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X-RAY CRYSTALLOGRAPHIC STUDIES OF HUMAN TRYPTOPHANYL-TRNA SYNTHETASE

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Introduction

Aminoacyl-tRNA synthetases (aaRSs) are a family of ancient enzymes that catalyze amino acid activation by ATP and the subsequent aminoacylation to its cognate tRNA in the first step of protein synthesis. The crucial role of aminoacyl-tRNA synthetases in maintaining the fidelity of the genetic code has motivated extensive study of the molecular basis of the specificity for cognate amino acid and tRNA. So far, most of the known structures of aaRSs are from bacteria or yeast. In order to understand the mechanism of aminoacylation by mammalian aaRSs, we are pursuing the structural and functional studies of human tryptophanyl-tRNA synthetase (hTrpRS). We have solved the ligand-free hTrpRS structure [1], and recently determined a series of structures of hTrpRS in complex with tRNA, substrates and substrate analog, representing a full set of structural snapshots of enzyme in the multi-step aminoacylation reaction. Comparison and analysis of these structures have provided insights into the catalytic mechanism of the aminoacylation reaction and the recognition mode of its substrates specificity.

Method and Results

Expression and purification of the wild-type hTrpRS were carried out as described previously [1]. Sparse-matrix crystallization screening with the Crystal Screen and Crystal Screen II kits (Hampton Research) was performed using the hanging-drop vapor diffusion method at 4°C. Crystals of hTrpRS in complex with ligands were mounted on a cryo-loop and flash-frozen in liquid nitrogen. Data collection was carried out using the ADSC CCD detector of BL6A or 5A at PF. Data processing and scaling were performed using the HKL2000 suite.

Crystals of the hTrpRS-Trp and hTrpRS-analog-ATP complexes both belong to tetragonal space group $P4_32_12$, with unit cell dimensions of a = b = 79.9Å and c = 382.3 Å, and a = b = 79.7Å and c = 383.2 Å, respectively. The refinement for hTrpRS-Trp (50-2.4 Å) has converged to final R-factor of 20.7%, with R-free value of 24.9%. The final R factor and R-free factor for hTrpRS-analog-ATP complex were reduced to 21.1% and 23.9, respectively.

Comparison of the crystal structures between the ligand-free and the substrate-complex forms has revealed that the extensive induced-fit conformational changes of domains and the local conformational changes within the substrates binding pocket occur around the active site upon substrate binding. The paper will be published in the near future.

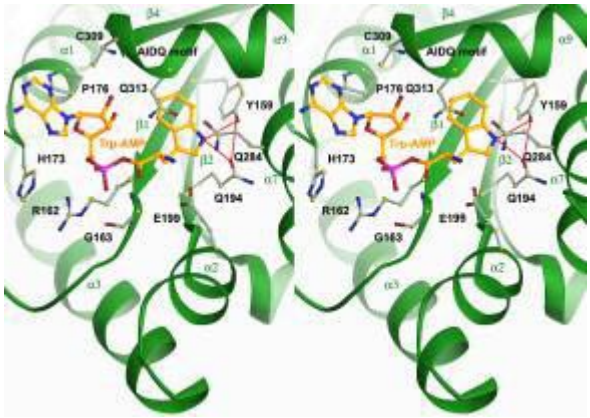

Fig. 1. Structure of the catalytic active site of hTrpRS

References

[1] Yu, Y. *et al* (2004). Crystal structure of human tryptophanyl-tRNA synthetase catalytic fragment. ***J. Biol. Chem.*** 279, 8378-8388.

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