A new tRNA-assisted mechanism of post-transfer editing by aminoacyl-tRNA synthetases

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Statement of the Problem: Aminoacyl-tRNA synthetases (aaRSs) maintain fidelity during protein synthesis by attaching amino acids to their cognate tRNAs. For many aaRSs, the required level of amino-acid specificity is achieved either by specific hydrolysis of misactivated aminoacyl-adenylate intermediate (pre-transfer editing) or by hydrolysis of the mischarged aminoacyl-tRNA (post-transfer editing). Both reactions are depend on a tRNA cofactor and required translocation to the editing site located in the separate domain. In this work we have studied molecular mechanisms of editing by synthetases from two different classes: Thermus thermophilus leucyl-tRNA synthetase (LeuRSTT) from class I and Enterococcus faecalis prolyl-tRNA synthetase (ProRSEF) from class II.

Methodology & Theoretical Orientation: To investigate the mechanism of post-transfer editing of norvaline by LeuRSTT and alanine by ProRSEF, we used molecular modeling, molecular dynamic (MD) simulations, quantum mechanical (QM) calculations, site-directed mutagenesis of the enzymes and tRNA modification. The transition states of the reactions were identified.

Findings: The results support a new tRNA-assisted mechanism of hydrolysis of misacylated tRNA which directly involves two water molecules. The most important functional element of this catalytic mechanism is the 2' or 3'-OH group of the terminal adenosine 76 of aminoacyl-tRNA, which forms an intra-molecular hydrogen bond with the carbonyl group of the misacylated residue. Bonding increases the electrophilic character of the carbon atom and strongly facilitates the subsequent nucleophilic attack by water molecule.

Conclusion & Significance: Class I LeuRS and class II ProRS with a different architecture of editing site have both tRNA-assisted mechanism of post-transfer editing in which free 2' or 3'-OH group of the substrate plays a key role in hydrolysis by forming an intra-molecular hydrogen bond with the substrate amino-acid carbonyl group. Proposed editing mechanism is significantly different from those described in the literature for class-I and class-II aaRSs.

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Advances in recent enzymology

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Canonical enzymology has been carried out under the pre-requisite conditions of [S] >> [E]. However, advances in analytical instrumentation allow us to investigate enzymes systems with minute quantities of both enzymes and substrates, of very high-affinity reactions, of membrane-bound enzyme-substrate interactions, and hydrophobic environments.

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