STABILITY OF POSTTRANSLOCATIVE COMPLEX

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In the course of elongation cycle the ribosome exists alternatively in two different functional states pretranslocative and posttranslocative ones. To model the posttranslocative complex at the first step we bind the analog of peptidyl—tRNA (AcPhe—$tRNA^{Phe}$) at the P sites and then ternary complex Phe — $tRNA^{Phe}$ elongation factor EF—Tu · GTP at the A sites of poly(U)-programmed 70S ribosomes. After the peptide bond formation catalyzed by peptidyltransferase center the majority of ribosomes contain a newly synthesized peptidyl-tRNAs at the A sites. In the presence of elongation factor EF-G $AcPhe_2-tRNA^{Phe}$ is translocated to the P site. Studying stability of this posttranslocative complex at 25°C we have observed a noticeable decrease of peptidyl—tRNA binding with the ribosome during prolonged incubation. The detailed analysis of this complex has shown that $AcPhe_2-tRNA^{Phe}$ doesn't leave the ribosome as a whole but is subjected to hydrolysis. We have proved this using different techniques. For example, in the experiment, where peptidyl-tRNA has two radioactive labels (both in the peptide and in the 3'-terminal Adenosine of tRNA molecule - Ac[^{3}H]Phe_2– [^{14}C]$tRNA^{Phe}$) we have found that the binding of [^{3}H] label decreases in parallel way with the amount of trichloroacetic acid (TGA)-precipitable Ac[^{3}H]Phe_2 (fig.1a, 1), whereas the amount of TGA-precipitable [^{14}C]$tRNA^{Phe}$ remains constant during the incubation time (fig.1a, 2). This means that we deal with the hydrolysis of ester bond in the $AcPhe^2-tRNA^{Phe}$ molecule.

The observed hydrolysis takes place only at the P sites of ribosomes. The release of peptidyl-tRNA from ribosome into the medium by the reduction of Mg^{2+} concentration prevents this process. Incubation of the ribosomes with the peptidyl—tRNA at the A sites (+ antibiotic hydromycin to inhibit spontaneous translocation) doesn't lead to the noticeable hydrolysis either. These results have allowed us to assume that the process analogous to termination occurs in the posttranslocative complex if it can not be involved in further steps of elongation. As neither termination factors nor special codons are present in the system, we deal with spontaneos termination [1]. And one can say that the capability to carry out termination is an intrinsic property of the 70S ribosomes.

The peptidyl—tRNA hydrolysis step of peptide chain termination is believed to be catalysed by peptidyltransferase, the enzymatic activity of the ribosome functioning in the peptide bond formation [2]. Thus, both of these processes may lead to the same consequences in respect to deacylated tRNA. The experiment with doubly labelled peptidyl—tRNA has shown an interesting fact. Namely, the deacylated tRNAs which have remained after the hydrolysis at the P sites of ribosome leave them during incubation (fig.*1b*, *2*). It seems to be due to the decrease of association constant of these tRNAs for the P sites. If this is

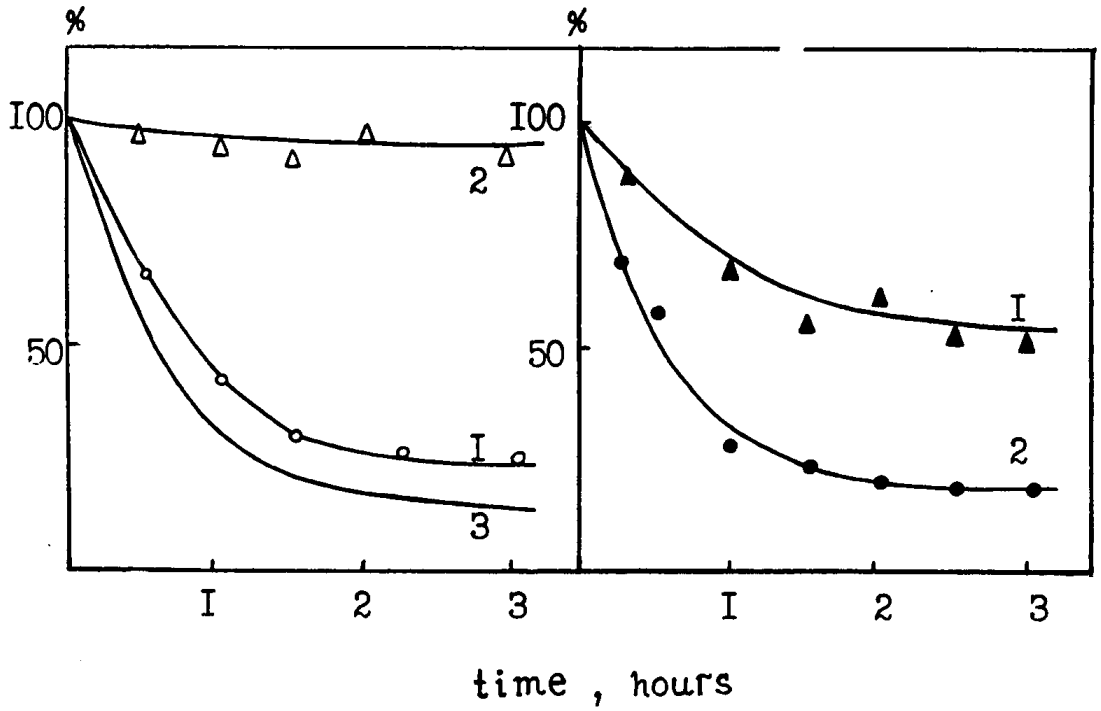

Fig.1. Stability of posttranslocative complex 70S·poly (U) ·Ac[^{3}H]Phe$_2$ — [^{14}C] tRNA^{Phe} at 25^{0} C. a. Amount of TGA-preciptable [^{3}H] (*1*) and [^{14}C] (*2*) material. b. Kinetics of Ac[^{3}H] Phe$_2$ (*1*) and [^{14}C] tRNA^{Phe} (*2*) binding. (*a,3* is the same with *b,1*)

the case, a vague moment of translocation can be explained. According to the Leder's hypothesis [3], the tRNA molecules have to move in the ribosome due to the dient of chemical potential: from the site with the lower affinity to the site with the higher one. But it has not been quite understood until now, why deacylated tRNA translocates from the P to the E site. It follows from the experiment on direct binding that deacylated tRNA has the highest affinity for the P site. If, however, the peptide bond formation as well as the hydrolysis of peptidyl—tRNA results in the reduction of deacylated tRNA affinity for the P site, direction of translocation of this tRNA becomes explainable.

References

1. Shapkina T.G., Semenkov Yu.P., Makhno V.I., Kirillov S.V. Preprint LNPI-1621. Leningrad. 1990. 14 p.
2. Tate P., Schulze H., Nierhaus K.H. //J.Biol.Chem. 1983. V.258. P.12810.
3. Leder P. //Adv.Protein Chem. 1973. V.27. P.213.
4. Semenkov Yu.P., Makarov E.M., Kirillov S.V. //Biopolimery i kletka. 1985. V.1. P.183.