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Structure of free tRNA^{Gly}(CCC) from *Pyrococcus horikoshii*

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1 Introduction

Gly-tRNA synthetase (GlyRS) belongs to Class IIa as the same as Pro-tRNA synthetase, Thr-tRNA synthetase and His-tRNA synthetase (ProRS, ThrRS and HisRS) which have the anticodon binding domain consisting of mixed α/β domain in the C-terminal. Three bases XGU, XGG and XCC (X stands for A, G, U or C) of the anticodon of tRNA^{Thr}, tRNA^{Pro} and tRNA^{Gly}, respectively are anticipated to be bound on three corresponding regions of the anticodon binding domain with the same topology. However, in the structure of "tRNA^{Thr}(CGU) bound" ThrRS [1], the bases of G35 and U36 arrange at neighborhood in parallel to each other with hydrogen bond, whereas in the structure of the "tRNA^{Pro}(CGG) bound" ProRS [2] in which the 3'-CCA end is far away from the active site in ProRS, G35 locates the similar position with G35 of the former but G36 is at the different position from U36. In order to clarify the three binding regions of the anticodon bases of tRNA^{Gly}, we determined the crystal structure of free tRNA^{Gly}(CCC) from *Pyrococcus horikoshii* at 3.0 Å resolution and constructed the model of tRNA^{Gly} bound GlyRS from *Thermus thermophilus* [3]. We discuss that the conformation change of tRNA^{Gly} occurs in the process of bound GlyRS on the basis of comparison of structures between free tRNA and tRNA bound aaRS.

2 Experiment

Crystallization was carried at 20°C by vapour diffusion in sitting drops. The droplet produced by merging 0.7 μL of 10mg/mL GlyRS and tRNA^{Gly} with molar ratio of 1:1 and 0.7 μL of the reservoir solution was equilibrated against the reservoir solution containing 100 mM imidazole pH 8.0, 200 mM $Ca(OAc)_2$, 10% PEG8000. The intensity data of needle-like shaped crystals were collected in Photon Factory beam-line 5A and Spring-8 BL32XU, and dataset processed using HKL2000 suite gave R merge of 7.4 % at 3.0 Å. The lattice unit size revealed that crystal is not complex but free tRNA^{Gly}. The crystal is tetragonal system and belongs to space group $I4_122$. The lattice constants are a=b=162.8 Å, c=49.91 Å and Z=16. The reasonable solution was obtained using Ec tRNA^{Asp} (PDB code 1C0A) as search model by molecular replacement method (PHASER of CCP4). The repeats of model buildings and refinements using COOT program and the REFMAC5 program gave R_{factor} of 0.25 (R_{free} of 0.28).

3 Results and Discussion

The stick model of Ph tRNA^{Gly} is shown in Fig.1. The 3' single-stranded ACCA end has the base stacking on the next base and forms a regular helical conformation. The regular helical structure is also observed in the ACCA end of free Sc tRNA^{Asp}(GUC) from *Saccaromyces serevisiae* [4]. Comparison of structures of free tRNA^{Gly}, free Sc tRNA^{Asp}(GUC) and free Sc tRNA^{Phe}(GAA) [5] with structures of tRNA^{Asp} bound AspRS [6] and tRNA^{Thr} bound ThrRS indicates that the L shape of free tRNA has open conformation (the angle between helical axis of the acceptor stem and the anticodon stem is larger) whereas that of tRNA bound aaRS has close conformation. In the anticodon loop of Ph tRNA^{Gly}(CCC), the bases of C35C36A37A38 have stacking form but the base of C34 deviates and the base of U33 directs outside. On the other hands, in free tRNA^{Asp} and free tRNA^{Phe}, the 34-38th bases have stacking form. The bend is observed between the 33th and 34th backbone in all free tRNAs. The docking model of Ph tRNA^{Gly}(CCC) on Th GlyRS shows the 3'CCA terminal and the anticodon bases are apart away from the active site and the binding region of GlyRS, respectively if tRNA does not change conformation from open to close form. The attaching of the inside of tRNA^{Gly} to GlyRS maybe induces the conformation change of tRNA.

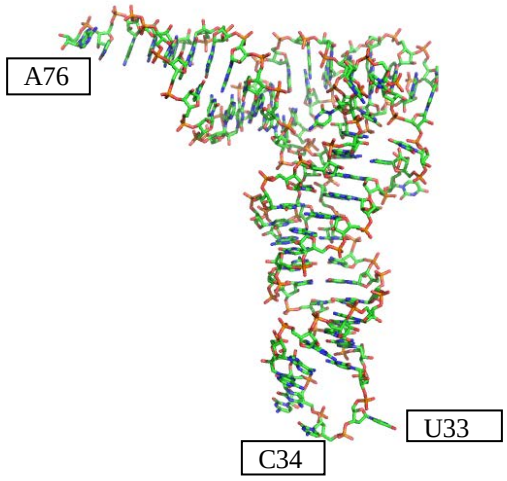

Fig. 1: Model of Ph tRNA^{Gly}

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