Kinetics of tRNA ribosome complex formation

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The kinetics of binding of yeast $tRNA^{Phe}$ to the P or A sites of poly(U) programmed E. coli ribosomes has been studied in fluorescence stopped flow experiments. Fluorescent derivatives of $tRNA^{Phe}$ have been used which carry proflavine in the anticodon or D loops.

Upon binding of the tRNA to the P site the anticodon label showed two kinetic steps, a fast one (relaxation time in the 100 ms range) and a slow one (relaxation time in the s range). Only the slow step was found to depend on the presence of poly(U). Both the deacylated and the N-Ac Phe-$tRNA^{Phe}$ derivatives showed the same kinetic behavior. The forward rate constants of the two steps (10^7 M^{-1} s^{-1} and 0.5 s^{-1}, respectively) suggest a two-step binding mechanism: an unspecific binding step is followed by a codon dependent rearrangement of the complex.

A qualitatively similar picture was obtained for A site binding of deacylated, aminoacylated, or the N-Ac Phe-$tRNA^{Phe}$ derivative (P site blocked with non-fluorescent $tRNA^{Phe}$). However, in this case the rearrangement which led to the stable complex retained on nitrocellulose filters took several min. When the aminoacylated $tRNA^{Phe}$ derivative was used the rate of the rearrangement step was increased dramatically by the addition of EF-Tu·GTP whereas the fast binding step was not affected appreciably. These results suggest that tRNA binding to the A site also occurs in at least two steps; one function of EF-Tu is to speed up the second step, i.e. the proper positioning of the tRNA in the A site.