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Crystallization of the seryl-tRNA synthetase:tRNA^{ser} complex of *Escherichia coli*

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Crystals of the complex between seryl-tRNA synthetase and tRNA$_2^{ser}$ from *Escherichia coli* have been obtained from ammonium sulphate solutions. The crystals are of the 1:2 enzyme:tRNA complex, belong to the space group $C222_1$, have cell dimensions of a = 128.9 Å, b = 164.9 Å, c = 127.3 Å and diffract anisotropically from 3.5 to 4.5 Å. An X-ray diffraction data set to 4 Å has been collected. The combination of molecular replacement using the refined structure of the catalytic domain of the native enzyme, data from a heavy atom derivative and solvent flattening was used to produce a map at 4 Å resolution. This shows that a tRNA molecule binds across the dimer, the anticodon stem and loop do not contact the protein and the helical arm of the enzyme contacts the TΨC loop and the long extra arm of the tRNA.

Crystallization; Serine; Aminoacyl-tRNA synthetase; tRNA; X-ray structure

1. INTRODUCTION

A principal goal of structural molecular biology is the elucidation of the nature of the specific interactions between biological macromolecules at the atomic level. The complexes formed by aminoacyl-tRNA synthetases and their cognate tRNAs are systems for both studying the interaction between RNA and protein and for understanding important recognition steps in maintaining fidelity in protein biosynthesis. An obvious means to study these interactions is high resolution X-ray diffraction analysis of suitable crystals. By this approach the interactions in the aminoacyl-tRNA synthetase:tRNA complexes for glutamine (from *Escherichia coli*) and aspartic acid (from yeast) have been described [1,2]. Recently the crystallization of similar complexes for the aspartic acid system from *E. coli* [3] and the serine system from *Thermus thermophilus* [4,5] have been reported.

We have previously determined the structure of the *E. coli* seryl-tRNA synthetase to high resolution [6]. A remarkable feature of this structure is a 60 Å helical arm-like domain that might be important for the recognition of *E. coli* serine isoacceptor tRNAs, which are characterised by the possession of long extra arms. Included in this group of tRNAs is the product of the *selC* gene, tRNA^{sec}, which is responsible for the incorporation of selenocysteine into proteins [7]. In the preceding report [8] we describe the cloning, overexpression and isolation of two tRNA^{ser}s and here we describe our studies on the crystallization of *E. coli* seryl-tRNA synthetase:tRNA complexes.

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Abbreviations: BAPPS, *N,N'*-bis(3-aminopropyl)-piperazine substituted Sepharose; PMSF, phenylmethyl sulphonyl fluoride; BD-cellulose, benzoylated DEAE-cellulose; MES, 4-morpholine-ethanesulphonic acid.

2. MATERIALS AND METHODS

2.1. *Seryl-tRNA synthetase*

Seryl-tRNA synthetase (SRSEC) was isolated from an overexpressing strain of *E. coli* as previously described [9].

2.2. *tRNA$_i^{ser}$*

Total tRNA (MRE 600) from Boehringer was dissolved in the smallest possible volume of buffer A (64.4 mM Tris, 50 mM HCl, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mM PMSF, 1 mM NaN_3, pH 7.6 at room temperature). This solution was fractionated on a column of (2.5 × 90 cm) of AcA 44 (IBF) equilibrated in the same buffer. The fractions containing tRNA^{ser} as measured by a standard charging assay with [^{14}C]serine [8], were pooled and precipitated with two volumes of ethanol, washed with 70% ethanol and dried; this treatment is mainly to remove pigment and any high or low molecular weight contaminents of the tRNA fraction. Based on the acceptance activity for serine a sample of the tRNA was dissolved in a solution of SRSEC in buffer A containing 50 mM NaCl such that the molar ratio of enzyme to tRNA^{ser} was about 2:1. This mixture was then fractionated by gel filtration on AcA 44 equilibrated in buffer A. The first peak of the chromatogram contained the enzyme and complexed tRNA^{ser}. Fractions from this peak were pooled and applied to a small column of DEAE- Sepharose CL-6B (Pharmacia) equilibrated in buffer A. This column was washed with start buffer and developed with a linear gradient of 0–0.5 M NaCl in buffer A. Separate peaks of synthetase (peak 1) and tRNA (peak 2) were obtained. The pool of tRNA fractions was then separated into isoacceptors by hydrophobic interaction chromatography on phenyl-Superose (Pharmacia) as described [8]. The tRNA of the major peak for serine acceptance was used in the crystallization trials and was asssumed to be tRNA$_1^{ser}$.

2.3. *$tRNA_2^{ser}$*

This was isolated as described in the accompanying report [8].

2.4. *$tRNA^{sec}$*

This tRNA was isolated from *E coli* strain FM420 harbouring the plasmid pMN81 as described [7] except that the first chromatographic separation was performed with BAPPS [10] instead of BD-cellulose.

2.5. *Crystallization*

Crystallization trials by the hanging drop/vapour diffusion procedure were performed on mixtures of seryl-tRNA synthetase with $tRNA^{ser}$ isolated from commercial total tRNA presumed to be $tRNA_1^{ser}$ [11], $tRNA^{sec}$, two fractions of $tRNA_2^{ser}$ and the amber supressor tRNA described in the proceeding report [8]. The trials were carried out with ammonium sulphate, sodium citrate and polyethylene glycol solutions at various pH values between 5.8–8.0 with MES, Tris, Tris-maleate and imidazole buffers.

2.6. *Crystallography*

Diffraction data were collected on a laboratory rotating anode source with graphite monochromator and a FAST detector (CNRS, Grenoble) or double-focussing mirrors and Mar-Research image-plate system (EMBL, Grenoble). Other data were collected on synchrotron beamline W32 at LURE [12] using a Mar-Research image-plate detector and a wavelength of 0.901 Å. Raw data were processed either using MADNES and the Kabsch profile fitting programmes PROCOR (FAST data) or the MOSFLM package for image-plate data [13] Subsequent data analysis was perfomed with the CCP4 program package (Daresbury Laboratory, England)

3. RESULTS

3.1. *Crystallization*

Crystals of the enzyme:tRNA complex were only obtained when the tRNA component was $tRNA_2^{ser}$. These crystals generally appeared in about 6 days in drops containing 5 mg SRSEC/ml and 3.9 mg tRNA/ml (stoichiometric ratio protein:tRNA = 1:2.4). 10 mM $MgCl_2$, 1 mM DTT, 0.1 mM PMSF, 1 mM NaN3 in 50 mM MES-NaOH buffer pH 6.5 equilibrated against 45–50% saturated ammonium sulphate solution in the same buffer. Other trials using other buffering agents but at the same pH gave similar results.

3.2. *Crystallography*

The crystal symmetry and Bravais lattice of the crystals was determined to be C-centred orthorhombic by means of the MADNES auto-indexing routine of the FAST system and confirmed by subsequent data analysis. The cell dimensions are a = 128.9 Å, b = 164.9 Å, c = 127.3 Å. The exact space-group is $C222_1$ (rather than C222) as determined during the molecular replacement procedure (see below). The crystals are rhombohedral prisms with the c-axis parallel to the long axis of the prism. They diffract anisotropically to about 3.5–4 Å in the directions a and c and only about 4.0–4.5 Å in the b direction. A striking feature of the diffraction pattern is the extremely intense diffuse scattering. The implied disorder may be correlated to the relatively poor diffraction quality.

A dataset has been collected to 4 Å resolution using 4 crystals on the wiggler beamline W32 at LURE with a wavelength of 0.901 Å, a crystal-image plate distance of 379 mm and a temperature of 2°C. From 24197 measured reflections 9824 unique reflections were obtained (average redundancy of 2.5) with an R_{merge} of 8.8% (24.8% in the highest resolution bin) and completeness of 83%. Since many of the most intense spots were absent from this data collection due to detector saturation, low resolution data was added from additional measurements on a laboratory source. The final dataset contained 10,550 independent reflections (89% complete to 4 Å resolution). Data were also collected on the laboratory source with an image-plate detector on a methylmercury acetate derivative obtained by co-crystallization. Using two crystals 3328 independent reflections were measured to 6 Å resolution (R_{merge} = 6%, 92% complete).

The refined 2.5 Å structure of seryl-tRNA synthetase from *E. coli* [6] was used as a search model for molecular replacement. Since the helical arms of the molecule are known to have a variable orientation they were excluded from the model. However, a straightforward cross rotation function did not yield a significant solution. Therefore a different stategy was employed.

The unit cell volume suggest that the asymmetric unit of the crystal contains one synthetase monomer and one tRNA molecule (corresponding to a V_m of 4.3 $Å^3$/Da). This implies that the molecular 2-fold axis is aligned with a crystallographic 2-fold axis. The problem then reduces to a simple rotation search about the possible 2-fold axes followed by a translation search along the axis. This procedure was performed with the programme BRUTE [14] for both possible space-groups. Only space-group $C222_1$ gave a significant solution (correlation of 0.50, for 5423 reflections betweeen 5 and 15 Å) which also gave a reasonable unit-cell packing. Program SIGMAA [15] was then used to calculate an electron density map from the model at 6 Å resolution. This showed clear density for the helical arm of the synthetase (which had been ommitted from the search model), but in a significantly different orientation to that found in the native structure. In addition, extra density was also seen into which one $tRNA_2^{ser}$ molecule (using a model built by E. Westhof) could be unambiguously placed. Upon re-adding the helical arm in the new orientation to the synthetase model, a rigid-body refinement using XPLOR [16] improved the correlation to 0.58 for the resolution range 5–15 Å.

The electron density map was improved by the following procedure. Phases from the initial model for the protein were used to calculate a difference map with the data from the methyl-mercury acetate derivative. This clearly revealed two mercury sites which corresponded to the positions at Cys-338 (13σ) and Cys-374 (6.5σ) as previously found for the ethyl-mercury chloride derivative of the native synthetase [6]. A anomalous difference map also showed the same sites. After phase refinement of the heavy-atom parameters, phases were calculated

using the mercury derivative isomorphous differences (mean figure of merit 0.40 for 2757 relections phased to 6 Å resolution). These were combined (using SIGMAA) with model phases, calculated to 4 Å resolution from the complete synthetase structure including the arm in its new orientation, giving a mean figure of merit of 0.49 for 10,648 reflections to 4 Å. From a new map at 4 Å resolution an envelope was calculated using the automatic procedure of Leslie [17]. An envelope of the atomic model, including a complete synthetase monomer and the Westhof $tRNA^{ser}$ model placed in the density, was also calculated. The union of the two envelopes (solvent content 57%, estimated solvent content in crystals 75%) was used in the solvent flattening procedure [18] and a final map calculated at 4 Å resolution. After the first cycle of solvent flattening the mean figure of merit was 0.70 for 10,648 reflections to 4 Å and converged to 0.89 after 8 cycles, although this value is clearly unrealistic. In the final map (see Fig. 1), in which no tRNA model has been used to derive phase information (except very indirectly via the envelope procedure), the almost complete backbone trace of the tRNA is clearly visible (Fig. 1). However, the present resolution is not sufficiently good to build an accurate model. Nevertheless the considerable deviations in the $tRNA_2^{ser}$ structure from the initial $tRNA_2^{ser}$ model obtained from E. Westhof (IBMC) are clearly visible.

4. DISCUSSION

The correctness of the structure determination is shown firstly by the location of the mercury sites at the same positions as found for the native synthetase and secondly by the clearly recognisable electron density for the tRNA molecule. At this stage, the following observations can be made on the structure of the complex.

(a) The asymmetric unit of the crystal contains one synthetase monomer and one tRNA molecule. The synthetase dimer carrying two tRNA molecules has exact 2-fold symmetry. The tRNA binds across the dimer, i.e. the 3′ end, enters the active site of one subunit, whereas the bulk of the tRNA is on the other subunit. The direction of entry of the tRNA into the active site conforms with that observed for the aspartyl-system, the other class 2 synthetase complex whose structure is known [2].

(b) The anticodon stem and loop are not in contact with the protein. This is consistent with several results showing that the anticodon is not important for synthetase recognition in the seryl-system [18]. Indeed in the crystals, two anticodon loops from two tRNA molecules related by a crystallographic 2-fold axis are in contact. A strong possibility (consistent with the low resolution electron density) is that the first two bases of the anticodon CGA form base-pairs with the self-com-

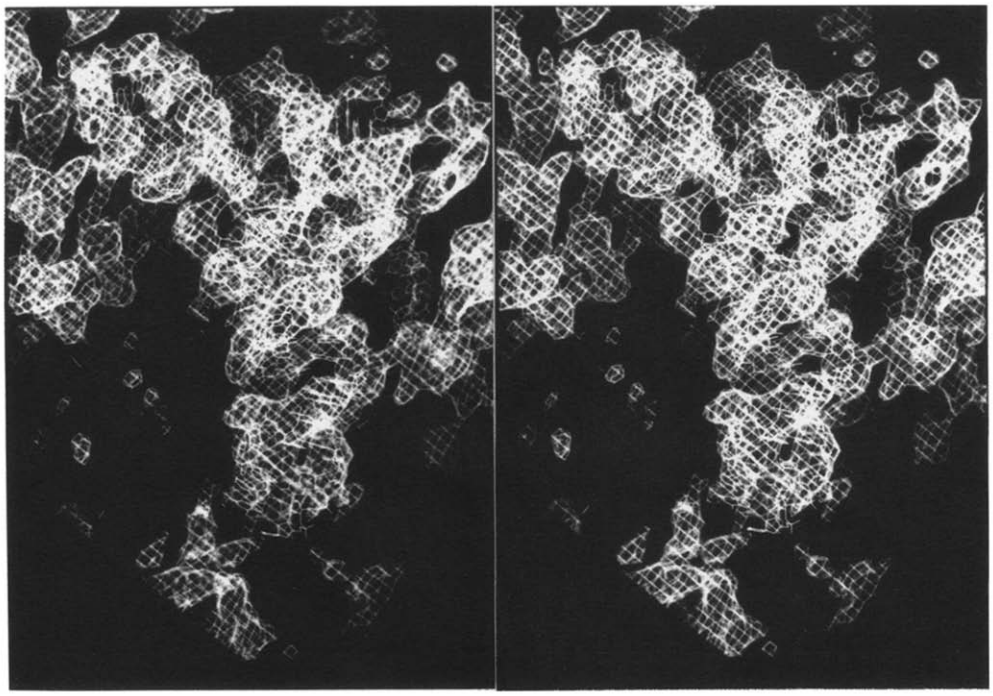

Fig. 1. Stereo picture of the final 4 Å resolution electron density map contoured at 1.2σ after phase combination and solvent flattening as described in the text. The L-shaped $tRNA^{ser}$ molecule is clearly visible as defined by the helical electron density of the backbone, with the acceptor stem entering the enzyme active site at top left and the anticodon loop at the bottom. The helical arm of the synthetase is seen at the top right of the figure. A model of the tRNA molecule is superimposed on the density.

plimentary bases of the second tRNA molecule. This hypothesis could explain the observation that the suppressor $tRNA^{ser}$ which is identical to $tRNA_2^{ser}$ except for having the anticodon CUA (in which the first two bases are not self-complimentary) has not been crystallized.

(c) The helical arm of the synthetase is inserted between, and makes contacts with, the TΨC loop and the long variable arm of the tRNA.

The model of the *E. coli* seryl-tRNA synthetase-tRNA complex outlined above is very similar to that found independently from a study at 2.9 Å resolution of crystals of the complex between seryl-tRNA synthetase and $tRNA^{ser}$ from *Thermus thermophilus* (Biou et al., manuscript in preparation: Yaremchuk et al. [5]).

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