INTERACTION OF DEACYLATED tRNA WITH P SITE OF ESCHERICHIA COLI RIBOSOMES. ROLE OF MODIFIED NUCLEOTIDE IN CODON-ANTICODON INTERACTION

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The method of anticodon loop replacement [1] has been used to make derivatives of yeast $tRNA^{Phe}_{G_mAAY}$ with the substitution at the 37 position ($tRNA^{Phe}_{GAAA}$), at the anticodon ($tRNA^{Phe}_{GCAG}$) and 37 position. A quantitative study of the interaction of various types of

yeast deacylated tRNA: $tRNA^{Phe}_{G_mAAY}$, $tRNA^{Phe}_{GAAA}$, $tRNA^{Phe}_{GCAG}$, $tRNA^{Phe}_{-Y}$, with P site of the [70S ribosome * poly(U)]-complex was carried out at different Mg^{2+} concentrations and temperatures. The presence and nature of the nucleotide, situated at the 3'-end of the anticodon is essential for such interactions in E.coli ribosomes.

At the P site: replacement of the Y base with unmodified adenosine decreases the interaction enthalpy from 39 kcal/mole to 24 kcal/mole, whereas its removal reduces the interaction enthalpy to 16 kcal/mole. Replacement of the second anticodon nucleotide, adenosine, with cystosine further reduces the enthalpy to 6 kcal/mole. In the absence of poly(U) the affinity of ($tRNA^{Phe}_{-Y}$), for the P site of the 70S ribosome is 5 times lower than the affinity of $tRNA^{Phe}_{G_mAAY}$ and $tRNA^{Phe}_{GCAG}$ [2].

At the A site: removal of the Y base from the Phe-$tRNA^{Phe}$ anticodon loop decreases the association constant 10-fold. In contrast to the P site, the removal of the Y base does not lead to a substantial change of the interaction enthalpy (enthalpy decreases from 10 kcal/mole to 7 kcal/mole). The presence of the Y base in the anticodon loop of the P site bound deacylated $tRNA^{Phe}$ increases the $tRNA^{Phe}_{-Y}$, affinity to A site two times, whereas such effect has not been found for Phe-$tRNA^{Phe}_{G_mAAY}$.

Thus in a ribosome a modified nucleotide stabilizes codon-anticodon interaction due to interaction with a codon-anticodon base stack, although values of the stabilization differ for A and P site. In addition, this decreases free energy of binding as a result of interaction of a modified nucleotide itself with a hydrophobic center of P site on a ribosome.

REFERENCES

1. *Bruce A.G., Uhlenbeck O.* Biochemistry, 1980. V.21. P.3921–3926.
2. *Katunin V., Soboleva N. et al.* Biochimie, 1994. V.76. P.51–57.