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Interaction of tRNA with the A and P sites of rabbit-liver 80S ribosomes and their 40S subunits

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The interaction between tRNA and rabbit liver 80S ribosomes and 40S subunits was studied using a nitrocellulose membrane filtration technique. Binding of the different tRNA forms (aminoacyl-, peptidyl- or deacylated) to poly(U)-programmed 40S subunits and 80S ribosomes was found to be a cooperative process. The association constants of AcPhe-tRNA^{Phe} for the A and P sites of 80S ribosomes and the cooperativity constant were measured at different temperature and Mg^{2+} concentration. The AcPhe-tRNA^{Phe} association constant for the P site was shown to be between 2×10^7 M^{-1} and 2×10^8 M^{-1} at 25–37 °C and 5–20 mM Mg^{2+}, while the affinity for the A site was 10–100-fold lower. The cooperativity constant was shown to decrease with the increase of incubation temperature and the decrease of Mg^{2+} concentration. The affinity of AcPhe-tRNA^{Phe} for the A site of 80S ribosomes was shown to depend upon the codon specificity of tRNA at the P site. The cooperativity of the tRNA interaction with 80S ribosomes was suggested to be mostly contributed by the association with the 40S subunit and result from the correct codon-anticodon pairing at the P site. The data presented imply a codon-anticodon interaction at the P site of eukaryotic 80S ribosomes.

Considerable information concerning the interaction of prokaryotic tRNA, mRNA and 70S ribosome has been accumulated. In the absence of elongation factors, all tRNA forms (aminoacyl-, peptidyl- or deacylated) have a higher affinity for the P site of the 70S ribosome than for the A site. Programmed 70S ribosomes are able to bind two molecules of aminoacyl- or peptidyl-tRNA. Codon-anticodon interactions at both ribosomal sites has been well documented [1]. The third site (E), specific for deacylated tRNA, has recently been reported for *Escherichia coli* ribosomes [2–4].

In contrast, surprisingly little data are available for eukaryotic ribosomes. Nothing is known about the thermodynamics of the tRNA interaction with the A and P sites of 80S ribosomes. We have shown [5, 6] that poly(U)-programmed 80S ribosomes and their 40S subunits are able to bind two tRNA molecules of aminoacyl- or peptidyl-tRNA. Here we present evidence that tRNA binding to the A and P sites of 80S ribosomes and 40S subunits is a cooperative process. This phenomenon appears to complicate the investigation of tRNA-ribosome interaction since it interferes with the correct modelling of different functional tRNA-ribosome complexes and the determination of tRNA association constants for the A and P sites. The affinity of tRNA for the A site of 80S ribosome was shown to depend on the correct codon-anticodon pairing at the P site. This leads to the speculation that the cooperativity results from the codon-induced interaction of tRNA molecules bound at the A and P sites of 80S ribosomes.

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Abbreviations. tRNA^{Phe}, deacylated phenylalanine tRNA; Phe-tRNA^{Phe}, phenylalanyl-tRNA^{Phe}; AcPhe-tRNA^{Phe}, *N*-acetyl-phenylalanyl-tRNA^{Phe}; tRNA^{-Phe}, total tRNA preparation free of phenylalanine tRNA; $\bar{\nu}^{\Sigma}$, the average number of tRNA molecules bound per ribosome or ribosomal subunit.

MATERIALS AND METHODS

Ribosomal subunits from rabbit liver were prepared according to the procedure described by Falvey and Staehelin [7] modified as in [8]. 80S ribosomes were obtained by the reassociation of 40S and 60S subunits at 1.1–1.2-fold excess of the latter. Enriched [^{14}C]Phe-tRNA^{Phe} (1.37 nmol/A_{260} unit), Ac[^{14}C]Phe-tRNA^{Phe} (1.59 nmol/A_{260} unit), [^{14}C]-tRNA^{Phe} (1.65 nmol/A_{260} unit) and tRNA^{-Phe} from *E. coli* as well as fractionated poly(U) (M_r = 30000) were prepared according to [9]. $\bar{\nu}^{\Sigma}$ values (i.e. the average number of tRNA molecules bound per ribosome or ribosomal subunit) were measured by the nitrocellulose membrane filtration technique [10].

tRNA binding to 80S ribosomes and 40S subunits

The incubation mixture contained in 50 µl buffer A (20 mM Tris/HCl pH 7.4, 100 mM NH_4Cl, 5–20 mM $MgCl_2$) 10 pmol 40S subunits, 11 pmol 60S subunits (for 80S ribosomes), 10 µg poly(U) and 0–60 pmol tRNA. In the preliminary experiments we have measured the kinetics of factor-free poly(U)-dependent AcPhe-tRNA^{Phe} binding to 80S ribosomes and 40S subunits. The maximum level of binding for 80S ribosomes and 40S subunits was reached within 1 h and 1.5–2 h at 0 °C and within 15 min and 30 min at 25 °C, respectively. In subsequent experiments the incubation was 2.5–3 h to ensure equilibrium conditions. The ribosome preparations were almost 100% active with respect to tRNA binding to both A and P sites and the formation of

diphenylalanine. The puromycin assay was performed as described in [11].

The effect of deacylated tRNA on AcPhe-tRNA^{Phe} binding to 80S ribosomes

10 pmol 40S subunits, 12 pmol 60S subunits, 6 μg poly(U) and 0–160 pmol tRNA^{Phe} or 0–9 nmol tRNA^{-Phe} were preincubated in 50 μl buffer A containing 10 mM $MgCl_2$ for 30 min at 25 °C. After the addition of 50 pmol Ac[^{14}C]Phe-tRNA^{Phe}, the incubation was continued for 60 min at 25 °C. To determine AcPhe-tRNA^{Phe} affinity for the A site of 80S ribosome the P site was occupied by tRNA^{Phe} (80, 110 or 160 pmol) or tRNA^{-Phe} (6 nmol); subsequently 0–60 pmol Ac[^{14}C]Phe-tRNA^{Phe} was added and the incubation was carried out for 90 min at 25 °C.

The calculation of the affinity constants, cooperativity constant and free energy of interaction

The affinity constants of tRNA for the A and P sites, cooperativity constant and free energy of interaction was estimated as described in [12]. The binding isotherm for tRNA association with the ribosome is given by:

$$\bar{\nu}^{\Sigma} = \frac{k_1 c + k_2 c^2}{1 + k_1 c + k_2 c^2},$$

where c is the concentration of free tRNA in solution, $k_1 = K^{A} + K^{P}$, $k_2 = K^{A} \cdot K^{P} \cdot K^{AP}$. In turn, K^{A} is the association constant of tRNA for the A site; K^{P} is the association constant of tRNA for the P site; K^{AP} is the cooperativity constant. k_1 and k_2 values can be estimated from the experimental curve. It is obvious, however, that various sets of K^{A}, K^{P} and K^{AP} correspond to the same binding curve. Therefore the three parameters can be calculated correctly only if one of them is determined by another experimental approach.

The equations describing the isotherm of tRNA binding to the A or P sites as well as the free energy of interaction with the ribosomal sites are given in [12].

RESULTS

Cooperativity of the interaction of tRNA with 40S ribosomal subunits and 80S ribosomes

The 40S ribosomal subunit from rabbit liver contains two tRNA-binding sites which are the functional parts of the A and P sites of 80S ribosome [5, 6]. Fig. 1 presents the Scatchard plots for the binding of aminoacyl-tRNA (A), peptidyl-tRNA (B) and deacylated tRNA (C) to poly(U)-programmed 40S subunits. The positive cooperativity of binding is strongly demonstrated by the shape of the curves (see for example [13]). The isotherm of Phe-tRNA^{Phe} binding to the 30S subunit containing two reciprocally independent tRNA-binding sites [14] is given in Fig. 1 (D). The comparison of this curve with curves A–C indicates that the cooperativity of tRNA association to the ribosomal sites is specific for eukaryotic ribosomes.

An analysis of the experimental curves is presented in Table 1. The affinity of different tRNA forms for the 40S subunit is approximately 10^7 M^{-1}. Assuming that tRNA binding constants are equal for both sites ($K^{A} = K^{P}$), the cooperativity appears to be 5–7 for different tRNA forms at 0 °C and 20 mM $MgCl_2$. In fact, under these experimental

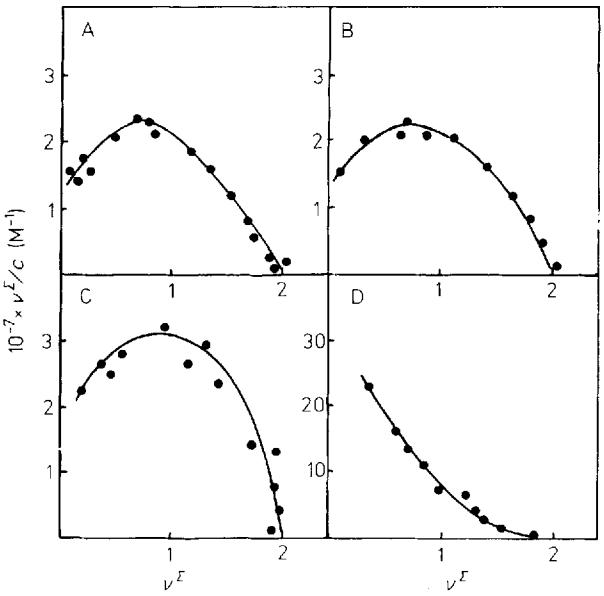

Fig. 1. *Isotherms of Ac[^{14}C]Phe-tRNA^{Phe} (A), [^{14}C]Phe-tRNA^{Phe} (B) or [^{14}C]tRNA^{Phe} (C) binding to poly(U)-programmed 40S subunits and of [^{14}C]Phe-tRNA^{Phe} binding to 30S subunits (D) represented in Scatchard plots.* The incubation was 2 h at 0 °C and 20 mM $MgCl_2$

conditions the addition of 60S subunits to the preformed 40S · poly(U) · Phe-tRNA^{Phe} complex results in the formation of diphenylalanine even in a large excess of ribosomes against tRNA, indicating that the affinity of at least aminoacyl-tRNA for both sites is approximately equal. In contrast, at 25 °C and 10 mM $MgCl_2$, the affinity constants differ, since a large proportion of the Phe-tRNA^{Phe} bound to the ribosome does not form diphenylalanine. Hence, the occupation of the ribosomal site (presumably P site) is not accompanied by diphenylalanine formation (data not shown). Assuming that $K^{A} = K^{P}$, the value of the cooperativity constant estimated is the minimum value of the cooperativity parameter. If it is assumed that K^{A} and K^{P} differ by one order of magnitude (the assumption which seems to be possible for 25 °C and 10 mM $MgCl_2$) the cooperativity constant is calculated to be at least 4.5.

Fig. 2 shows the binding isotherms of AcPhe-tRNA^{Phe} to poly(U)-programmed 80S ribosomes. The positive cooperativity of tRNA binding to both ribosomal sites is again demonstrated by the shape of the curves. As mentioned above, one of the association parameters should be determined independently for the correct estimation of all three affinity constants. This raises the problems of selective inhibition of tRNA binding to the definite ribosomal site. Different approaches have been applied for selective inhibition of tRNA binding to the A and P sites of prokaryotic ribosomes. These include the use of site-specific antibodies, the identification of P site binding by the puromycin reaction, and site discrimination on the basis of different affinities of tRNA forms for ribosomal sites.

The estimation of tRNA affinity constants for the A and P sites of 80S ribosome and the cooperativity constant

The first approach used to discriminate between the A and P sites of eukaryotic ribosomes was the application of site-specific antibiotics. Tetracycline and edeine are the classical

Table 1. *Association parameters of different tRNA forms with 40S subunits*

tRNA form	Temp.	$[MgCl_2]$	$10^{-7} \times K^A + K^P$	$-\Delta G_t^\circ$	K^{AP}	
					a	b
	°C	mM	M^{-1}	kJ/mol (kcal/mol)		
AcPhe-tRNA^{Phe}	25	10	2.2	81.1 (19.4)	2	4.5
AcPhe-tRNA^{Phe}	0	20	1.5	76.1 (18.2)	7	20
tRNA^{Phe}	0	20	3.3	78.6 (18.8)	5	13
Phe-tRNA^{Phe}	0	0	1.4	75.7 (18.1)	6	19

K^{AP} has been determined as (a) the minimum value of the cooperativity constant estimated from the assumption that $K^A = K^P$ and (b) as the value of the cooperativity constant estimated from the assumption that K^A and K^P differ by one order of magnitude

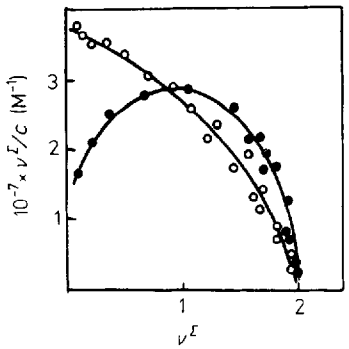

Fig. 2. *Isotherms of Ac[^{14}C]Phe-tRNA^{Phe} binding to poly(U)-programmed 80S ribosomes represented in Scatchard plots at 0°C and 20 mM $MgCl_2$ (●) or at 25°C and 10 mM $MgCl_2$ (○)*

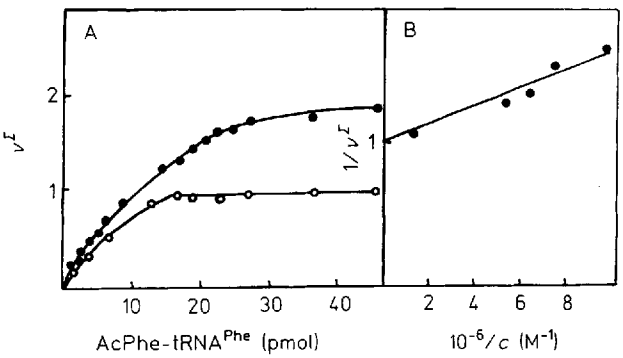

Fig. 3. *The estimation of tRNA affinity constants for the A and P sites of the 80S ribosome.* (A). Isotherm of Ac [^{14}C]Phe-tRNA^{Phe} binding to 80S ribosomes at 25°C and 10 mM $MgCl_2$ (●) and the increase of Ac[^{14}C]Phe-tRNA^{Phe} reaction with puromycin during titration (○). (B). The determination of Ac[^{14}C]Phe-tRNA^{Phe} affinity for the A site of 80S ribosomes ($K^A \cdot K^{AP}$). The P site is occupied by Ac[^{14}]Phe-tRNA^{Phe}

inhibitors of tRNA binding to the A and P sites of prokaryotic ribosomes [15]. Both antibiotics are known to bind to eukaryotic ribosomes [16, 17]. Our results indicate, however, that, in contrast to prokaryotic ribosomes, tetracycline inhibits tRNA binding nonspecifically to both sites on 80S ribosome. Similarly, edeine was found to inhibit tRNA binding to both ribosomal sites. The inhibitory effect of edeine may, however, be overcome by the addition of excess tRNA, indicating that edeine decreases tRNA affinity for both ribosomal sites (data not shown). Thus neither tetracycline nor edeine are applicable for the discrimination of tRNA binding to a definite ribosomal site.

Another possibility was to estimate tRNA affinity constants for the A and P sites of 80S ribosomes using the puromycin assay. Fig. 3 presents the isotherm of AcPhe-tRNA^{Phe} binding to 80S ribosomes at 25°C and 10 mM $MgCl_2$ and the increase of the puromycin reaction of AcPhe-tRNA^{Phe} during the titration. According to the puromycin assay, half of the AcPhe-tRNA^{Phe} bound to the ribosome in the presence of tRNA excess is at the P site. The remaining tRNA is obviously associated with the A site. Fig. 3 (A) demonstrates that at low AcPhe-tRNA^{Phe} concentrations tRNA binds preferentially to the P site. For the determination of tRNA affinity for the A site we have chosen the part of the isotherm which corresponds to the plateau of the puromycin reaction, indicating that all accessible P sites are occupied and AcPhe-tRNA^{Phe} binds therefore to the A site of the ribosome. In this case the plot $1/\bar{\nu}^E$ vs $1/c$ is linear and the affinity constant of AcPhe-tRNA^{Phe} for the A site can be estimated from the slope of the plot (Fig. 3B). Since the P site occupation results in the increase of tRNA affinity for the A site by the cooperativity parameter, the constant estimated in this case appears to be $K^A \cdot K^{AP}$. At 25°C and 10 mM $MgCl_2$, $K^A \cdot K^{AP}$ equals 1.6×10^7 M^{-1}.

The puromycin reaction characterizes tRNA binding to the P site. It is not applicable, however, for the determination of tRNA affinity for the P site. Because of positive cooperativity, P site binding of tRNA depends on both the affinity for the P site and the adsorption on the A site. Nevertheless, at large excess of the ribosomes tRNA binds preferentially to the P site. The level A site binding is negligible. This allows the affinity constant of AcPhe-tRNA^{Phe} for the P site of 80S ribosomes with a free A site to be calculated. It appears to be 3.5×10^7 M^{-1} at 25°C and 10 mM $MgCl_2$.

According to the binding isotherm presented in Fig. 3 (A), $K^A + K^P = 3.8 \times 10^7$ M^{-1}, $K^A \cdot K^P \cdot K^{AP} = 5.4 \times 10^{14}$ M^{-2}. If $K^P = 3.5 \times 10^7$ M^{-1} according to the puromycin reaction, $K^A = 3 \times 10^6$ M^{-1}, $K^{AP} = 5$. On the other hand, if $K^A \cdot K^{AP} = 1.6 \times 10^7$ M^{-1}, $K^A = 4 \times 10^6$ M^{-1}, $K^P = 3.4 \times 10^7$ M^{-1}, $K^{AP} = 4$. This indicates that the two approaches for the estimation of affinity constants give similar results. The values of the affinity constants and the free energy of interaction at different temperatures and $MgCl_2$ concentrations are presented in Table 2. The cooperativity constant appears to decrease with increasing incubation temperature and decreasing magnesium concentration.

The effect of cognate and noncognate deacylated tRNA on AcPhe-tRNA^{Phe} binding to poly(U)-programmed 80S ribosomes

Figs 4(A) and 5(A) demonstrate the inhibition of AcPhe-tRNA^{Phe} binding to poly(U)-programmed ribosomes by

Table 2. *The affinity constants of AcPhe-tRNA^{Phe} for the A and P sites of 80S ribosome, cooperativity constant and free energy of interaction at different temperatures and Mg^{2+} concentrations*

Temp.	[$MgCl_2$]	K^A	K^P	K^{AP}	$-\Delta G_t^\circ$	$-\Delta G_A^\circ$	$-\Delta G_P^\circ$
°C	mM	M^{-1}			kJ/mol (kcal/mol)		
25	5	3×10^5	2.4×10^7	1	72.7 17.4)	30.9 (7.4)	41.8 (10.0)
25	10	4×10^6	3.4×10^7	4	83.6 (20.0)	38.9 (9.3)	44.7 (10.7)
25	20	5×10^6	1.6×10^8	13	90.7 (21.7)	41.4 (9.9)	49.3 (11.8)
0	10	4×10^5	5×10^6	15	69.8 (16.7)	31.8 (7.6)	38.0 (9.1)
37	10	3×10^6	3.0×10^7	1	82.8 (19.8)	38.5 (9.2)	44.3 (10.6)

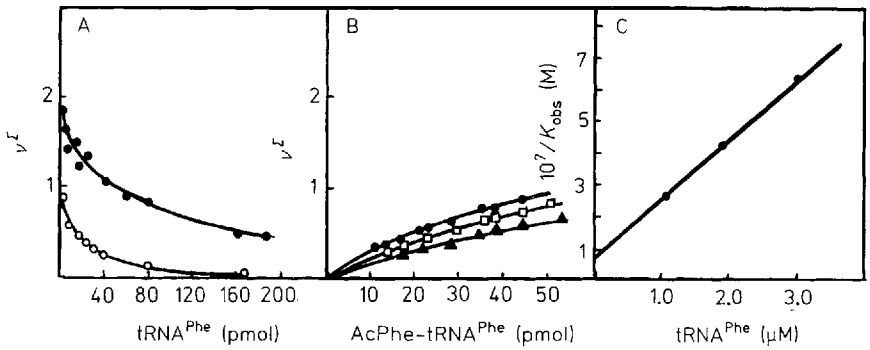

Fig. 4. *Effect of cognate tRNA^{Phe} on Ac[^{14}C]Phe-tRNA^{Phe} binding to poly(U)-programmed 80S ribosomes.* (A). Inhibition of Ac[^{14}C]Phe-tRNA^{Phe} binding (●) and the reaction with puromycin (○) by tRNA^{Phe}. (B). Isotherms of Ac[^{14}C]Phe-tRNA^{Phe} binding to 80S ribosomes in the presence of 80 pmol (●), 110 pmol (□) or 160 pmol (▲) of tRNA^{Phe}. (C) The dependence of Ac[^{14}C]Phe-tRNA^{Phe} affinity for the A site on the concentration of tRNA^{Phe}. The value of the constants are estimated from the isotherms presented in B

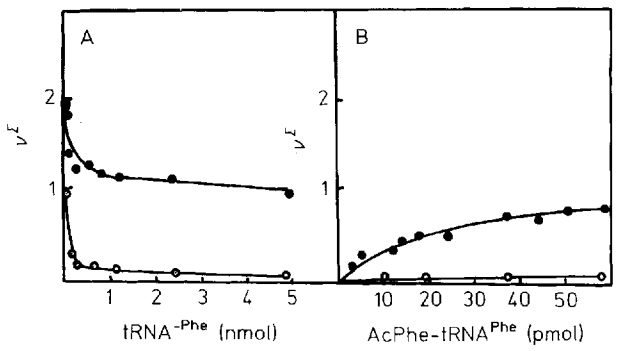

Fig. 5. *The effect of noncognate tRNA^{-Phe} on Ac[^{14}C]Phe-tRNA^{Phe} binding to poly(U)programmed 80S ribosomes.* (A) Inhibition of Ac[^{14}C]Phe-tRNA^{Phe} binding (●) and the reaction with puromycin (○) by tRNA^{Phe}. (B) Isotherm of Ac[^{14}C]Phe-tRNA^{Phe} binding to the A site of 80S ribosomes with tRNA^{-Phe} at the P site (●) and the reaction with puromycin (○)

tRNA^{Phe} and tRNA^{-Phe}. Cognate tRNA^{Phe} is shown to inhibit AcPhe-tRNA^{Phe} binding to both ribosomal sites. According to the puromycin assay, small concentrations of tRNA^{Phe} inhibit AcPhe-tRNA^{Phe} binding to the P site preferentially. The 50% decrease in AcPhe-tRNA^{Phe} binding does not however, correspond to complete inhibition of the puromycin reaction (Fig. 4 (A), indicating that tRNA^{Phe} affects both A and P site binding. At high tRNA^{Phe} concentrations (>70 pmol/50 μl incubation mixture), AcPhe-tRNA^{Phe} binding to the P site is completely inhibited (there is no puromycin reaction). The affinity constant of AcPhe-tRNA^{Phe} for the A site in the presence of different tRNA^{Phe} concentrations is given by the equation [18]:

$$1/K_{obs} = 1/K_p + K_t \cdot t/K_p,$$

where K_{obs} is the affinity of AcPhe-tRNA^{Phe} for the A site measured at the concentration t of the inhibitor; K_t and K_p are the affinity constants of tRNA^{Phe} and AcPhe-tRNA^{Phe} for the A site in the absence of the inhibitor. Therefore, the AcPhe-tRNA^{Phe} affinity constant for the A site may be estimated from the ordinate intersection of the linear plot of $1/K_{obs}$ vs t. Fig. 4 (B) represents the isotherms of AcPhe-tRNA^{Phe} binding to the A site of the ribosome in the presence of different tRNA^{Phe} concentrations. The dependence of AcPhe-tRNA^{Phe} affinity for the A site on the concentration of tRNA^{Phe} is demonstrated in Fig. 4 (C). Since deacylated tRNA binds to the A, P and E sites, the concentration of deacylated tRNA free in solution (t) was corrected for the amount of tRNA^{Phe} bound to the P and E sites according to tRNA^{Phe} affinity constants (data not shown). Thus with cognate tRNA^{Phe} in the P site, the affinity of AcPhe-tRNA^{Phe} for the A site appears to be 1.3×10^7 M^{-1} at 25 °C and 10 mM $MgCl_2$.

In contrast to tRNA^{Phe}, noncognate tRNA^{-Phe} inhibits exactly half of the AcPhe-tRNA^{Phe} binding to 80S ribosomes (Fig. 5A). The puromycin assay indicates that the inhibition of AcPhe-tRNA^{Phe} binding occurs at the P site of the ribosome. This provides the possibility of estimating the affinity of AcPhe-tRNA^{Phe} for the A site of the ribosome with tRNA^{-Phe} at the P site (Fig. 5B). In this case the affinity constant of AcPhe-tRNA^{Phe} for the A site appears to be 3×10^6 M^{-1} at 25 °C and 10 mM $MgCl_2$. Noncognate tRNA^{-Phe} does not influence AcPhe-tRNA^{Phe} binding to the

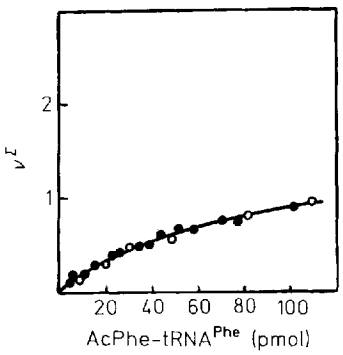

Fig. 6. *Isotherm of Ac[^{14}C]Phe-tRNA^{Phe} binding to 80S ribosomes (●) and the reaction with puromycin (○) in the absence of poly(U)*

A site of poly(U)-programmed ribosomes. Therefore the low value of the affinity constant does not result from competition between the two tRNA forms for A site binding.

The interaction of AcPhe-tRNA^{Phe} with 80S ribosomes in the absence of template

Fig. 6 demonstrates that, in the absence of poly(U), 80S ribosome binds one molecule of AcPhe-tRNA^{Phe}. The puromycin reaction indicates that AcPhe-tRNA^{Phe} is bound to the P site in this case. The affinity constant of AcPhe-tRNA^{Phe} for the P site in the absence of poly(U) equals 1.5×10^6 M^{-1} at 30 °C and 10 mM $MgCl_2$, the free energy of interaction ΔG_P° being −35.9 kJ/mol.

DISCUSSION

The data presented provide evidence for the positive cooperativity of tRNA interaction with the A and P sites of 80S ribosomes. The binding energy is mostly contributed by the 40S subunit: the total free energy of tRNA association with the 40S subunit ($\Delta G_t^\circ = -81.1$ kJ/mol) or the 80S ribosome ($\Delta G_t^\circ = -83.6$ kJ/mol) is very similar. The cooperativity of tRNA association is characteristic for both 80S ribosomes and 40S subunits. We did not succeed in determining tRNA affinity for the binding sites of the 40S subunit. On the one hand, edeine and tetracycline appeared to be inapplicable for the selective inhibition of tRNA binding. On the other hand, the use of functional tests is rather limited in this case. The puromycin reaction is slow for eukaryotic ribosomes [19] and the addition of 60S subunits influences 40S subunit affinity constants during the time of the puromycin reaction. The test of diphenylalanine formation is complete in 2–5 min, thus indicating site occupation in the preformed complex 40S · poly(U) · Phe-tRNA^{Phe}. The analysis of diphenylalanine formation may, however, provide only qualitative information. According to the results of this test, the ratio of the A and P site binding constants is assumed to be similar for 80S ribosomes and 40S subunits. The A and P site binding constants for 80S ribosome differ by one order of magnitude at 25 °C and 10 mM $MgCl_2$ (Table 2). If our assumption is correct and the ratio of the A and P site binding constants for the 40S subunit is approximately the same value, the cooperativity constant for the 40S subunit ($K^{AP} \approx 4-5$) appears to be similar to that for the 80S ribosome. The positive cooperativity of 40S subunit association is revealed for different tRNA forms. These data indicate that the cooperativity of tRNA association with 80S ribosome may be mostly contributed by the 40S subunit and tRNA regions interacting with it.

The data on the affinity of tRNA for the ribosomal sites may provide significant information towards understanding different molecular events in protein biosynthesis. Two different approaches were adopted to solve the problem. The first was to estimate AcPhe-tRNA^{Phe} affinity constants for the A and P sites on the basis of the puromycin reaction. The second was to discriminate between the ribosomal sites on the basis of different affinities of tRNA forms (deacylated and peptidyl-tRNA) for the A and P sites. Deacylated tRNA is a potent inhibitor of aminoacyl- or peptidyl-tRNA binding to the ribosome. Cognate tRNA^{Phe} inhibits the association of tRNA acylated forms with both A and P sites [20, 21]. Noncognate tRNA^{-Phe} is able to bind to the P site of 80S ribosomes [22] and affect the P site association of aminoacyl-tRNA [23]. We have determined AcPhe-tRNA^{Phe} affinity constants for the A site of 80S ribosomes with cognate or noncognate deacylated tRNAs at the P site. The affinity of AcPhe-tRNA^{Phe} for the A site is similar to tRNA^{Phe} or AcPhe-tRNA^{Phe} at the P site. In contrast, with noncognate tRNA^{-Phe} at the P site the affinity of AcPhe-tRNA^{Phe} for the A site decreases by the cooperativity parameter. These data lead to the speculation that the cooperativity of tRNA association with A and P sites results from correct codon-anticodon pairing at both ribosomal sites.

Earlier Labuda et al. [24] found that binding of cognate codons to tRNA in solution induces dimerization of the codon · tRNA complex. The proposed dimerization of tRNA at the ribosome would contribute to the efficiency and fidelity of translation. In accordance with [24], the codon-dependent cooperativity of tRNA association with the A and P sites may be interpreted as the codon-induced interaction of two tRNA molecules on the ribosome. However, the cooperativity cannot be attributed solely to tRNA dimerization, since the codon-induced dimerization of the same tRNA species (tRNA^{Phe} from *E. coli*) has not been observed for 70S ribosomes. This indicates that the mechanisms of tRNA interaction may be somewhat different for prokaryotic and eukaryotic ribosomes and implies an active role of 80S ribosomes in codon-induced cooperativity of tRNA interaction with the A and P sites.

The dependence of AcPhe-tRNA^{Phe} affinity for the A site on codon specificity of tRNA bound to the P site implies codon-anticodon pairing at the P site of eukaryotic ribosome. The affinity of AcPhe-tRNA^{Phe} for the P site of 80S ribosomes increases 20–30-fold with the addition of the template. These results conflict with the conclusion [22, 23] that tRNA binding to the P site of 80S ribosomes does not depend on the presence of the cognate codon. Noncognate tRNA was shown to inhibit codon-dependent binding of fMet-tRNA$_f^{Met}$ to the P site of 80S ribosomes; both deacylated and fMet-tRNA$_f^{Met}$ were able to bind to the P site in the absence of the cognate codon. All these data indicate that tRNA affinity for the P site is rather high even in the absence of the cognate codon. Accordingly, we have found that the affinity of AcPhe-tRNA^{Phe} for the P site in the absence of template equals 1.5×10^6 M^{-1} at 30 °C and 10 mM $MgCl_2$. Therefore under certain experimental conditions codon-anticodon interaction of the P site would not be observed.

Thus, the binding of tRNA to the A and P sites of eukaryotic ribosomes is characterized by positive cooperativity which is mostly contributed by the interaction of tRNA

with the 40S subunit and results from correct codon-anticodon pairing at the P site.

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