*M* potassium phosphate pH 6.8 and the proteins were eluted with a linear gradient of 10–200 m*M* potassium phosphate pH 6.5. The enzyme eluted at 90 m*M* salt was dialyzed against 20 m*M* Tris–HCl pH 7.5 and concentrated to 14 mg ml^{-1} ($\varepsilon_{280\text{nm}}$ = 55 900 M^{-1} cm^{-1}) by centrifugation in a Vivaspin concentrator (Sartorius). This protocol yielded 20 mg pure enzyme. Since the protein precipitates at 277 K when the concentration exceeds 2.5 mg ml^{-1}, it was stored at 298 K. Its purity and homogeneity was checked by activity assay and gel electrophoresis under denaturing conditions.

2.2. Crystallization

Initial crystallization conditions were searched by sparse-matrix screening (Jancarik & Kim, 1991) using the vapour-diffusion technique in hanging drops at 293 K. 4 µl drops were equilibrated over 1 ml reservoirs. A total of 296 conditions were covered with four screens (Hampton Research Screens I and II, Molecular Dimensions Limited 3D Structure Screen and Emerald Biostructure, Wizard I and II). For each condition, 2 µl AspRS2 solution (14 mg ml^{-1}) was mixed with 2 µl reservoir solution. Reproducible crystals were obtained with the streak-seeding technique using a dog whisker and large crystals (Fig. 1) were grown by macroseeding (Stura, 1999).

2.3. Diffraction measurements

Prior to data collection, suitable native crystals were soaked in a cryobuffer composed of mother liquor mixed with 20%(*v*/*v*) glycerol. Crystals were then mounted in a nylon loop and flash-cooled in liquid ethane at 120 K.

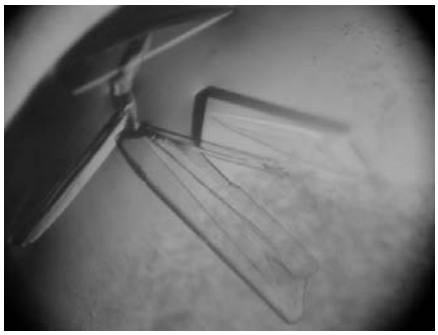

Figure 1
Crystals of *T. thermophilus* AspRS2.

X-ray data were collected at 100 K either on a DIP2000 Enraf–Nonius area detector using a rotating-anode generator (wavelength 1.542 Å, crystal-to-detector distance 160 mm) or on beamline ID14-2 at the European Synchrotron Radiation Facility (ESRF, Grenoble). With the rotating-anode X-ray source, a total range of 91° was covered with 1.0° oscillation per frame. Data were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1996). Synchrotron data were collected by maintaining the crystal in a nitrogen-gas stream at 100 K, with incident radiation at a wavelength of 0.933 Å and a crystal-to-detector distance of 200 mm. The diffraction spots were recorded on an ADSC-Q4 CCD detector with a 1.0° oscillation and a 12 s exposure per CCD image over a range of 100°. Data were processed as above and the indexed intensities were converted to structure factors using *TRUNCATE* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994) without any σ cutoff.

3. Results and discussion

Of the 296 crystallization conditions tested, only one gave crystals, namely condition No. 29 from the Emerald Biostructure Wizard I kit containing 10%(*v*/*v*) PEG 8000, 200 m*M* NaCl and 100 m*M* CHES buffer pH 9.5. Small crystals grew in about two weeks from solution, but only a few of the assays were successful. Reproducible crystallization was obtained by streak-seeding using microcrystals and drops having compositions close to that of the equilibrated drops. The drops were produced by mixing 2 µl of a twice-concentrated reservoir solution (with twice-concentrated content of the initial screen solution No. 29) with 2 µl of a solution containing enzyme (14 mg ml^{-1}) in 20 m*M* Tris–HCl pH 7.5. Small crystals grew in less than 1 d from a precipitate. Optimized conditions were searched by slightly varying the concentration of the crystallizing agent (±10% around the initial condition). Large crystals suitable for X-ray diffraction analysis were obtained by macroseeding under similar conditions. These crystals were of elongated prismatic shape and grew within 3 d. We note the rather unconventional crystallization condition of AspRS2 at pH 9.5, in contrast to other synthetases which crystallize in the pH range 6.0–8.0 (*e.g.* Boeglin *et al.*, 1996; Poterszman *et al.*, 1993; Sauter *et al.*, 1999).

Table 1
Statistics of X-ray data measurement for *T. thermophilus* AspRS2 crystals.

Values in parentheses correspond to the resolution shell 2.50–2.59 Å.

	'In-house' data	ESRF data
Resolution range (Å)	40–3.0	40–2.5
No. of unique reflections	24685	39070
R_{sym} (%)	7.8 (23.9)	4.5 (24.2)
Completeness (%)	99.1 (99.3)	93.3 (88.39)
$\langle I/\sigma(I)\rangle$	17.8 (6.1)	18.1 (3.8)
Multiplicity	3.5	2.9

AspRS2 crystals belong to an orthorhombic space group, with unit-cell parameters a = 57.3, b = 121.9, c = 166.9 Å and Z = 4. Systematic extinctions [$I/\sigma(I) < 3$] of $h00$ (with $h = 2n + 1$), $0k0$ (with $k = 2n + 1$) and $00l$ (with $l = 2n + 1$) reflections indicate that the space group is $P2_12_12_1$. AspRS2 is a homodimer with M_r = 96 000 and subunits containing 422 amino-acid residues; assuming one dimer in the asymmetric unit, the packing density V_{M} is 3.03 Å^3 Da^{-1} and the solvent content is 57.9%. These values are in good agreement with those of other proteins (Matthews, 1968).

Diffraction data were collected using a rotating-anode generator and a synchrotron X-ray source (Table 1). The most complete data set at the highest resolution (resolution range 40–2.50 Å) was collected with synchrotron radiation. 111 711 reflections were reduced to 39 070 unique reflections. Overall, R_{sym} was 4.5% on intensities ($R_{\text{sym}} = \sum |I - \langle I\rangle| / \sum I$) and completeness was 93.3%. Initial attempts to solve the crystal structure of AspRS2 were performed by molecular replacement with the program *AMoRe* (Navaza, 1994) in the resolution range 10–3.5 Å. The dimeric AspRS of *P. kodakaraensis* (Schmitt *et al.*, 1998), which shows 41% identity with *T. thermophilus* AspRS2 and similar functional properties (Becker *et al.*, 2000), served as the structural model. The rotation function calculated with a 58 Å integration radius gave two major peaks related by a twofold non-crystallographic axis, in agreement with the presence of a dimer in the asymmetric unit. Subsequently, the calculation of the translation function gave one major peak with a correlation

$$C = \frac{\sum(|F_o| - \langle|F_o|\rangle)(|F_c| - \langle|F_c|\rangle)}{[\sum(|F_o| - \langle|F_o|\rangle)^2 \sum(|F_c| - \langle|F_c|\rangle)^2]^{1/2}}$$

of 19.8%, while the next solution exhibited a correlation of 15.2%. After rigid-body refinement using the *CNS* package (Brunger

et al., 1998) in the resolution range 10–3.5 Å to optimize rotational and translational parameters of the first solution, the *R* factor was 52.4%. Model building and refinement of the AspRS2 structure are in progress.

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