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Crystal structural analysis of the complex of tRNA^{Asp} mutant and Arginyl-tRNA synthetase from *Pyrococcus horikoshii*

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Introduction

The aminoacyl-tRNA synthetases exhibit *in vitro* fidelity of one error per 10^4 to 10^5 codons. Surprisingly, it was reported [1] that *Saccharomyces cerevisiae* arginyl-tRNA synthetase (ArgRS) *in vitro* arginylates a non-cognate tRNA^{Asp}(**GUC**) transcript with about 10 fold less efficiency than a cognate tRNA^{Arg}(**UCU**) transcript and further that the k_{cat}/K_m value of tRNA^{Asp} **U**34 mutant is the same as that of tRNA^{Arg}. These facts reveal that in the anticodon-binding domain of ArgRS, the base **U**34 of tRNA^{Asp}(**UUC**) mutant binds at the site accepting **U**34 of tRNA^{Arg}(**UCU**). The fact that tRNA^{Met}(**CAU**) is also arginylated by *E. coli* ArgRS [2] expects that the bases of the anticodon **C**34, **A**35, **U**36 interact with sites accepting **C**34, **C**35 and **G**36 bases of tRNA^{Arg}, respectively. Taking these reports into consideration, the bases of **U**35 and **C**36 of tRNA^{Asp}(**UUC**) mutant also interact with sites accepting 35th and 36th bases, respectively. tRNA^{Asp}(**GUC**) is not common to the second base **C**35 and the third base **G**36/**U**36 of the anticodon of tRNA^{Arg} but this paper [1] proposed 'slipped' recognition set hypothesis that in *S. cerevisiae* tRNA^{Asp}(**GUC**), **C**36-G37 may be used in place of **C**35-**G**36 of tRNA^{Arg}. The relative orientation of **G**37 base for **C**36 base in *S. cerevisiae* tRNA^{Asp}(**GUC**) [3] is quite different from the relative orientation of **G**36/**U**36 base for **C**35 base in *S. cerevisiae* tRNA^{Arg}(**ICG**) [4] and *Pyrococcus horikoshii* tRNA^{Arg}(**CCU**) [5] (Figure 1). Therefore, the 'slipped' recognition set hypothesis is less possible. In order to prove that bases of the anticodons of tRNA^{Asp} mutants interact with sites accepting **C**34, **C**35, **U**36 bases of tRNA^{Arg}, we tried crystal structure analysis of the complex of *P. horikoshii* ArgRS and tRNA^{Asp} mutants.

Results

Experiment

The D loop of all *P. horikoshii* tRNA^{Asp} mutants was substituted by that (AGCCA^{17a}GGAC^{20a}A) of *P. horikoshii* tRNA^{Arg}(CCU) such that tRNA^{Asp} does not occur steric obstacle with ArgRS. The nucleotides of anticodon loop of tRNA^{Asp} are CU**GUC**AC, whereas those of tRNA^{Arg}(CCU) are CU**CCU**AA. The genes of tRNA^{Asp} mutants with the anticodon of **UUC**, **GUC**, **CCU** were cloned with the T7 promoter into the vector pUC119. The crude transcript was purified by Resource Q column. The crystallizations of complexes of ArgRS and tRNA^{Asp} mutants were tried at the same condition as that of the complex of tRNA^{Arg}. Crystals of the complex of tRNA^{Asp}(**CCU**) hybrid containing the tRNA^{Arg} body and the tRNA^{Arg} anticodon loop grew. The crystal was exposed at 100 K on beamline AR-NE3A at the Photon Factory. The reflections were observed at 4.5 Å resolution but not enough to analyze.

We also constructed the model of tRNA^{Asp}(**GUC**) and its mutants, **C**34-**C**35-**U**36, **U**34-**U**35-**C**36 on the basis of the structures of complexes of tRNA^{Arg}(**ICG**) and *S. cerevisiae* ArgRS [4] and of tRNA^{Arg}(**CCU**) and *P. horikoshii* ArgRS [5] (Figure 1). The anticodon stem and the D stem of tRNA^{Asp}(**GUC**) [3] were superimposed into those of *S. cerevisiae* tRNA^{Arg}(**ICG**) bound a ArgRS. Then the backbone of the anticodon loop of tRNA^{Arg}(**ICG**) is kept and the bases are replaced by those of tRNA^{Asp}(**GUC**). **U**35 and **C**36 are inserted into hydrophobic pockets accepting **C**35 and **G**/**U**36 in ArgRS, respectively.

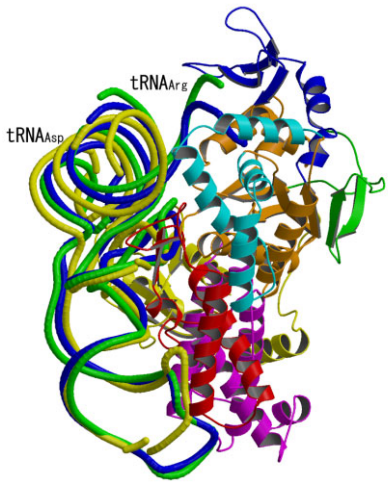

Figure 1. Model of tRNA^{Asp}(**GUC**) (thin) [3] and tRNA^{Arg} (**ICG**) (thick) [4] superimposed into tRNA^{Arg}(CCU) (thin) bound *P. horikoshii* ArgRS [5]

References

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