Crystal structural analysis of the complex of tRNA$^{\text{Asp}}$ 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$^{\text{Asp}}$(GUC) transcript with about 10 fold less efficiency than a cognate tRNA$^{\text{Asp}}$(UCU) transcript and that the $k_{\text{cat}}/K_m$ value of tRNA$^{\text{Asp}}$ U34 mutant is the same as that of tRNA$^{\text{Asp}}$. These facts reveal that in the anticodon-binding domain of ArgRS, the base U34 of tRNA$^{\text{Asp}}$(UUC) mutant binds at the site accepting U34 of tRNA$^{\text{Asp}}$(UCU). The fact that tRNA$^{\text{Asp}}$(CAU) is also arginylated by *E. coli* ArgRS [2] expects that the bases of tRNA$^{\text{Asp}}$ (thick) [3] and of tRNA$^{\text{Asp}}$(CCU) [4] (Figure 1). The anticodon stem and D stem of tRNA$^{\text{Asp}}$(GUC) [3] were superimposed into those of *S. cerevisiae* tRNA$^{\text{Asp}}$(CCU) bound a ArgRS. Then the backbone of the anticodon loop of tRNA$^{\text{Asp}}$(ICG) is kept and the bases are replaced by those of tRNA$^{\text{Asp}}$(GUC). U35 and C36 are inserted into hydrophobic pockets accepting C35 and G/U36 in ArgRS, respectively.

We also constructed the model of tRNA$^{\text{Asp}}$(GUC) and its mutants, C34-C35-U36, U34-U35-C36 on the basis of the structures of complexes of tRNA$^{\text{Asp}}$(ICG) and *S. cerevisiae* ArgRS [4] and of tRNA$^{\text{Asp}}$(CCU) and *P. horikoshii* ArgRS [5] (Figure 1). The anticodon stem and D stem of tRNA$^{\text{Asp}}$(GUC) [3] were superimposed into those of *S. cerevisiae* tRNA$^{\text{Asp}}$(ICG) bound a ArgRS. Then the backbone of the anticodon loop of tRNA$^{\text{Asp}}$(ICG) is kept and the bases are replaced by those of tRNA$^{\text{Asp}}$(GUC). U35 and C36 are inserted into hydrophobic pockets accepting C35 and G/U36 in ArgRS, respectively.

**Results**

*Experiment*

The D loop of all *P. horikoshii* tRNA$^{\text{Asp}}$ mutants was substituted by that (AGCCAAGGAC$^\text{Asw}$) of *P. horikoshii* tRNA$^{\text{Asw}}$(CCU) such that tRNA$^{\text{Asw}}$ does not occur steric obstacle with ArgRS. The nucleotides of anticodon loop of tRNA$^{\text{Asw}}$ are CUGUCAC, whereas those of tRNA$^{\text{Asw}}$(CCU) are CUCCUAA. The genes of tRNA$^{\text{Asw}}$ 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$^{\text{Asw}}$ mutants were tried at the same condition as that of the complex of tRNA$^{\text{Asw}}$. Crystals of the complex of tRNA$^{\text{Asw}}$(CCU) hybrid containing the tRNA$^{\text{Asw}}$ body and the tRNA$^{\text{Asw}}$ 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.

*References*


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