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Crystal structure analyses of yeast tyrosyl-tRNA synthetase

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Introduction

Aminoacyl-tRNA synthetases (aaRSs) play a central role in the assembly of amino acids into polypeptide chains. By selection of the correct amino acid for acylation of a specific tRNA, they provide the key for translation of the genetic code. The 20 aaRSs are divided into two classes of 10 enzymes each. Tyrosyl-tRNA synthetase (TyrRS) is a class I enzyme, but is unusual in that it is a functional dimer, a feature only shared with tryptophanyl-tRNA synthetase amongst class I aaRSs. Since there are differences of the tRNA-recognition modes by TyrRSs between the eukaryotic/archaeal and prokaryotic systems, three-dimensional structures of archaeal, eukaryotic, or prokaryotic TyrRSs complexed with their cognate tRNA have long been awaited.

Recently, crystal structure analyses of prokaryotic and archaeal TyrRSs complexed with their cognate $tRNA^{Tyr}$ have been reported. On the other hand, crystal structure of eukaryotic (human) TyrRS lacking the C-terminal domain has been reported, however, no structures are available for eukaryotic TyrRS complexed with its cognate $tRNA^{Tyr}$.

To understand the structure-function relationships and the tRNA-recognition mode of eukaryotic TyrRS, we initiated the crystal structure analysis of yeast TyrRS (yTyrRS). Here we report the preliminary X-ray crystallographic studies of recombinant yTyrRS complexed with its cognate $tRNA^{Tyr}$.

Experimental

Crystallization

The expression and purification of recombinant yTyrRS were performed as described [1]. The purification of $tRNA^{Tyr}$ was performed in a way similar to the method as described [2]. A tyrosyl-adenylate (Tyr-AMP) analog was prepared as described [3].

Crystallization was carried out at 293 K by the hanging-drop vapor diffusion method. In the best case, a droplet was prepared by mixing an equal volume of the ternary complex solution [10 mg/ml yTyrRS (ca. 0.2 mM), 5 mM Tyr-AMP analog, 0.2 mM $tRNA^{Tyr}$, and 40 mM KCl in 20 mM Tris buffer pH 7.5] and a reservoir solution containing 25 % (v/v) polyethylene glycol 400 (PEG400) and 100 mM $CaCl_2$ in 100 mM Tris buffer at pH 7.5. Crystals with typical dimensions of about 0.2 x 0.2 x 1.0 mm^3 could be grown in 2 weeks [4].

X-ray data collection

The data collection was performed by rotation method at 100 K using an ADSC Quantum4R CCD detector with synchrotron radiation (λ = 1.00 Å at beamline 6A of the Photon Factory). The Laue group was found to be 4/*mmm* and the unit-cell dimensions were $a = b = 63.85$ Å, $c = 330.3$ Å, and $\alpha = \beta = \gamma = 90°$. Only reflections with h =2n, k = 2n, and l = 4n were observed along the (h 0 0), (0 k 0), and (0 0 l) axes, respectively, indicating a tetragonal space group $P4_{1(3)}2_12$. An assumption of one molecule each of yTyrRS and $tRNA^{Tyr}$ (one-half of a 2:2 complex) per asymmetric unit leads to an empirically acceptable V_M value of 2.55 $Å^3$/Da, corresponding to a solvent content of 52 %. The current best diffraction data from a native crystal were collected up to 2.5 Å.

Results and Discussion

Initially, we tried to solve the structure of yTyrRS by molecular replacement techniques. The structures of several TyrRSs complexed with or without their cognate $tRNA^{Tyr}$s deposited in the Protein Data Bank, having ca. 10-30 % sequence identity with yTyrRS, were used as a search model. Since these attempts failed, we prepared a Se-Met substituted yTyrRS using LeMaster medium and *E. coli* B834(DE3). Phase determination by the multi-wavelength anomalous diffraction method is in progress.

References

[1] S. Ohno et al., J. Biochem. 130, 417 (2001).
[2] T. Ohyama et al., J. Biochem. 97, 29 (1985).
[3] T. Moriguchi et al., J. Org. Chem. 65, 8229 (2000).
[4] Y. Kusakabe et al., in preparation.

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