Photoreactive tRNA Derivatives as a Tool for Investigation of Protein - Nucleic Acid Interactions

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Photoreactive $tRNA^{Phe}$ derivatives containing s^4U residues have been prepared by using T7 RNA polymerase transcription system. The substitution of 16 U residues with s^4U ($[16s^4U]$-$tRNA^{Phe}$) resulted in 14-fold reduction of catalytic efficiency of aminoacylation with T. thermophilus phenylalanyl-tRNA synthetase (PheRS). $[1s^4U]$-$tRNA^{Phe}$ was prepared by random incorporation of s^4U residues followed by affinity electrophoresis isolation. s^4U-monosubstituted tRNA molecules were aminoacylated with kinetic parameters similar to those of the wild-type $tRNA^{Phe}$ transcript. s^4U-containing $tRNA^{Phe}$ derivatives have been cross-linked to PheRS and the specificity of the labeling has been proved. The stoichiometry of labeling suggests thermophilic PheRS to be a functional dimer. Both α and β subinits of the $\alpha_2\beta_2$ enzyme were labeled, with the products of simultaneous coupling of both subunits to $[16s^4U]$-$tRNA^{Phe}$ being formed. The preferential labeling of α subunits indicates the enzyme to contact with tRNA at positions not resolved in the crystal structure of the complex [1]. $tRNA^{Phe}$-s^4U-75 has been prepared using tRNA nucleotidyl transferase. The nucleotide at the position 75 has been shown to form base specific contacts with β-subunit of Th. thermophilus PheRS. $tRNA^{Phe}$ sites contacting with the enzyme have been identified using various techniques. The D stem-loop, the T stem-loop and the variable loop of $tRNA^{Phe}$ are shown to interact with small (α) subunits of the enzyme. The acceptor end and anticodon loop are found to contact with large (β) subunits of the enzyme. The structural data gained using s^4U-monosubstituted tRNA transcripts are specially useful to get interrelation of tRNA•enzyme contacts and their functional significance since monosubstituted $tRNA^{Phe}$ analogs cross-linked to PheRS retain their aminoacylation ability [2]. Substrate competence of tRNA cross-linked to aminoacyl-tRNA synthetase has been shown for the first time.

1. Y. Goldgur, L. Mosyak, L. Reshetnikova, V. Ankilova, O. Lavrik, S. Khodyreva and M. Safro . (1997) Structure, 5: 59-68.

2. Moor N.A., Favre A., Lavrik O.I. (1998) FEBS Lett, 427: 1-4.

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