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Number of tRNA binding sites on 80 S ribosomes and their subunits

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The ability of rabbit liver ribosomes and their subunits to form complexes with different forms of $tRNA^{Phe}$ (aminoacyl-, peptidyl- and deacylated) was studied using the nitrocellulose membrane filtration technique. The 80 S ribosomes were shown to have two binding sites for aminoacyl- or peptidyl-tRNA and three binding sites for deacylated tRNA. The number of tRNA binding sites on 80 S ribosomes or 40 S subunits is constant at different Mg^{2+} concentrations (5–20 mM). Double reciprocal or Scatchard plot analysis indicates that the binding of Ac-Phe-$tRNA^{Phe}$ to the ribosomal sites is a cooperative process. The third site on the 80 S ribosome is formed by its 60 S subunit, which was shown to have one codon-independent binding site specific for deacylated tRNA.

80 S ribosome; 40 S subunit; 60 S subunit; tRNA binding site

1. INTRODUCTION

To understand different molecular events in protein biosynthesis, the number of tRNA binding sites on the ribosome should be known exactly. As far as this problem is concerned notable progress has been achieved for prokaryotic ribosome. 70 S ribosome is supposed to possess three tRNA binding sites: in addition to the classic A and P sites there is a third one specific for deacylated tRNA [1–4]. The A and P sites are formed by both ribosomal subunits. Binding energy is mostly contributed by the small subunit. Consequently, the 30 S subunit can bind simultaneously two molecules of aminoacyl-, peptidyl- or deacylated tRNA. The large subunit contains parts of the A and P sites (in the peptidyltransferase center) [5]. The third tRNA binding site was also shown to exist on 50 S subunits which differs at least from the P site. The affinity constant of the former site for deacylated tRNA is relatively high ($K_a = 10^7\ M^{-1}$) [6]. This site was suggested to contribute the main part of the third site on 70 S ribosome [6,7].

The problem of elucidation of the number of tRNA binding sites on the eukaryotic ribosome and its subunits is much worse. As can be seen from [8] the small ribosomal subunit contains one tRNA binding site, presumably the A site. As far as deacylated tRNA is concerned, the large ribosomal subunit has two binding sites characterized by different values of the affinity constant. The high affinity site was suggested to correspond to the P site. The second site possessing a lower affinity constant was suggested to constitute a part of the A site [9]. All experimental data concerning 80 S ribosome are discussed in terms of the classic A+P model. The third ribosomal site was observed on the eukaryotic ribosomes by Wettstein and Noll [10]. So far, however, no results confirming their data have been reported.

Rat liver active 40 S subunits were recently reported to be able to bind two molecules of each tRNA form in the presence of poly(U) [11]. Our present work shows that: (i) the 80 S ribosome contains two tRNA binding sites for aminoacyl- or

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peptidyl-tRNA, whereas for deacylated tRNA there are three binding sites; (ii) the 60 S subunit contains one binding site for deacylated tRNA with the affinity constant $5 \times 10^6\ M^{-1}$ and is almost inactive in Phe-tRNA^{Phe} and Ac-Phe-tRNA^{Phe} binding.

2. MATERIALS AND METHODS

Ribosomal subunits from rabbit liver were obtained according to [12], except that polysomes were preincubated for 50 min with all the components of protein synthesis, thereafter for 10 min with puromycin (0.5 mM) and then made up with KCl to 0.6 M of monovalent cations. 80 S ribosomes were prepared from the subunits under 2-fold excess of large subunits. The excess of 60 S subunits was taken into account in further calculations.

Enriched [^{14}C]Phe-tRNA^{Phe} (1650 pmol/A_{260} unit), Ac-[^{14}C]Phe-tRNA^{Phe} (1370 pmol/A_{260} unit) and [^{14}C]tRNA^{Phe} (1430 pmol/A_{260} unit) from *Escherichia coli* as well as fractionated poly(U) (M_r = 30000) were prepared according to [13]. All experiments were performed in buffer TAM (0.02 M Tris-HCl, pH 7.4; 0.02 M $MgCl_2$; 0.1 M NH_4Cl; 0.001 M EDTA). $\bar{\nu}^{\Sigma}$ values (i.e. the number of tRNA molecules bound per one ribosome or ribosomal subunit) were measured by the nitrocellulose membrane filtration technique. To determine the number of diphenylalanines synthesized per 80 S ribosome the samples were treated as in [14]. Other experimental conditions are described in the legends to the figures.

3. RESULTS AND DISCUSSION

Recently the rat liver ribosomal small subunit has been reported to contain two tRNA binding sites [11]. We have observed that rabbit liver 40 S ribosomal subunit is also able to bind two molecules of aminoacyl-, peptidyl- or deacylated tRNA (fig.1A). The decreasing Mg^{2+} concentration (20–5 mM) affects the affinity of tRNA for these sites while their number remains constant. Fig.1A indicates that tRNA binding to both sites is codon dependent. The addition of 60 S subunits to the preformed (40 S·poly(U)·(Phe-tRNA^{Phe})$_2$) complex does not result in the formation of an additional site on the 80 S ribosome. The number of diphenylalanines synthesized per 40 S subunit was close to 0.9 under the excess of 60 S subunits (fig.1B) and varied from 0.7 to 1.0 for different 60 S preparations, which indicates that the two sites on the 40 S subunit are of functional relevance and consequently are parts of the A and P sites of the 80 S ribosome.

The 80 S ribosome reassociated from subunits

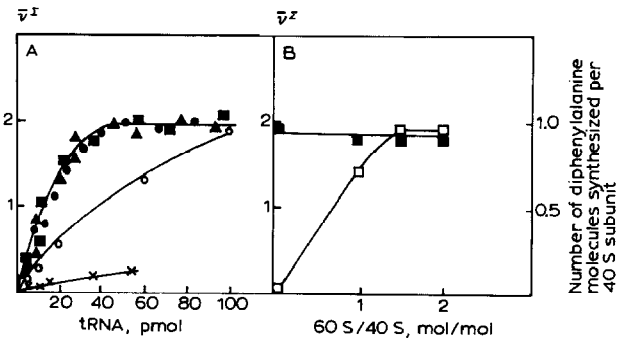

Fig.1. (A) Titration of 40 S subunits with different tRNA^{Phe} forms. The incubation mixture contained in 50 μl of buffer TAM, 10 pmol 40 S subunits, 6 μg poly(U) and increasing amounts of [^{14}C]Phe-tRNA^{Phe} (■), Ac[^{14}C]Phe-tRNA^{Phe} (●) or [^{14}C]tRNA^{Phe} (▲). Ac[^{14}C]Phe-tRNA^{Phe} binding at 6 mM Mg^{2+} (○). Ac[^{14}C]Phe-tRNA^{Phe} binding without poly(U) (×). Incubation was performed for 90 min at 0°C. (B) Effect of 60 S subunits on [^{14}C]Phe-tRNA^{Phe} binding (■) and diphenylalanine formation (□). The incubation mixture contained in 20 μl of buffer TAM 10 pmol 40 S subunits, 6 μg poly(U) and 52 pmol [^{14}C]Phe-tRNA^{Phe}. After the incubation for 90 min at 0°C the increasing amounts of 60 S subunits were added in 30 μl and the samples were incubated for 10 min at 25°C.

binds two molecules of aminoacyl-tRNA. Peptidyl-tRNA also binds to both sites (fig.2A). Thus, the exclusion principle proposed by Rheinberger et al. [15] does not seem to be suitable either for prokaryotic [16] or for eukaryotic ribosomes.

The existence of the third tRNA binding site on the 80 S ribosome (fig.2A) is in good agreement with [10]. Fig.2B indicates that the third site on the 80 S ribosome is formed by its 60 S subunit, since the addition of the large subunit to preformed (40 S·poly(U)·(tRNA^{Phe})$_2$) complex results in the appearance of the third tRNA binding site. On the other hand, the addition of 60 S subunits to an analogous complex with peptidyl-tRNA does not result in the formation of an extra site on the 80 S ribosome (fig.2C).

The decreasing Mg^{2+} concentration (20–5 mM) only leads to the decrease of tRNA affinity for the ribosomal sites, which is also the case for the small ribosomal subunit.

Double reciprocal or Scatchard plots indicate (fig.3A,B) that tRNA interaction with two sites on the ribosome is a cooperative process.

Large subunits contain one binding site for deacylated tRNA with the affinity constant 5 ×

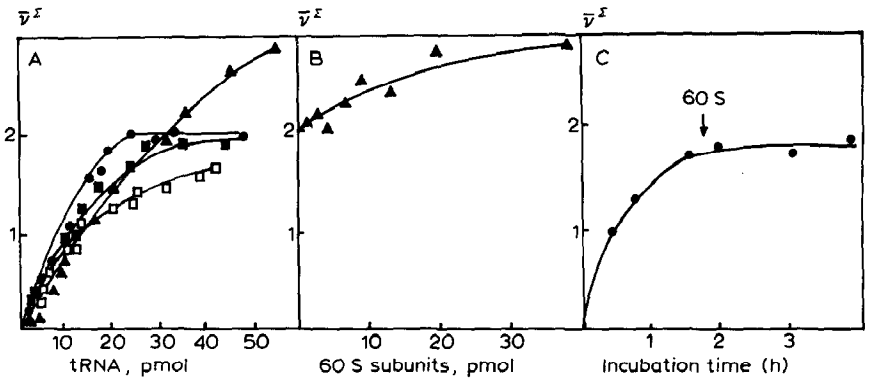

Fig.2. (A) Titration of 80 S ribosomes with different $tRNA^{Phe}$ forms. The incubation mixture contained in 50 μl of buffer TAM, 10 pmol 80 S ribosomes, 6 μg poly(U) and increasing amounts of [^{14}C]Phe-$tRNA^{Phe}$ (■), Ac[^{14}C]Phe-$tRNA^{Phe}$ (●) or [^{14}C]$tRNA^{Phe}$ (▲). Ac[^{14}C]Phe-$tRNA^{Phe}$ binding at 10 mM Mg^{2+} (□). The incubation was for 60 min at 0°C. (B) Effect of 60 S subunit on E site formation. The mixture contained 10 pmol 40 S subunits, 6 μg poly(U) and 64 pmol [^{14}C]$tRNA^{Phe}$ in 50 μl of buffer TAM. After incubation for 90 min at 0°C the increasing amounts of 60 S subunits were added and the samples were incubated for the next 60 min. (C) Kinetics of Ac[^{14}C]Phe-$tRNA^{Phe}$ binding to ribosome. The mixture contained 10 pmol 40 S subunits, 4 μg poly(U) and 98 pmol Ac[^{14}C]Phe-$tRNA^{Phe}$ in 20 μl of buffer TAM. After 100 min of incubation at 0°C 20 pmol, the 60 S subunits were added to 30 μl and kinetic measurements were continued.

10^6 M^{-1} (fig.4A,B) but are almost inactive in Phe-$tRNA^{Phe}$ and Ac-Phe-$tRNA^{Phe}$ binding within the same range of tRNA concentrations. $tRNA^{Phe}$ binding to the large subunit is codon independent (fig.4A). Buisson et al. [9] have shown that rat liver 60 S subunits have two binding sites for deacylated tRNA. The stronger site with the affinity constant of 2.3×10^9 M^{-1} was interpreted as the P site; the weaker one with $K_a = 10^8$ M^{-1} as part of the A site. However, the analysis of the experimental points from [9] has led us to the conclusion that the affinity constants were overestimated by 3 to 4 orders of magnitude. Thus, within the tRNA concentrations used in our experiments, only the high affinity site can be observed on the 60 S subunits. As mentioned above, both the 60 S high affinity site and the third site on the 80 S ribosome are specific for deacylated tRNA and the appearance of the 80 S site is due to addition of 60 S subunits. These data strongly suggest that the 60 S

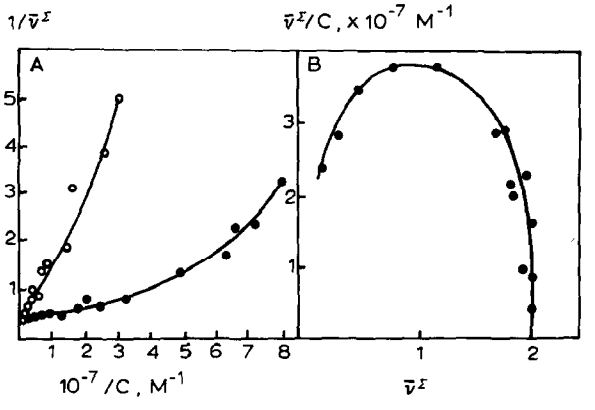

Fig.3. Titration curves of Ac[^{14}C]Phe-$tRNA^{Phe}$ binding to ribosomes. (A) Double reciprocal plots at 20 mM Mg^{2+} (●) and 10 mM Mg^{2+} (○). (B) Scatchard plots at 20 mM Mg^{2+}. The experimental data are from fig.2A.

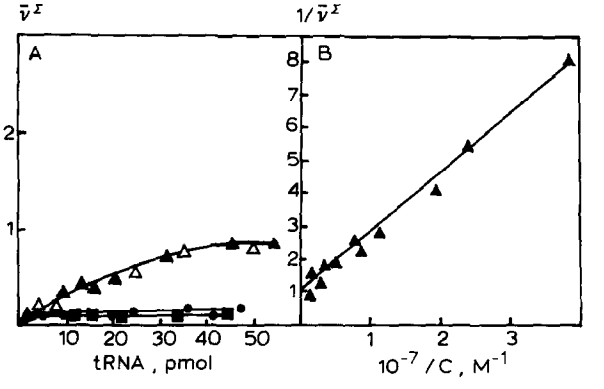

Fig.4. (A) Titration of 60 S subunits by different tRNA forms. The mixture containing 8 pmol 60 S subunits and increasing amounts of [^{14}C]Phe-$tRNA^{Phe}$ (■), Ac[^{14}C]Phe-$tRNA^{Phe}$ (●), [^{14}C]$tRNA^{Phe}$ (▲) or [^{14}C]$tRNA^{Phe}$ with 6 μg of poly(U) (△) was incubated for 60 min at 0°C. (B) The experiment for [^{14}C]$tRNA^{Phe}$ from A represented in double reciprocal plots.

site either coincides with the 80 S third site or is its main part rather than part of A and P sites.

The present study shows that in spite of considerable differences between prokaryotic and eukaryotic ribosomes they seem to be similar both in the number of tRNA binding sites and in their distribution between ribosomal subunits.

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