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Mechanism of codon-anticodon interaction in ribosomes. Direct functional evidence that isolated 30S subunits contain two codon-specific binding sites for transfer RNA

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ABSTRACT

30S subunits were isolated capable to bind simultaneously two molecules of Phe-tRNA^{Phe} (or N-Acetyl-Phe-tRNA^{Phe}), both poly(U) dependent. The site with higher affinity to tRNA was identified as P site. tRNA binding to this site was not inhibited by low concentrations of tetracycline (2×10^{-5}M) and, on the other hand, N-Acetyl-Phe-tRNA^{Phe}, initially prebound to the 30S·poly(U) complex in the presence of tetracycline, reacted with puromycin quantitatively after addition of 50S subunits. The site with lower affinity to tRNA revealed features of the A site: tetracycline fully inhibited the binding of both Phe-tRNA^{Phe} and N-Acetyl-Phe-tRNA^{Phe}. Binding of two molecules of Phe-tRNA^{Phe} to the 30S·poly(U) complex followed by the addition of 50S subunits resulted in the formation of (Phe)$_2$-tRNA^{Phe} in 75-90% of the reassociated 70S ribosomes.

These results prove that isolated 30S subunits contain two physically distinct centers for the binding of specific aminoacyl- (or peptidyl-) tRNA. Addition of 50S subunits results in the formation of whole 70S ribosomes with usual donor and acceptor sites.

INTRODUCTION

Many authors observed earlier functional heterogeneity of 30S subunits. For example, exclusion of monovalent ions from the incubation medium causes a two-fold decrease of the percentage of active subunits[1]; the binding of aminoacyl-tRNA to 30S subunits is characterized by two-step concentration sensitivity to N-ethylmaleimide[2]; the kinetics of initiation complex formation, in a system with N-Acetyl-Phe-tRNA and poly(U), is heterogeneous[3]; inhibition by tetracycline, which is a general diagnostic assay for the A site of ribosomes, never yields unambiguous results: its action varies from very weak (25%)[4] to nearly quantitative (70%)[5]. Two different suggestions can be made according to these results: i) 30S subunits contain two

distinct sites for tRNA; ii) 30S subunits contain only one site, but are structurally heterogeneous. One cannot discriminate between these possibilities, because only small fractions of 30S subunits were active in experiments mentioned above.

In our previous publication we reported about detailed study of nonenzymatic interaction of aminoacyl-tRNA with 30S subunits [6]. If a fraction of poly(U) of moderate molecular weight was used, then all ternary complexes 30S·poly(U)·Phe-$tRNA^{Phe}$ were homogeneous in stability and all subunits were active in the binding of one molecule of aminoacyl-tRNA per subunit.

In the course of further investigations, we noticed a well pronounced heterogeneity of subunits: dissociation of ternary complexes did not conform to first order kinetics; inhibition by tetracycline was variable and depended on aminoacyl-tRNA concentration. Furthermore, after a slight modification in the procedure of subunits isolation, we succeeded in preparation of 30S subunits with a maximal binding capacity up to 1.5-2.0 molecules of tRNA per subunit.

In this work we investigated 30S subunits, which revealed a poly(U)-dependent binding of two molecules of aminoacyl- (or peptidyl-) tRNA. This result is a direct evidence for the existence of two physically distinct tRNA-binding centers on a 30S subunit; their relationship to the donor (P) and acceptor (A) sites of whole 70S ribosomes was examined.

MATERIALS AND METHODS

Ribosomes

Cells of strain E.coli MRE-600, stored in liquid N_2, were suspended in two volumes of buffer I (0.02 M Tris-HCl, pH 7.1; 0.02 M $MgCl_2$; 0.2 M NH_4Cl; 0.001 M EDTA) and disrupted by a two-fold passage through a French press. The suspension was centrifuged 30 min at 30,000xg, then ribosomes were pelleted from the crude extract by high-speed centrifugation during 6 h at 100,000xg. The pellet was resuspended in buffer I, and the ribosomes were purified by centrifugation through a sucrose cushion (1.1 M) in buffer II (0.02 M Tris-HCl, pH 7.1; 0.01 M $MgCl_2$ 0.5 M NH_4Cl) according to Staehelin and Maglott[10]. Then puri-

fied 70S ribosomes were resuspended in buffer 3 (0.02 M Tris-HCl, pH 7.I; 0.00I M $MgCl_2$; 0.2 M NH_4Cl; 0.002 M 2-mercaptoethanol), and the separation into subunits was performed in a zonal rotor according to Eikenberry et al [9] (exponential sucrose gradient 7.4-38% in buffer 3). To the fractions of 30S and 50S subunits (with no more than 2% crosscontaminations) $MgCl_2$ was added till a concentration 20 mM, and then - 0.65 volumes of 95% ethanol. After 5 min standing in the cold the precipitate was quickly centrifuged (5 min at 5,000xg); then the subunits were resuspended in buffer I, reactivated 20 min at 40°, and stored in liquid N_2.

Preparations of tRNA and poly(U)

Isolation of ^{14}C-Phe-tRNA^{Phe} with a content of ^{14}C-Phenylalanyne I500 pmole/A_{260} unit, as well as poly(U) with an average molecular weight about 30,000 daltons, were described earlier [11,12]. N-Acetyl-Phe-tRNA^{Phe} was prepared from enriched ^{14}C-Phe-tRNA^{Phe} according to Rappoport and Lapidot [13].

Assays

Incubation mixtures contained in 200-400 μl of buffer I: I0 pmole 30S subunits, I0 μg poly(U) and 4-80 pmole ^{14}C-Phe-tRNA^{Phe} (or N-Acetyl-Phe-tRNA^{Phe}). If 30S subunits contain two independent centers for the binding of tRNA, then the overall binding may be described by the equation:

$$\bar{\nu}^{\Sigma} = \bar{\nu}^{p} + \bar{\nu}^{a} = \frac{M^{p} \cdot K^{p} \cdot C}{I + K^{p} \cdot C} + \frac{M^{a} \cdot K^{a} \cdot C}{I + K^{a} \cdot C}$$

where $\bar{\nu}^{\Sigma}$ is an average number of tRNA molecules bound per subunit, $\bar{\nu}^{p}$ and $\bar{\nu}^{a}$ - those bound in the P and A sites respectively, M^{p} and M^{a} - fractions of active sites, K^{p} and K^{a} - assotiation constants of tRNA with P and A sites and C - concentration of free tRNA in solution. M^{p}, M^{a}, K^{p} and K^{a} values were calculated as described earlier [6].

The assay mixtures were incubated for 60 min at 0° and filtered through nitrocellulose filters according to Nirenberg and Leder [14]. In experiments with TET (tetracycline) the 30S·poly(U) complex was initially preincubated with the antibiotic

during 30 min according recommendations in [15]; then tRNA was added in a buffer containing the same concentration of TET. The amounts of N-Acetyl-Phe-tRNA^{Phe} reacting with puromycin (PM) were measured by extraction of N-Acetyl-Phe-puromycin (Ac-^{14}C-Phe-PM) into ethylacetate according to Leder and Burzstyn [16].

RESULTS

As can be seen from Fig.1A, at high concentrations of Phe-tRNA^{Phe} $\bar{\nu}^{\Sigma}$ tends to 2.0 (-o-), i.e. every subunit is able to bind simultaneously two molecules of aminoacyl-tRNA. Some binding exists in the absence of poly(U); as shown earlier, the nonspecific, "basal" interaction between tRNA and 30S subunits in the absence of messenger is characterized by a low association constant of order of 10^5-10^6 M^{-1} (Grajevskaja et al [17]).

TET inhibits the maximal binding by a factor of 2; $\bar{\nu}^{p}$ va-

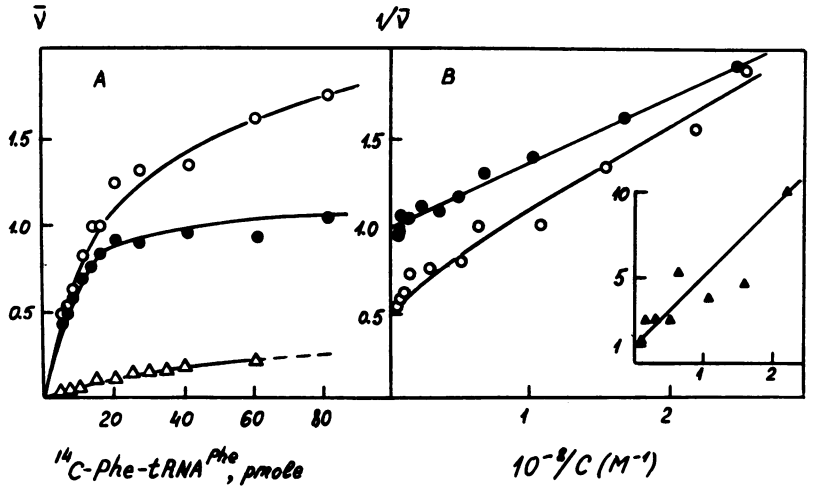

Fig.1. Titration of the 30S·poly(U) complex by ^{14}C-Phe-tRNA^{Phe}. A - dependence of $\bar{\nu}$ on the amount of ^{14}C-Phe-tRNA^{Phe} added: in the presence of TET (-●-); in the absence of TET (-o-); control without poly(U) (-Δ-). B - $1/\bar{\nu}$ versus I/C plots calculated from the data of section A: in the absence of TET (-o-, for $1/\bar{\nu}^{\Sigma}$); in the presence of TET (-●-, for $1/\bar{\nu}^{p}$). Insert: $1/\bar{\nu}^{a}$ versus I/C plot calculated as described in "Materials and Methods".

lue tends to unity (-●-). Results of this experiment in inverse coordinates are represented in Fig.1B. TET-resistant binding is well approximated by a straight line with the parameters $M^p=1$ and $K^p=2.5\times10^8 M^{-1}$. TET-sensitive binding is also characterized by $M^a=1$, but appeared weaker: $K^a=3\times10^7 M^{-1}$ (insert in Fig.1B).

Concentrations of TET throughout this work were extremely low, $2-3\times10^{-5}$M. At these concentrations nearly one molecule of antibiotic binds to the ribosome ($K_{ass}\simeq10^6 M^{-1}$), which is responsible for the selective inhibition of tRNA binding to the A site [18,19]. Hence, we conclude that TET-sensitive binding takes place on that portion of the A site which belongs to the 30S moiety of ribosome.

We observe here apparently "paradoxical" situation: TET inhibits the binding of exactly one molecule of aminoacyl-tRNA if $C \to \infty$; on the other hand, inhibition of the overall binding depends strongly on aminoacyl-tRNA concentration (from nearly zero to maximum 50%). This fact is easily explained by the considerable difference between K^p and K^a values.

To characterize functionally TET-resistant binding, we made a similar experiment with an analogue of peptidyl-tRNA - N-Acetyl-Phe-$tRNA^{Phe}$. In this case we also observe that $\bar{\nu}^{\Sigma}\to2$ (Fig.2) but K^p and K^a values were somewhat lower - $3\times10^7 M^{-1}$ and approximately $6\times10^6 M^{-1}$ respectively.

If the 30S subunit contains a portion of the P site, and the latter is equivalent to our TET-resistant site, then the addition of 50S subunits must result in the formation of Ac-^{14}C-Phe-PM provided that both tetracycline and puromycin are present in the incubation mixture.

This is the case, as shown in Fig.3. After 30 min incubation of N-Acetyl-Phe-$tRNA^{Phe}$ with the 30S·poly(U) complex (in the presence of TET) $\bar{\nu}^p$ tends to unity; addition of 50S subunits does not change the binding (-o-). After the formation of a ternary complex puromycin was added, and kinetics of Ac-^{14}C-Phe-PM synthesis was measured at 30°. We see that more than 90% of peptidyl-tRNA is competent in the reaction with puromycin (-●-). It should be noted, that the yield of the puromycin reaction can be overestimated as a consequence of exchange of deacylated $tRNA^{Phe}$ with a second molecule of peptidyl-tRNA, and repe-

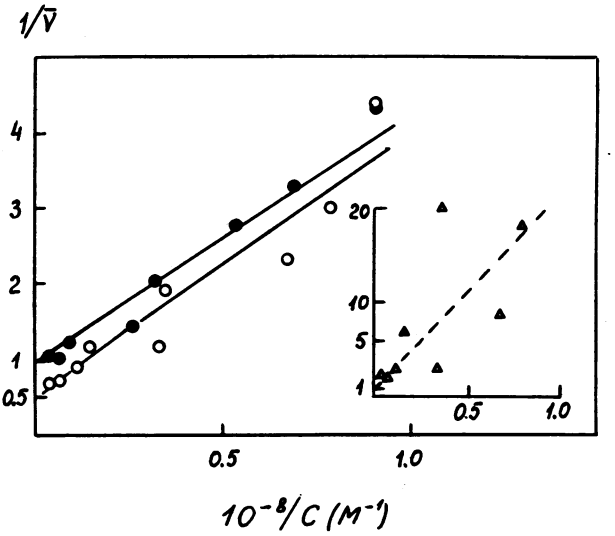

Fig.2. Titration of the 30S·poly(U) complex by N-Acetyl-Phe-tRNA^{Phe} (presented as I/$\bar{\nu}$ versus I/C plots): in the absence of TET (-o-, for I/$\bar{\nu}^{A}$); in the presence of TET (-●-, for I/$\bar{\nu}^{P}$). Insert: I/$\bar{\nu}$ a versus I/C.

ated formation of peptidyl-puromycin on the same ribosome [20]. To prevent this side-effect, an excess of deacylated nonspecific tRNA (tRNA^{-Phe}) was added simultaneously with puromycin [21]. We see, actually, that tRNA^{-Phe} decreases slightly the yield of Ac-^{14}C-Phe-PM, and a 15-fold excess is sufficient for its maximal action (-■-). One can conclude from these results that N-Acetyl-Phe-tRNA^{Phe} was initially prebound to a part of the P site and the latter is equivalent to the TET-resistant site.

Another approach to the functional identification of both sites on the 30S subunit is demonstrated in Fig.4. A 30S·poly(U) complex charged with two molecules of ^{14}C-Phe-tRNA^{Phe} was formed, and 1.5 excess of 50S subunits was added; no stimulation of aminoacyl-tRNA binding was observed, as both vacant sites were already filled (-o-). In the course of prolonged incubation aliquotes of the reaction mixture were taken to detect the formation of (Phe)$_2$-tRNA^{Phe} by paper chromatography. In this experiment, as a result of the high concentration

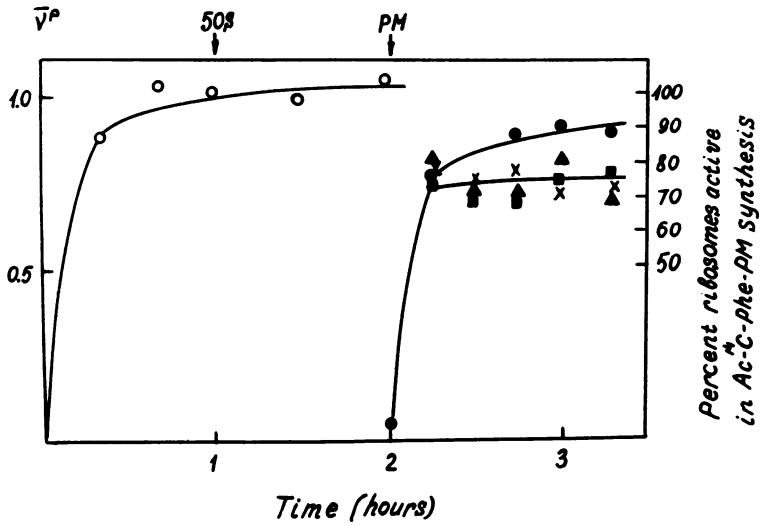

Fig.3. Identification of the TET-resistant site on 30S subunits Initial mixture contained in 7 ml of Buffer I: 350 pmole of 30S subunits, 350 μg poly(U) and 1600 pmole N-Acetyl-Phe-tRNA^{Phe} in the presence of TET (final concentration 3×10^{-5} M). During incubation at 0° aliquotes (200 μl) were taken to measure the kinetics of peptidyl-tRNA binding (step I, -o-). After 60 min incubation a 1.5-fold excess of 50S subunits was added, and the measurement of kinetics was continued for another hour (step II, -o-). Then the reaction mixture was divided into several portions, puromycin was added (final concentration 2×10^{-4}M) without or with different amounts of tRNA^{Phe}, and the kinetics of Ac-^{14}C-Phe-PM formation was measured at 30° (step III): in the absence of tRNA^{Phe} (-●-); in the presence of a 15-fold (-■-), 30-fold (-▲-) and 50-fold excess of tRNA^{Phe} (-x-).

of aminoacyl-tRNA and, at the same time, low excess of it (see legend to Fig.4), 82% of ^{14}C-Phe-tRNA^{Phe} was bound, but 18% remained in solution. For this reason we subtracted 18% of the total ^{14}C-radioactivity, recovered in the "Phe" peak on the chromatograms, and then calculated the fraction of 70S ribosomes bearing $(Phe)_2$-tRNA^{Phe}. We see, from Fig.4 and Fig.6, that 75% of ribosomes are competent in peptide bond formation at 0° (-●-) and 90% - at 30° (-▲-). So far as $(Phe)_2$-tRNA^{Phe} was unable to react with puromycin (data not shown), it means that we have a practically homogeneous population of ribosomes

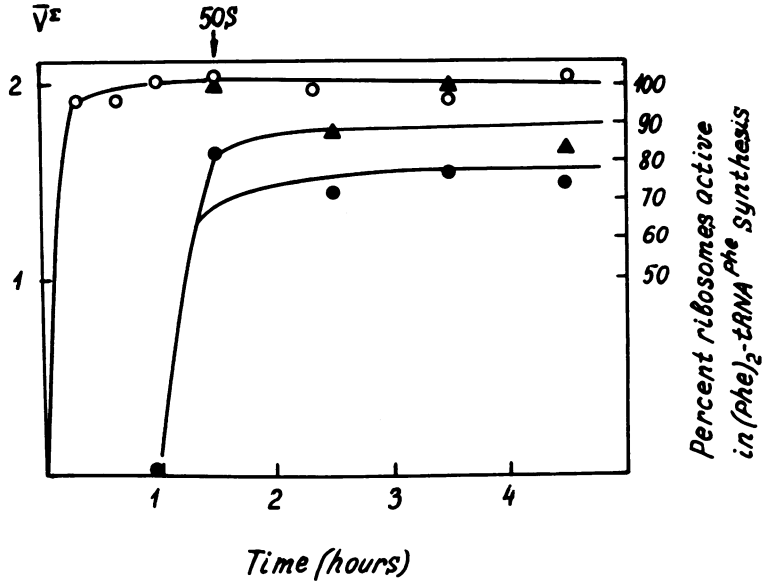

Fig.4. Determination of the fraction of 70S ribosomes active in $(Phe)_2$-$tRNA^{Phe}$ synthesis. Initial mixture contained in 800 μl of buffer I: 600 pmole 30S subunits, 80 μg poly(U) and 1460 pmole ^{14}C-Phe-$tRNA^{Phe}$. During incubation at 0° aliquotes were taken to measure the kinetics of binding of aminoacyl-tRNA (stepI,-o-). After I h incubation a I.5-fold excess of 50S subunits was added, and the reaction mixture was divided into several portions. The first one was used for the further measurement of kinetics of binding (step II,-o-) and kinetics of of Phe_2-$tRNA^{Phe}$ formation, both at 0° (-•-, see for details legend to Fig.6). The second portion was used for a similar measurement of kinetics of $(Phe)_2$-$tRNA^{Phe}$ formation at 30° (-▲-). The third portion was used for sedimentation analysis of the complex (see Fig.5).

in pretranslocation state. A minor fraction of ribosomes bears two separate molecules of aminoacyl-tRNA; obviously their peptidyltransferase centers are inactive. Again we draw the conclusion that the two binding sites for tRNA on 30S subunits form conventional A and P sites after the addition of 50S subunits. In a control experiment we observe that 30S subunits charged with two molecules of Phe-$tRNA^{Phe}$, are readily and quantitatively reassociated with vacant 50S subunits (Fig.5).

In Fig.7 $I/\bar{\nu}$ versus I/C plots are shown for Phe-$tRNA^{Phe}$

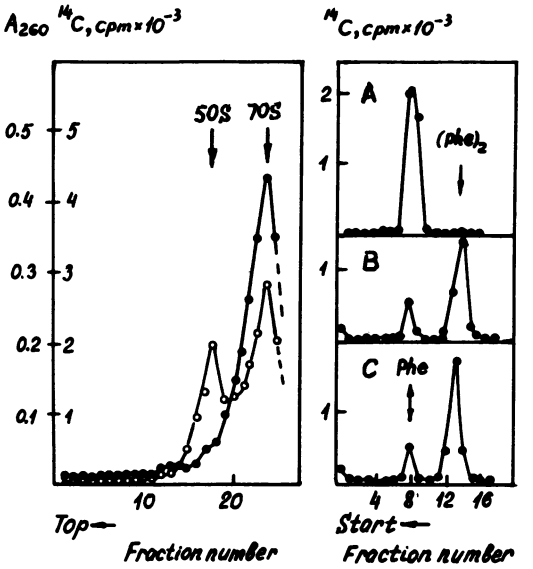

Fig.5 Fig.6

Fig.5. Sedimentation of 70S·poly(U)·(Phe-tRNA^{Phe})$_2$ complex. 200 μl of incubation mixture (see for details legend to Fig.4) were run through a sucrose gradient 5-20%. Sedimentation was performed in a SW-65 Ti rotor (Spinco Model L 5-65) at 40,000 RPM and 5° during 2.5 h. Fractions were analysed for A_{260} (-o-) and ^{14}C-radioactivity (-●-).

Fig.6. Determination of the fraction of 70S ribosomes active in (Phe)$_2$-tRNA^{Phe} synthesis. To the 30 μl aliquotes of the reaction mixture (see for details legend to Fig.4) NH_4OH was added to a final concentration I M; then they were incubated 45 min at 37° and neutralized by 2N CH_3COOH to pH 5. Ascending chromatography was performed using FN-15 paper (GDR) in a system: n-butanol/acetic acid/water (4:1:5, v/v/v). A - control: ^{14}C-radioactivity from the complex 30S·poly(U)·(Phe-tRNA^{Phe})$_2$ after 60 min incubation (the end of step I, Fig.4). B - ^{14}C-radioactivity from the complex 70S·poly(U)·(Phe-tRNA^{Phe})$_2$ after 60 min incubation at 0° (step II, Fig.4). C - the same as B, but at 30°. Similar chromatograms after 2,3 and 4 h incubation are not shown.

at 10 and 5 mM Mg^{2+}. Data computed from this Fig. and Fig.1 are summarized in Table I. We see, that the affinity of aminoacyl-tRNA to the P site of a 30S·poly(U) complex is higher at all concentrations of Mg^{2+} studied.

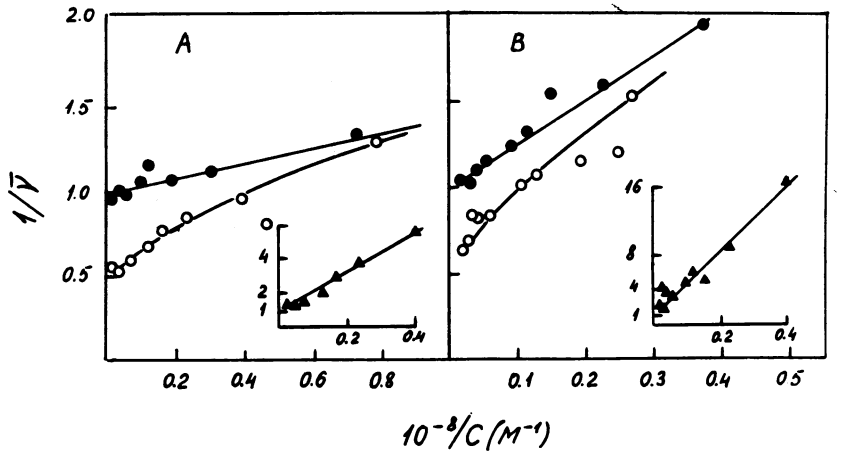

Fig.7. Titration of the 30S·poly(U) complex by ^{14}C-Phe-tRNA^{Phe} at different concentrations of Mg^{2+} ions. All details are the same as in Fig.I. A - IO mM Mg^{2+}, B - 5 mM Mg^{2+}. Inserts: $I/\bar{\nu}$ a versus I/C plots for TET-sensitive binding.

DISCUSSION

a) How many sites for transfer RNA does contain a 30S subunit?

It is established and generally accepted that a 70S ribosome contains two well defined sites: P (donor) and A (acceptor). The situation is much more complicated in case of 30S subunits. Different and contradictory results were reported, that 30S subunit contains: i) one, A site [22]; ii) one, P site

Table I. K^p, K^a, M^p and M^a values calculated from the data of Fig.I and Fig.7.

Expt. N°	Mg^{2+}, mM	$K^p \times 10^{-8}$, M^{-1}	M^p	$K^a \times 10^{-7}$, M^{-1}	M^a
I	20	2.5	I	2.2	I
2	IO	2.4	I	0.9	I
3	5	0.4	I	0.25	I

[23,24]; iii) both A and P sites [2]; iv) one "hybrid" site [25].

At recent few years a vast number of investigations were performed, using the technique of chemical modification of ribosomal proteins, to identify those which contribute to the formation of tRNA-binding centers on ribosome and, particularly, on their 30S subunit. It appeared that up to 15 S-proteins participate in this function - a "disheartening" number [26] for the proper binding of one small molecule of transfer RNA. We shall not discuss this problem - it is well reviewed by Johnson et al [28], Fanning et al [26], Pongs [27]. It is essentially that several groups of authors [26,27], taking into account topographycal models of 30S subunits [29,30], came to the conclusion, that they must contain two physically distinct sites for transfer RNA.

Our results are a direct evidence for the two-site model of 30S subunits. Functional tests showed that none of the sites observed had artifact origin because: i) both sites are messenger-dependent; ii) the site with a higher affinity to tRNA was identified as a P site: it was not influenced by low concentrations of TET and, on the other hand, N-Acetyl-Phe-$tRNA^{Phe}$, initially prebound to the 30S·poly(U) complex in the presence of TET, reacted quantitatively with puromycin after the addition of 50S subunits; iii) the site with lower affinity to tRNA revealed features of A site as binding of aminoacyl-tRNA (as well as peptidyl-tRNA) was totally prevented by TET; iv) the binding of two molecules of Phe-$tRNA^{Phe}$ to the 30S·poly(U) complex followed by the addition of 50S subunits resulted in the formation of $(Phe)_2$-$tRNA^{Phe}$ almost in all ribosomes.

Surprisingly, the interaction of tRNA with the P site, at all concentrations of Mg^{2+}, appeared stronger, than with the A site. One could think that this result is in conflict with the firmly established fact that, in the course of protein synthesis, aminoacyl-tRNA binds only - by definition - to the decoding, or acceptor site of the ribosome. This contradiction can be resolved by the following way: i) initiation is the only step in protein biosynthesis, where 30S subunits function in isolated state; they contain a binding site for initiator tRNA,

which coinsides or closely overlaps with the P site [27]. It is interesting to note that in the absence of initiation factors this site does not discriminate between an analogue of initiator peptidyl-tRNA, N-Acetyl-Phe-$tRNA^{Phe}$, and aminoacyl-tRNA. Moreover, the affinity of aminoacyl-tRNA to the P site is higher than that of peptidyl-tRNA (compare Fig.I and Fig.2).
ii) on the other hand, during the elongation step the binding of aminoacyl-tRNA takes place only to the entire 70S ribosome, exclusively in posttranslocation state; the P site is occupied by peptidyl-tRNA, and only the "weak" A site is vacant. It is likely that the elongation factor $EF\text{-}T_u$ is involved in aminoacyl-tRNA selection to increase the affinity of the cognate aminoacyl-tRNA to the A site of ribosome.

Thus, both 30S and 50S subunits contain parts of P and A sites of the ribosome. In a simplified model system (30S·poly(U)·$tRNA^{Phe}$) tRNA has a higher affinity to the P site. This difference is even pronounced in the case of a 70S·poly(U)·$tRNA^{Phe}$ complex.

b) Problem of active ribosomes

In virtually all of the experiments so far reported, only a small fraction of 30S subunits, even thermally reactivated, was active in codon-dependent tRNA binding: $\bar{\nu}^{\Sigma}$ value varied, as a rule, from 0.I to 0.5 instead of $\bar{\nu}^{\Sigma}_{max} = 2.0$. It means that, in the course of separation and purification of subunits, their P and A sites were irreversibly and to a great extent inactivated. All reasons of subunits inactivation are far from being understood, but some of them were recently elucidated. For example, i) application of high centrifugal fields, in buffers with I mM Mg^{2+} and 0.5-I.0 M NH_4^+, causes additional dissociation of some S-proteins off 30S subunits. It decreases the affinity of Phe-$tRNA^{Phe}$ to the 30S·poly(U) complex, but does not change its active fraction [7]; ii) an increase of NH_4^+ concentration (other conditions being equal) during the purification of 30S subunits yields the same effect [8]; iii) incubation of 30S subunits in media with low Mg^{2+} concentration (I mM) and too low NH_4^+ concentrations (I00-20 mM) leads to

irreversible inactivation of a considerable fraction of 30S subunits [8]. For this reason we used routinely concentrations of NH_4Cl within 200-500 mM in all stages of subunits isolation beginning with cells disruption (see,for details,"Materials and Methods").

There exist also another, seeming source of low activity of 30S subunits - the underestimation of the real fraction of active ones. For a correct determination of $\bar{\nu}^{\pm}_{max}$ values it is necessary: i) to use enriched preparations of specific aminoacyl- or peptidyl-tRNA's, as the presence of deacylated tRNA's inhibits maximal binding; ii) to use fractionated messenger RNA's (if synthetic ones are used) of moderate molecular weight to avoid the formation of aggregates of 30S subunits with too long polynucleotide chains; iii) it is desirable to represent the binding isotherms in inverse coordinates for correct extrapolation of $\bar{\nu}^{\pm}$ to maximal values when $C \to \infty$.

In conclusion, we must emphasize, that 100% activity of 30S subunits in binding tests, as well as linearity of $I/\bar{\nu}$ versus I/C plots,does not yet mean adequately that these subunits are structurally homogeneous. This question was discussed in detailes in our preceding paper [7].

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REFERENCES

1 Pestka,S., Nirenberg,M. (1966) J.Mol.Biol. 21, 145-161.
2 Ginzburg,I., Miskin,R., Zamir,A. (1973) J.Mol.Biol. 79, 481-493.
3 Blumberg,B., Nakamoto,T., Goldberg,J. (1975) Biochemistry 14, 2889-2894.
4 Suzuka,I., Kaji,H., Kaji,A. (1966) Proc.Natl.Acad.Sci.USA 55, 1483-1490.
5 Igarashi,K., Kaji,A. (1970) Eur.J.Biochem. 14, 41-48.
6 Kirillov,S., Makhno,V., Semenkov,Yu. (1978) Eur.J.Biochem. 89, 297-304.
7 Kirillov,S., Makhno,V., Peshin,N., Semenkov,Yu. (1978) Mol.Biol. (Mosc.) 12. 602-611.
8 Kirillov.S.,Peshin.N.(1979) Mol.Biol.(Mosc.) 13,752-759.
9 Eikenberry,E., Bickle,T., Traut,R., Price,C. (1970) Eur.J.Biochem. 12, 113-116.

I0 Staehelin,T., Maglott,D. (I97I) in Methods in Enzymology vol.XX, part C, pp 449-455, Academic Press, New-York.
II Semenkov,Yu., Makhno,V., Kirillov,S. (I976) Mol.Biol. (Mosc.) I0, 754-763.
I2 Kirillov,S., Makhno,V., Semenkov,Yu. (I976) Dokl.Acad. Nauk SSSR 229, 488-49I.
I3 Rappoport,S., Lapidot,Y. (I974) in Methods in Enzymology, vol XXIX, part E, pp 685-693, Academic Press, New-York.
I4 Nirenberg,M., Leder,P. (I964) Science I45, I399-I405.
I5 Černa,I., Rychlik,I., Pulkrabek,P. (I969) Eur.J.Biochem. 9, 27-35.
I6 Leder,P., Burzstyn,H. (I966) Bioch.Biophys.Res.Communs. 25, 233-237.
I7 Grajevskaja,R., Odintsov,V., Saminsky,E., Bresler,S. (I973) FEBS Lett. 33, II-I4.
I8 White,J., Cantor,C. (I97I) J.Mol.Biol. 58, 397-4I5.
I9 Streltsov,S., Kukhanova,M., Yursky,Y., Krajevsky,A., Beljanskaya,J.,Victorova,L., Treboganov,A., Gottikh,B. (I975) Mol.Biol. (Mosc.) 9, 9I0-92I.
20 de Groot,N., Panet,A., Lapidot,Y. (I970) Eur.J.Biochem. I5, 2I5-22I.
2I Ivanov,Yu., Grayevskaya,R., Saminsky,E. (I979) Eur.J. Biochem., submitted.
22 Randall-Haselbauer,H., Kurland,C. (I972) Mol.Gen.Genet. II5, 234-244.
23 Kaji,A., Igarashi,K., Ishitsuka,M. (I968) in Cold Spring Harbor Symp.Quant.Biol., vol. XXXIV, pp I67-I77.
24 Weissbach,H., Redfield,B., Yamasaki,E., Brot,N. (I972) Arch.Biochem.Biophys. I49, 560-568.
25 Pestka,S. (I972) J.Biol.Chem. 247, 4669-4678.
26 Fanning,T., Cantrell,M., Shih,C., Craven,G. (I978) Nucl.Acids Res. 5, 933-950.
27 Pongs,O. (I978) in Transfer RNA, pp 78-I04, MIT Press.
28 Johnson,A., Fairclough,R., Cantor,C. (I977) in Nucleic acids-Protein Recognition, pp 469-490, Academic Press, New-York.
29 Tischendorf,G., Zeichhardt,H., Stoffler,G. (I975) Proc. Natl.Acad.Sci.USA 72, 4820-4824.
30 Lake,J., Kahan,L. (I975) J.Mol.Biol. 99, 63I-644.