

CRYSTAL STRUCTURES OF LEUCYL-tRNA SYNTHETASE COMPLEXED WITH tRNA^{Leu}

Leucyl-tRNA synthetase (LeuRS) is one of the 20 enzymes that comprise the essential family of aminoacyl-tRNA synthetases (aaRSs). It is responsible for aminoacylating the aliphatic amino acid leucine to up to six different tRNA^{Leu} isoacceptors, each with a different anticodon. Although common aaRSs strictly recognize the anticodon for cognate tRNA selection, LeuRSs do not recognize the anticodon in tRNA^{Leu}, possibly because the anticodons are so divergent. Instead, LeuRS strictly recognizes the adenosine residue at position 73 (A73). This tRNA position is known as the “discriminator”, and is recognized by many aaRSs. In addition, the archaeal LeuRSs and most of the eukaryal LeuRSs recognize the long variable arm of tRNA^{Leu}, which is characteristic to tRNA^{Leu}. But how LeuRS recognizes the discriminator A73 and the long variable arm was unknown.

Another interesting feature of LeuRS is that it has a proofreading (editing) enzymatic activity. The aminoacylation domain mis-recognizes the near cognate amino acids, such as isoleucine and methionine, and mischarges them to the 3'-end of tRNA^{Leu}. If these misformed Ile-tRNA^{Leu} and Met-tRNA^{Leu} were delivered to the ribosome and used in protein synthesis, mutant proteins are produced.

To prevent such mutant protein production, the LeuRS “editing domain” hydrolyzes the mis-formed aminoacyl-tRNA and contributes to high-fidelity aminoacylation. The previously determined crystal structure of tRNA-free archaeal *Pyrococcus horikoshii* LeuRS revealed that aminoacylation and editing sites are separated by more than 35 Å [1]. How the aminoacylated 3'-end of tRNA is delivered from the aminoacylation to editing sites was unknown.

To elucidate these mechanisms, we determined the crystal structure of *P. horikoshii* LeuRS in complex with tRNA^{Leu} at 3.2 Å resolution, using the **BL26B1** and **BL41XU** beamlines (Fig. 1) [2]. The protruding C-terminal domain (light green in Fig. 1) of LeuRS reaches the tip of the long variable arm. Some residues at the C-terminal extremity recognize the tip bases of the long arm in a sequence specific manner. Thus, we clarified the recognition mechanism of the long variable arm.

The tRNA 3'-terminal adenosine is located in the aminoacylation site. Thus, our tRNA-complex structure represents the “aminoacylation complex”. The editing domain swings from its tRNA-free position to avoid clashing with tRNA, and thereby lets the CCA end bend and reach the aminoacylation active site.

In the asymmetric unit of the crystal, there are two

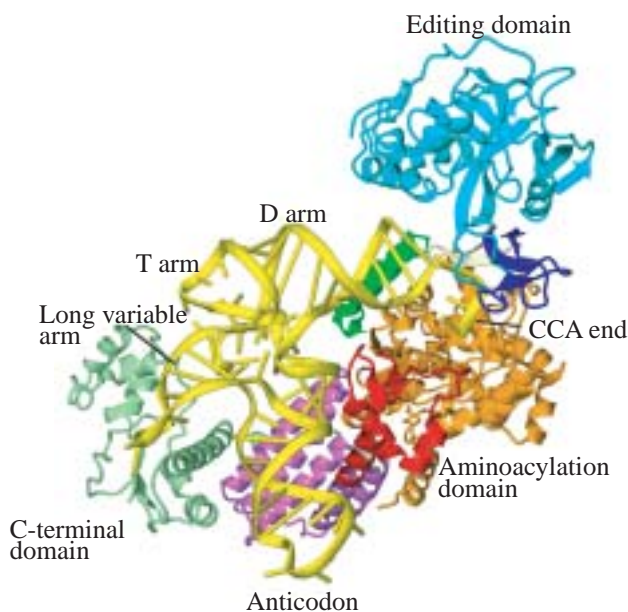


Fig. 1. Ribbon model of *P. horikoshii* LeuRS-tRNA^{Leu} complex structure. tRNA^{Leu} is shown in yellow. LeuRS is shown in seven colors according to the domains.



Fig. 2. Superposition of two LeuRS-tRNA^{Leu} complexes (complexes A and B) in asymmetric unit. Complex A is color-coded as in Fig. 1. tRNA and the editing domain of complex B are in pink. The other parts of the LeuRS structures are almost the same between the two complexes.

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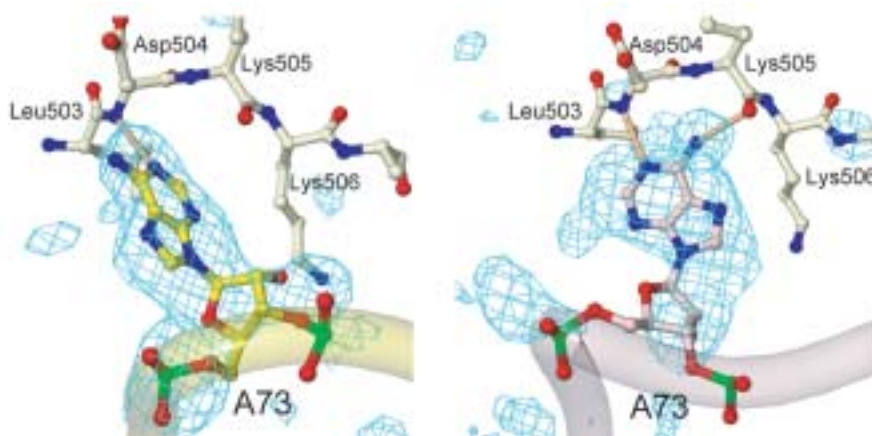


Fig. 3. Distinct conformations of A73. Omit electron density map for discriminator base A73 in complexes A (a) and B (b).

LeuRS-tRNA^{Leu} complexes. Between the two complexes, the tRNA 3'-region assumes distinct conformations (Fig. 2) that allow A73 to be specifically recognized, but via different mechanisms (Fig. 3). One conformation (in complex A) is the canonical "aminoacylation state" (shown in yellow in Figs. 2, 3, and 4), with the tRNA 3'-region bound deeply in the aminoacylation active site. In contrast, the other conformation (in complex B) appears to be a snapshot of the "intermediate state", in which the misaminoacylated 3'-end has partially relocated to the editing domain (shown in pink in Figs. 2, 3, and 4).

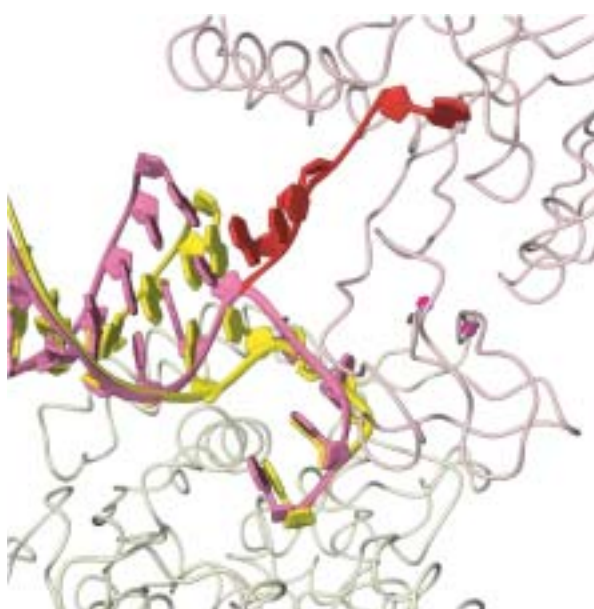


Fig. 4. Aminoacylation (complex A, yellow), intermediate (complex B, pink), and post-transfer editing (model, red) conformations. LeuRS in complex B is shown by a white line, with the editing domain in pink.

The "editing state" model, in which the 3'-end is located in the editing domain with the rest of the tRNA remains bound to the enzyme body, can be constructed on the basis of only the complex B structure, but not on the complex A structure. The discriminator base A73 is recognized via two different but switchable mechanisms (Fig. 3), indicating its important role in switching the tRNA conformation.

The structures suggest that after the aminoacylation reaction in the complex A "aminoacylation state", the conformation of the discriminator A73 is switched first. Then the tRNA 3'-end undergoes conformational change, and eventually becomes the complex B "intermediate state". After that, the 3'-end is released out of the aminoacylation site, and is bound to the editing site. The near-cognate amino acid is hydrolyzed, if present.

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