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Structure of a complex of *Pyrococcus horikoshii* arginyl-tRNA synthetase and $tRNA^{Arg}$ with large variation of codon usage for different species

Michiko Konno*[1], Tomomi SUMIDA[1], Yukie Mori[1] & Ryouko Takasaka[1]
[1]Ochanomizu University, Department of Chemistry, 2-1-1 Otsuka, Bunkyo-ku,Tokyo 112-8610 Japan

Introduction

Aminoacyl-tRNA synthetases catalyze aminoacylation reaction from amino acid, tRNA and ATP to aminoacyl-tRNA and AMP in the presence of Mg^{2+} ion. Comparison of codon usage among archaebacteria *Pyrococcus horikoshii* (Ph), prokaryote *E. coli* (Ec), *Thermus thermophilus* (Tt) and eukaryote yeast reveals that codons of Arg, Ile and Gly have large variation for these species. The main codon usages of Arg are CGX (X stands for U/C/A/G) for Ec, CGG/AGG for Tt and CGX/AGA/AGG for yeast, while AGA/AGG for Ph are 98 %. We have determined a crystal structure of a complex of Ph arginyl-tRNA synthetase (ArgRS) and $tRNA^{Arg}$ (CCU) and compared this structure with crystal structures of yeast ArgRS and Tt ArgRS and in order to clarify common binding region of three bases of anticodon compared with MetRS, IleRS and ValRS of class Ia, the anticodon binding domains of which have the same helix-bundle structure as that of ArgRS.

Experiments and Results

Experiments

E. coli BL21(DE3) was transformed with a plasmid of pET28c/*ArgRS* constructed from genome of Ph. The crude protein was purified on Ni-NTA superflow column and with an AKTA purifier FPLC system through columns Resource Q HitrapHeparin and hydroxyapatite. The template DNA for tRNA was prepared in a large scale with PCR by using the plasmid pUC119/ $tRNA^{Arg}$(CCU). *In vitro* transcription of the template DNA was carried out with T7 RNA polymerase and the crude transcript was purified. Crystals of a complex of Ph ArgRS and $tRNA^{Arg}$ (CCU) and a complex containing AMP-PNP were obtained by hanging drop vapor diffusion method. Intensity data were collected at 100 K on NW12 beam line and processed by HKL2000 and SCALEPACK. The structure was solved by molecular replacement method with MOLREP by using Tt ArgRS (PDB:1IQ0) as a search model. The structure of tRNA (ICG) binding yeast ArgRS (PDB:1F7V) was used as the model. Refinements of this structure gave R factor of ca. 40%. The low electron density maps in N-terminal domain and tRNA revealed large deviation of this domain of Ph ArgRS from Tt and yeast ArgRSs and large confor mational change of Ph tRNA from yeast tRNA. The repeats of the model building and refinements using O and CNS gave finally R_{factor} = 0.215 (R_{free} = 0.259) at 2.0 Å resolution for the ternary complex and R_{factor} = 0.206 (R_{free} = 0.266) at 2.3 Å for the binary complex.

Result and Discussion

The ribbon model of a complex of Ph ArgRS and tRNA is shown in Fig. 1. The biochemical experiments of mutants of MetRS and its crystal structure [1] reveal that three bases of the anticodon bind on the surface of helix I and helix III and the loop connecting helix III-IV (III-IV loop). It was reported that the first base of anticodon interacts with III-IV loop from the mutant experiments of its loop of IleRS and MetRS. The crystal structures of each cognate tRNA binding on MetRS from *Auifex aeolicusy* [2], IleRS from *S. aureus* [3] and Tt ValRS [4], respectively reveal that the base of the second anticodon Ade35 of three tRNAs resides in close proximity of conserved Asn on helix I. On the other hands, in the complex of Ph ArgRS and tRNA, the base of the second anticodon C35 form two hydrogen bonds with CO and NH of main chains of III-IV loop and overlaps in parallel to the aromatic ring of Tyr587 on helix III (Fig. 2). Three bases of anticodon for tRNA is far away from Tyr509 conserved among all investigated ArgRSs, which locates the position corresponding to that of conserved Asn on helix I of MetRS, IleRS and ValRS. ArgRS has additional N-terminal domain and the base of Ade20 of D loop of tRNA interacts with Tyr85 and Asn87 in N-terminal domain (Fig. 1). In the crystals, this interaction may deviate tRNA from the initial binding location.

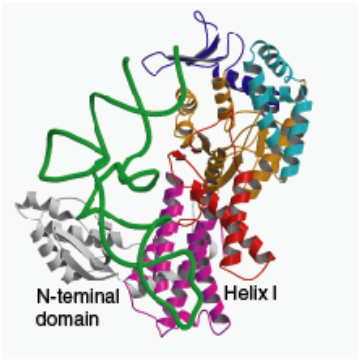

Fig.1 Ribbon model of Ph ArgRS and tRNA

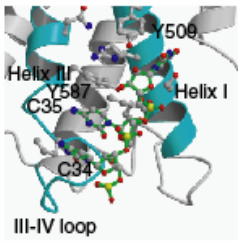

Fig.2 anticodon binding domain and tRNA

References

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* konno@cc.ocha.ac.jp