THERMODYNAMICS OF TRANSLOCATION AND FUNCTIONAL ROLE OF E SITE

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The exit function was suggested from the very beginning for a third abbitional site (E) for tRNA binding in ribosome [1 — 3], but there was no direct evidence. If the E site really has an exit function, i. e. deacylated iRNA moves from the P site into the solution via the E site in a translocation process, its occupancy by deacylated tRNA has to inhibit the rate of translocation. Thus the strategy of the experiments is to measure the rate of translocation at different levels of the E site occupancy by both cognate and noncognate deacylated tRNA.

SCHEME OF EXPERIMENTS

1. Preparation of pretranslocation complex

Two types of pretranslocation complexes were used in this study.

a) 70S ribosomes were programmed by $AUG(U)_n$ and contained formylmethionine tRNA on the P site and fMet-Phe-tRNA on the A site.

b) 70S ribosomes were programmed by poly(U) and contained phenylalanine tRNA on the P site and AcPhe-Phe-tRNA on the A site.

The pretranslocation complexes were prepared as follows: first a complex was of 70S with poly(U) and N-AcPhe-tRNA or $AUG(U)_n$ and fMet-tRNA. Then the Phe-tRNA (the ratio of the Phe-tRNA to the 70S ribosomes was equal to 1.2) and, if indicated, a catalytical amount of EF-Tu were added to the complex. In all the cases the dipeptides were formed on the ribosome due to very fast peptide bond formation after filling both A and P sites with tRNAs, and the occupation of sites by tRNA was determined.

II. Measurements of translocation kinetics

Puromycin and tetracycline (1 mM and 0.1 mM) were added to the pretranslocation complex to prevent additional Phe-tRNA binding to the posttranslocational complex and after 5 minutes EF-G and GTP were added and aliquots were diluted ten-fold volume of 0.1 M Na acetate, pH 5.5 to stop the translocation and to determine the amount of the posttranslocation complex by ethylacetate extraction of the reaction product.

KINETICS OF TRANSLOCATION

The kinetics of the translocation of fMet-Phe-tRNA and Ac-Phe-tRNA were studied at different temperatures. The values of the rate constant of translocation are summarized in table 1.

Table 1

Absolute rate constant of translocation

	k*1000 (1/s)			
Temperature (°C)	10	17	24	31
poly(U)		3.2	5.2	14.3
$AUG(U)_n$	2.9	5.9	12.8	33.2

Table 2

Thermodynamical parameters of translocation

	ΔG*	ΔH*	ΔS*
Messenger	kcal/mol	kcal/mol	kal/mol deg
poly(U)	20.3 ± 2.7	19.2 ± 2.5	−3
$AUG(U)_n$	19.8 ± 2.0	20.4 ± 1.8	2

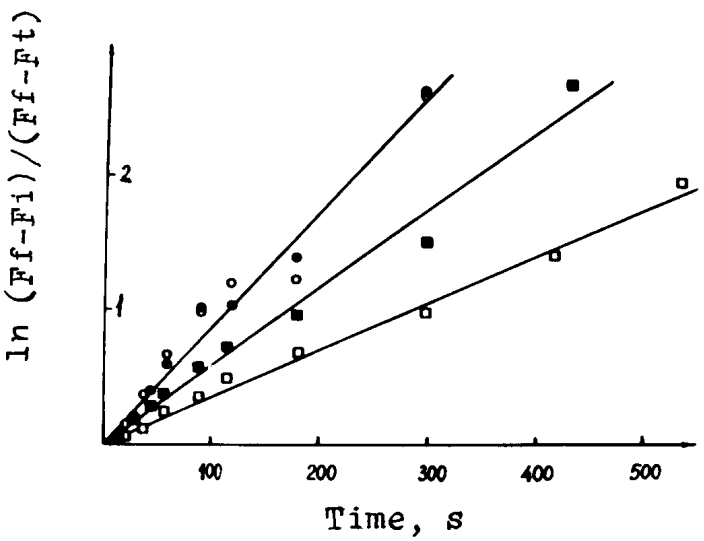

Fig. Inhibition of translocation rate by E site bound deacylated tRNA:
(○, ●) — without addition of deacylated tRNA; (□) — — with addition of phenylalanine tRNA; (■) — with addition of nonphenylalanine tRNA

The rate constant increases with pH increase and its extrapolation to the optimal pH and temperature qives a value close to 2 1/s which is consistent with the data of Robertson et al [4].

The results presented in figure show that deacylated tRNA bound to the E site of the pretranslocation complex inhibits the rate of translocation, while the amount of translocating ribosomes remains constant.

CONCLUSIONS

1. The thermodynamical parameters of the translocation of peptidyl-tRNA synthesised on the ribosome are close to those determined for a model system with deacylated tRNA on the P site and AcPhe-tRNA on the A site.
2. The rate constant of translocation exponentially depends on pH.
3. The occupancy of the E site of the pretranslocational complex by both cognate and noncognate deacylated tRNA inhibits the rate of translocation that proves the involvement of the E site in the translation process.

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