NW12A, 5A, 17A, NE3A/2009G670

X-Ray structure analysis of *Aeropyrum pernix* threonyl-tRNA synthetase lacking a *cis*-editing domain

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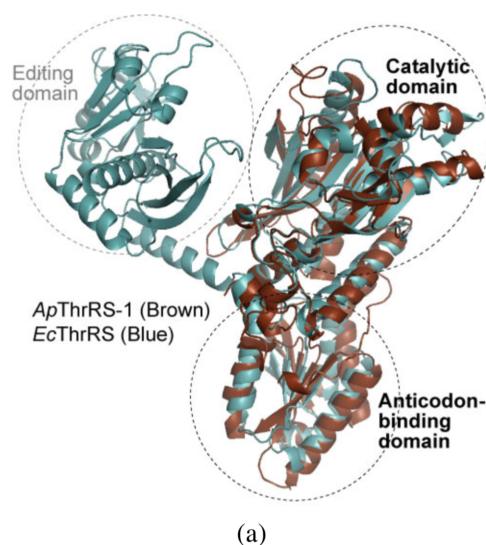
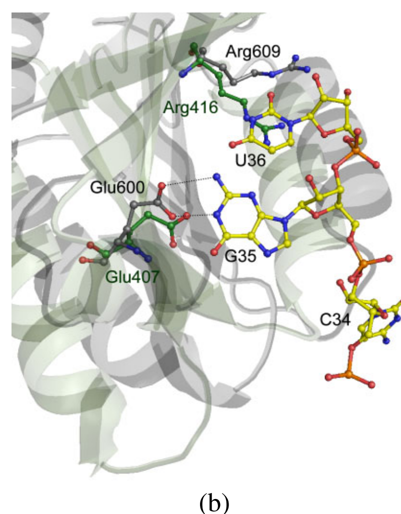
In protein synthesis, threonyl-tRNA synthetase (ThrRS) must recognize threonine (Thr) from the twenty kinds of amino acids and the cognate $tRNA^{Thr}$ from different tRNAs in order to generate Thr-$tRNA^{Thr}$. In general, an organism possesses one kind of gene corresponding to ThrRS. However, it has been recently found that some organisms have two different genes for ThrRS in the genome, suggesting that their proteins ThrRS-1 and ThrRS-2 function separately and complement each other in the threonylation of $tRNA^{Thr}$; one for catalysis and the other for *trans*-editing of misacylated Ser-$tRNA^{Thr}$. In order to clarify their three-dimensional structures, X-ray analyses of two putatively assigned ThrRSs from *Aeropyrum pernix* (*Ap*ThrRS-1 and *Ap*ThrRS-2) have been performed.

These proteins were overexpressed in *E. coli*, purified and crystallized. The crystal structure of *Ap*ThrRS-1 has been successfully determined at 2.3 Å resolution.

The two *Ap*ThrRS-1 molecules are related by the crystallographic two-fold symmetry and associate with each other to form a dimer. This is a characteristic feature of the members of class II ARSs, and is also the case in *Ec*ThrRS and *Sa*ThrRS. Superimposition of the *Ap*ThrRS-1 subunit onto that of *Ec*ThrRS is shown in Fig. 1(a). In *Ap*ThrRS-1, the N-terminal domain corresponds to the catalytic domains of *Ec*ThrRS. In addition, the C-terminal domains of *Ap*ThrRS-1 superimpose well onto the anticodon binding domains of *Ec*ThrRS. Therefore, it is apparent that the editing domains of *Ec*ThrRS are completely missing in *Ap*ThrRS-1. These structural features show that ThrRS-1 catalyzes only the aminoacylation of the cognate tRNA, suggesting the necessity of the second enzyme ThrRS-2 for *trans*-editing. In the anticodon binding domain, the interacting residues are well conserved in *Ap*ThrRS-1[see Fig. 1(b)]. Since the N-terminal sequence of *Ap*ThrRS-2 is similar to the sequence of the editing domain of ThrRS from *Pyrococcus abyssi*, *Ap*ThrRS-2 has been expected to catalyze deaminoacylation of a misacylated serine moiety at the CCA terminus.



(a)



(b)

Fig. 1. Superimposition (a) of the subunit structures between *Ap*ThrRS-1(brown) and *Ec*ThrRS (blue) and (b) of the anticodon binding domains of *Ap*ThrRS-1 (green) and *Ec*ThrRS (gray) complexed with $tRNA^{Thr}$ (yellow). The tRNA two bases, G35 and U36, interact with the conserved Glu and Arg residues.

[1] Satoru Shimizu, *et al.*, *Acta Crystallogr.*, **F64**, 903-910 (2008).

[2] Satoru Shimizu, *et al.*, *J. Mol. Biol.*, **394**, 286-296 (2009).