Comparative study of the interaction of polyuridylic acid with 30S subunits and 70S ribosomes of *Escherichia coli*

V.I.Katunin, Yu.P.Semenkov, V.I.Makhno and S.V.Kirillov

B.P.Konstantinov Nuclear Physics Institute of the Academy of Sciences of the USSR, Gatchina, Leningrad district 188350, USSR

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ABSTRACT

Fractionated polyuridylic acid with an average chain length of 55 nucleotides forms binary complexes with 30S subunits with a stoichiometry of 1:1. These complexes are heterogeneous in stability. The more stable one, is characterized by an association constant $K_2 = 5.5 \times 10^9 \text{ M}^{-1}$, and the less stable - by $K_1 = 10^6 \times \text{M}^{-1}$, at 20 mM Mg^{2+} , 200 mM NH_4^+ and 0° C. The main reason for this heterogeneity is the presence or absence of the ribosomal protein S1 in the subunits. Decrease of Mg^{2+} concentration down to 5 mM hardly changes the K_2 values but reduction of the NH_4^+ concentration to 50 mM results in a 25-fold increase of K_2 . Association constants K_2 for the stable complex, i.e. in the presence of S1 protein, were measured at different temperatures (0 - 30°C) and the thermodynamic parameters of binding (ΔH° , ΔS° , ΔG°) were determined.

Analogous experiments were made with 70S ribosomes. K_2 values as well as ΔH° , ΔS° , ΔG° appeared the same both for 30S and 70S ribosomes in all conditions examined. This is strong evidence that the 50S subunits do not contribute to the interaction of poly(U) with the complete 70S ribosomes.

INTRODUCTION

Interaction of messenger RNA with ribosomes is one of the central events during protein biosynthesis. It is well established that each ribosome contains one binding site for mRNA and several S-proteins, as well as 16S RNA, contribute to the formation of the mRNA-binding centre (1-5). It seems important to develop a thermodynamic approach to study the molecular events of mRNA-ribosome and mRNA-tRNA-ribosome interactions.

Here we report quantitative results on the interactions in different conditions of a widely used synthetic messenger poly(U) with both 30S subunits and 70S ribosomes. The contribu-

tion of SI protein to these interactions was studied.

MATERIALS AND METHODS

Buffer 1: 0.02 M Tris-HCl, pH 7.1 containing 0.02 M $MgCl_2$, 0.001 M EDTA, 0.2 M NH_4Cl (or lower if indicated). Buffer 2: 0.1 M Tris-HCl, pH 8.3, containing 0.005 M $MgCl_2$, 0.001 M EDTA. Fractionated 3H -poly(U). A mixture for the synthesis of 3H -poly(U) contained in 50 ml of buffer 2: 230 mg of 3H -UDP with a specific activity 17 count/min pmole of uridine and 300 units of polynucleotidephosphorylase (PNP, EC. 2.7.7.8). After 2 h incubation at 37°C, 3H -poly(U) was deproteinized twice by phenol extraction and, after two-fold precipitation by 96% ethanol, dissolved in water.

The conditions necessary for a quantitative study of poly(U)-ribosome interaction are: 1) a narrow molecular weight distribution of poly(U) to know its molar concentration ; 2) a poly(U) with moderate molecular weight to avoid the formation of aggregates with several ribosomes.

For this reason poly(U) was subjected to partial alkaline hydrolysis (0.1 N KOH, 15 min at 25°C) and then fractionated on Sephadex G-100. The fraction with a distribution coefficient 0.2 - 0.35 and an average molecular weight 18,000 daltons (as determined by sedimentation analysis (6)) was chosen. Ribosomes and tRNA. Isolation of 30S and 50S subunits, as well as preparation of enriched I^4C -Phe-tRNA^{Phe} and I^2C -Phe-tRNA^{Phe} with a content of Phe 1500 pmole/ A_{260} unit, were described earlier (7,8).

Ribosomal SI protein. To obtain SI protein, 30S subunits were dialysed 24 h at 4°C against 0.001 M Tris-HCl pH 7.4 , as was described by van Duin and van Knippenberg (9). The mixture was layered on a cushion of 20% sucrose in the same buffer and centrifuged for 4.5 h at 100,000 g. The upper layer of supernatant contained pure SI protein, as determined by electrophoresis in polyacrylamide gel in the presence of SDS (10), Fig I , rod 3). Pelleted 30S subunits were depleted of this protein (compare Fig I, rods I and 2). The concentration of SI protein was determined by the reaction with Amidoblack IOB, according to Weissman and Scheffner (11).

Fig.I. Acrylamide-gel electrophoresis in the presence of SDS. 1- standard 30S subunits, 2- SI-depleted 30S subunits, 3 - SI protein.

Binding of ^3H -poly(U) to ribosomes. Free poly(U), unlike other polynucleotides is readily adsorbed on nitrocellulose filters. To measure the association constants of poly(U) to ribosomes, we must separate the binary complex from free poly(U). For this purpose nitrocellulose filters were treated with alkali (12). After 25 min of treatment with 0.5 M KOH at 26°C the Synpore filters preserved up to 65-70% of their ability to bind both 30S and 70S ribosomes, but less than 0.5% of free poly(U).

Adsorption isotherms were studied by two methods: in the first one the volumes of the incubation mixture were constant, but the amounts of ^3H -poly(U) added were different; in the second one the assay mixtures contained equal amounts of ^3H -poly(U), but their volumes were varied. In all conditions used 15 min incubation were sufficient to attain equilibrium.

70S ribosomes were reassociated from isolated 30S and 50S subunits; control sedimentation revealed that at least 95% of the 30S subunits were converted into 70S ribosomes if a 20% excess of 50S subunits was present. Hence, we adopted the amount of 70S ribosomes in the incubation mixture equal to that of 30S subunits.

Materials. ^3H -UDP was purchased from the Institute of Applied

Chemistry (Leningrad, USSR), cold UDP - from " Reanal " (Hungary) and nitrocellulose filters (with pore diameter $0.4 \mu m$) from " Sympore " (CSSR). PNP was a gift of Dr. V. Chernayenko. 3H and ^{14}C -radioactivities were counted in the scintillation counter " Isocap- 300 " (Nuclear Chicago) with an efficiency 30% and 60% respectively.

It was assured for calculations that 1 A_{260} unit corresponds to 75 pmole of 30S subunits, 37 pmole of 50S subunits and 24 pmole of 70S ribosomes and the molar extinction of uridine residues in poly(U) is equal to 8.8×10^3 (13).

RESULTS

Heterogeneity of 30S · poly(U) complexes. If both components of the system are homogeneous, than the reaction of poly(U) with 30S subunits can be described by a Langmuir's isotherm:

$$\bar{n} = \frac{N K [U]}{1 + K [U]}$$

where $\bar{n}$ is the average number of poly(U) molecules bound per one subunit, N - the number of binding sites for poly(U), $[U]$ - the concentration of free poly(U) and K - the association constant of the complex. In reverse coordinates we expect a rectilinear plot of $1/\bar{n}$ versus $1/[U]$:

$$1/\bar{n} = 1/N + 1/N K [U]$$

If two types of binary complexes with different stabilities are formed, overall binding is described by the equation:

$$\bar{n} = \bar{n}_1 + \bar{n}_2 = \frac{N_1 K_1 [U]}{1 + K_1 [U]} + \frac{N_2 K_2 [U]}{1 + K_2 [U]}$$

where N_1 and N_2 - are the fractions of subunits forming respectively weak and stable complexes, K_1 and K_2 - their association constants.

In reality we always find the latter situation: plots of $1/\bar{n}$ on $1/[U]$ are nonlinear, but can be readily approximated by the sum of two rectilinear graphs, as shown on Fig.2. (curve I). Every subunit is able to bind one molecule of messenger, as $\bar{n} = \bar{n}_1 + \bar{n}_2$ tends to unity. The stable component is characterised by $N_2 = 0.57$ and $K_2 = 2.3 \times 10^8 M^{-1}$; for

Fig.2. Titration of 30S subunits by ^3H -poly(U). A. The assay mixture contained in $200\text{ }\mu\text{l}$ of buffer I: 10 pmole 30S subunits and 400 pmole ^3H -poly(U). 1 - standard 30S subunits isolated in sucrose gradient, containing 1 mM MgCl_2 and 500 mM NH_4Cl , 2 - the same as 1, but concentration of NH_4Cl during isolation of subunits was 200 mM, 3 - SI-depleted 30S subunits. B. 1 - to the assay mixture marked I on Fig. 2A (curve I) increased amounts of ^{12}C -Phe-tRNA were added and poly(U) - binding was measured. 2 - stimulation of ^3H -poly(U) binding to 70S ribosomes by ^{12}C -Phe-tRNA. The assay mixture contained in $200\text{ }\mu\text{l}$ of buffer I: 10 pmole 70S ribosomes, 25 pmole ^3H -poly(U) and 0 - 25 pmole ^{12}C -Phe-tRNA.

the weak component corresponding values equal : $N_1 = 0.43$ and $K_1 = 1.5 \times 10^6 \text{M}^{-1}$. At moderate concentrations of poly(U) (point I, curve I) $\bar{n}$ approximates to unity, if a sufficient concentration of aminoacyl-tRNA is added (Fig. 2B, curve I). We observe here a tRNA-dependent binding of mRNA. Similar results were described earlier by Hatfield and Nirenberg, in a system with short oligonucleotides and aminoacyl-tRNA's (14).

Experiments described above were performed with standard preparations of 30S subunits, which were isolated from 70S ribosomes in a buffer with 1 mM Mg^{2+} and 500 mM NH_4^+ . When the concentration of NH_4Cl was reduced to 200 mM (other conditions being identical), the content of the more stable component increased to 80% (Fig. 2A, curve 2). If subunits exhausted for SI protein were used (see " Materials and Methods "),

then N_2 values decreased to 0.17 (Fig. 2A, curve 3).

This result is a strong argument in favour of the explanation that heterogeneity of 30S · poly(U) complexes is due to a deficiency of SI protein in the subunits. This protein is called "fractional" because it is never found in stoichiometric amounts in isolated subunits (9,15,16). If labelled, it readily exchanges between ribosomes and free protein in solution (17). For this reason its content in 30S subunits can be varied widely depending on the particular methods and conditions of subunits isolation. This alone may explain the difference between curve 1 and 2. (Fig. 2A).

In Fig. 3 we see that addition of SI protein stimulates the binding of ^3H -poly(U) to 30S subunits. As this protein is quantitatively adsorbed on nitrocellulose filters (12) and forms binary complexes with poly(U) in the absence of ribosomes (18), the true value of ^3H -poly(U) bound to 30S subunits is the difference between corresponding ordinates of curve 1 and curve 2. The resulting stimulation of binding is shown in Fig. 3B. We see that 40 pmole of SI protein per 100 μl of the mixture are sufficient for stoichiometric binding of ^3H -poly(U)

Fig.3. Stimulation of ^3H -poly(U) binding to 30S subunits by SI protein. A. The assay mixture contained in 100 μl of buffer I: 10 pmole 30S subunits, 25 pmole ^3H -poly(U) and 1 - 40 pmole SI protein (1). 2 - the same as 1 except 30S subunits. B. The quantity of ^3H -poly(U) in the 30S · poly(U) complex ($\bar{n}$) was calculated from curves 1 and 2.

to 30S subunits at low concentration of the former.

The experiment presented in Fig. 4 was identical with that in Fig. 2, but saturating amounts of SI protein were added in all probes. As expected, the less stable component completely disappeared (curve 2). This means that SI protein increases the association constant of poly(U) to 30S subunits at least 100-fold.

Measurement of ^3H -poly(U)- SI protein interaction. The "background " curve 2 in Fig. 3A is in fact the dependence of SI . poly(U) complex formation on SI protein concentration. It is possible to titrate a constant amount of SI protein by increasing quantities of ^3H -poly(U). Then we obtain a linear plot of $I/\bar{v}$ versus $I/[U]$ (Fig. 5) ($\bar{v}$ - is the mean number of ^3H -poly(U) molecules bound per SI molecule) and determine the maximal amount of ^3H -poly(U) bound per molecule of SI protein $\bar{v}_{\max} = 0.26$. It means that only 26% of the SI protein present in solution was active in the binding. The association constant of SI protein with ^3H -poly(U) (chain length about 55 nucleotides) is equal to $(4.7 \pm 0.4) \times 10^7 \text{ M}^{-1}$. This value is in good agreement with the data of Gassen et al. (19) for polynucleotides of about the same chain length $(4 \times 10^7 \text{ M}^{-1})$.

Fig.4. Titration of 30S subunits by ^3H -poly(U). 1 - without SI protein (from Fig. 2A, curve 1), 2 - in the presence of saturating amounts of SI protein.

Fig.5. Titration of SI protein by ^3H -poly(U). The assay mixture contained in $100\ \mu\text{l}$ of buffer I: 40 pmole of SI protein and 4-30 pmole ^3H -poly(U). After 15 min incubation at 0°C the mixture were filtered through alkali pretreated nitrocellulose filters.

Effect of external conditions on the stability of 30S·poly(U) complexes. The effect of Mg^{2+} and NH_4^+ concentrations, temperature and para-chlormercuribenzoate (PCMB) on the stability of 30S·poly(U) complexes was studied only for 30S subunits saturated with SI protein.

As can be seen from the data presented in Table I (line I-4, 7 - 9), a decrease of Mg^{2+} concentration from 20 mM to 5 mM does not change significantly the binding constant of poly(U) to 30S subunits at 0°C and 30°C .

Fivefold reduction of NH_4^+ concentration (from 250 mM to 50 mM) results in a 25-fold increase of the association constant of poly(U) with 30S subunits (from $5 \times 10^7\ \text{M}^{-1}$ to $1.3 \times 10^9\ \text{M}^{-1}$, see Fig.6 and Table I (line 10 - 13)). Fig.7 shows these results as a $\log K_2$ versus $\log [\text{NH}_4^+]$ plot. We see that $\log K_2$ is a linear function of $\log [\text{NH}_4^+]$.

Fig.8 shows the temperature dependence of the association

Table I. Influence of external conditions on the stability of binary complexes 30S · poly(U).

exper.	pH	temperature °C	NH ₄ ⁺ mM	Mg ²⁺ mM	K ₂ M ⁻¹
I	7.1	0	200	20	(6.8 ± 1.1) × 10 ⁹
2	"	"	"	15	(4.6 ± 0.4) × 10 ⁹
3	"	"	"	10	(6.5 ± 1.1) × 10 ⁹
4	"	"	"	5	(1.8 ± 0.2) × 10 ⁹
5	"	"	100	20	(1.7 ± 0.4) × 10 ¹⁰
6	"	"	50	"	(~ 1) × 10 ¹¹
7	"	30	200	"	(8.2 ± 2.0) × 10 ⁷
8	"	"	"	15	(1.1 ± 0.3) × 10 ⁸
9	"	"	"	10	(1.2 ± 0.3) × 10 ⁸
10	"	"	250	20	(5.0 ± 1.0) × 10 ⁷
11	"	"	150	"	(3.7 ± 0.7) × 10 ⁸
12	"	"	100	"	(4.7 ± 0.5) × 10 ⁸
13	"	"	50	"	(1.3 ± 0.3) × 10 ⁹
14	"	25	200	"	(3.2 ± 0.5) × 10 ⁸
15	"	21	"	"	(5.4 ± 0.8) × 10 ⁸
16	"	11	"	"	(2.3 ± 0.8) × 10 ⁹
17	6.6	30	"	"	(8.4 ± 1.3) × 10 ⁷
18	7.4	"	"	"	(9.7 ± 1.7) × 10 ⁷
19	8.1	"	"	"	(8.8 ± 1.5) × 10 ⁷

constant of 30S · poly(U) complexes. Using Vant Hoff's equation:

$$\log K = - \frac{\Delta H^{\circ}}{2.303 R T} + \frac{\Delta S^{\circ}}{2.303 R}$$

where ΔH° and ΔS° are standard enthalpy and entropy changes during complex formation, R - the gas constant, we calculated the thermodynamic parameters ΔH° and ΔS° of poly(U) binding to 30S subunits and total 70S ribosomes (Table 3).

Fig.9 demonstrates the independence of the stability of 30S · poly(U) complexes of pH in the range 6.6 - 8.1.

PCMB treatment decreases Phe-tRNA binding to 70S ribosomes (20), affecting some SH-containing proteins which contribute

Fig.6. Influence of different conditions on the association constant of stable 30S · poly(U) complexes. A. The assay mixture contained in 0.2 - 35 ml: 10 pmole of 30S subunits, 10 - 15 pmole 3H -poly(U). 1 - binding at 0°C in buffer I, 2 - the same as 1, but with 100 mM NH_4Cl , 3 - the same as 1, but with 50 mM NH_4Cl . B. Titration of 30S subunits by 3H -poly(U) at 30°C buffer I. 1 - concentration of NH_4Cl 250 mM, 2 - 200 mM, 3 - 100 mM, 4 - 50 mM.

Fig.7. Effect of ionic strength on poly(U) binding to 30S subunits and 70S ribosomes. 1 - at 0°C, 2 - at 30°C, - Δ - 30S subunits, - $\circ$ - 70S ribosomes.

Fig.8. The temperature stability of 30S·poly(U) and 70S·poly(U) complexes. 1 - 200 mM NH_4Cl , 2 - 50 mM NH_4Cl , Δ - 30S subunits, $\circ$ - 70S ribosomes.

to the aminoacyl-tRNA binding center. In turn, the treatment of 30S subunits by PCMB at 2×10^{-4} M for 5 min results in a drop of poly(U) binding (Fig.10). PCMB affects only the stable complex decreasing its association constant by three orders of magnitude (from $5 \times 10^9 \text{ M}^{-1}$ to $2.6 \times 10^6 \text{ M}^{-1}$). Thus practically all PCMB treated 30S subunits bind poly(U) with $K_I = 2.6 \times 10^6 \text{ M}^{-1}$ i.e. almost the same association constant as SI-depleted 30S subunits (Fig.2A). It is worth noting that after PCMB treatment there is always some 8 - 17% of stable complexes left

Fig.9. Effect of pH on the stability of 30S·poly(U) complexes. Binding was performed at 30°C in buffer I.

Fig.10. Influence of PCMB on the binding of poly(U) to 30S subunits. 1 - adsorption isotherm of ^3H -poly(U) to 30S subunits pretreated by PCMB at 2×10^{-4} M for 5 min at 0°C . 2 - without PCMB treatment (from Fig.6A, curve 1). Incubation mixture contained in $200\ \mu\text{l}$ of buffer I: 10 pmole of 30S subunits, 2 - 200 pmole ^3H -poly(U). Incubation at 0°C for 15 min.

(results not shown).

Poly(U) binding to 70S ribosomes. Fig.2B (curve 2) shows that every 70S ribosome is active in poly(U) binding. The presence of 50S subunits does not contribute to the association constant. Titration curves $1/\bar{n}$ versus $1/[U]$ for 70S ribosomes are almost identical to those for 30S subunits with nearly the same ratio of loose to stable components. The value K_1 and K_2 for 70S ribosomes and 30S subunits are very close, e.g. at 200 mM NH_4Cl , 20 mM MgCl_2 and 0°C : K_1 (70S) $\sim 10^6\ \text{M}^{-1}$, K_2 (70S) $= (5.5 \pm 0.7) \times 10^9\ \text{M}^{-1}$, K_1 (30S) $= 1.5 \times 10^6\ \text{M}^{-1}$, K_2 (30S) $= (6.8 \pm 1.1) \times 10^9\ \text{M}^{-1}$.

The influence of external conditions on 70S $\cdot$ poly(U) complexes is shown in Fig.6,7,8,11 and Table 2 and 3 (only for the stable component). All data for 70S ribosomes nearly coincide with those for 30S subunits.

DISCUSSION

Poly(U) directed cell-free systems are widely used for the investigation of protein biosynthesis. For the quantitative study of the mechanism of codon-anticodon interaction in

Fig.II. Titration of 70S ribosomes by ^3H -poly(U). The assay mixture contained, in 0.2 - 35 ml : 10 pmole of 70S ribosomes, 10 - 15 pmole of ^3H -poly(U). 1 - measurement of binding at 0°C in buffer I, 2 - the same as 1, but with 100 mM NH_4Cl , 3 - the same as 1, but with 50 mM NH_4Cl .

Table 2. Influence of external conditions on the stability of binary complexes 70S · poly(U).

exper.	temperature $^\circ\text{C}$	NH_4^+ mM	Mg^{2+} mM	$K_2 \text{ M}^{-1}$
I	0	200	20	$(5.5 \pm 0.7) \times 10^9$
2	"	"	10	$(7.9 \pm 1.6) \times 10^9$
3	"	"	5	$(6.7 \pm 1.3) \times 10^9$
4	"	400	20	$(3.1 \pm 0.6) \times 10^9$
5	"	150	"	$(9.0 \pm 3.1) \times 10^9$
6	"	100	"	$(2.5 \pm 0.7) \times 10^{10}$
7	"	50	"	$(5.0 \pm 1.4) \times 10^{10}$
8	20	"	"	$(4.6 \pm 0.7) \times 10^9$
9	30	250	"	$(7.1 \pm 1.2) \times 10^7$
10	"	200	"	$(2.2 \pm 0.3) \times 10^8$
11	"	150	"	$(1.6 \pm 0.4) \times 10^8$
12	"	100	"	$(7.4 \pm 1.7) \times 10^8$
13	"	50	"	$(1.0 \pm 0.2) \times 10^9$
14	25	200	"	$(1.3 \pm 0.3) \times 10^8$
15	20	"	"	$(7.6 \pm 1.4) \times 10^8$
16	11	"	"	$(1.2 \pm 0.3) \times 10^9$

Table 3. Thermodynamic quantities of poly(U) binding to 30S subunits and 70S ribosomes of *Escherichia coli*.

	ΔH° , $\frac{\text{kcal}}{\text{mole}}$	ΔS° , $\frac{\text{cal}}{\text{mole degr.}}$
30S subunits 200 mM NH_4^+	$-(23 \pm 3)$	$-(39 \pm 10)$
70S ribosomes 200 mM NH_4^+	$-(19 \pm 4)$	$-(27 \pm 13)$
70S ribosomes 50 mM NH_4^+	$-(21 \pm 2)$	$-(26 \pm 6)$

ribosomes it is very important to be sure that every active ribosome is attached to a poly(U) molecule. For this purpose it is necessary to fulfill the criterion $K \times [U] \gg 1$ (21), where K - is the association constant of poly(U) with ribosomes and $[U]$ - the concentration of poly(U). We used poly(U) with a mean molecular weight of 18,000 daltons and detected the formation of two types of 30S · poly(U) complexes with different association constants: $K_1 = 1.5 \times 10^6 \text{ M}^{-1}$ and $K_2 = 2.7 \times 10^8 \text{ M}^{-1}$. The ratio of both components depends on the conditions of isolation and washing of the 30S subunits. For example, if we change the concentration of NH_4Cl in the sucrose gradient during subunit isolation from 500 to 200 mM, it results in an increasing amount of SI protein in the subunits. Hence the fraction of stable complexes changes from 0.57 to 0.8. For SI-depleted 30S subunits only 0.17 of the stable component was formed (Fig. 1A). A direct experiment (Fig 4) confirmed that the main reason for the heterogeneity of 30S · poly(U) complexes is the absence or presence of SI protein in the subunits. The ribosomal protein SI plays a central role in protein synthesis (22). It is required for the binding and translation of poly(U) and natural messenger RNA's (2,9,18, 22). It is a "fractional" protein but was found in approximately stoichiometric amounts in polysomes, i.e. functional

ribosomes. Some data on the SI function in protein biosynthesis seem controversial. Van Duin and Kurland (2) showed that SI improved binding of Phe-tRNA to 30S subunits because of stimulation of poly(U) binding to the subunits. On the other hand Fanning et al. (23) presented some evidence that SI is a tRNA-binding protein, and Lind et al. (24) showed that SI is not required for oligo(U) binding to 30S subunits, but is very efficient in oligo(U) or poly(U)-stimulated binding of Phe-tRNA to 30S subunits.

The results of our earlier studies (2I) and the direct measurements presented in this paper indicate that SI protein modifies strongly the association constant of poly(U) with 30S subunits and 70S ribosomes but does not affect Phe-tRNA binding as such. The deficiency of SI protein can be fully compensated by a high molar concentration of poly(U). Then the criterion $K \times [U] \gg I$ is fulfilled and the binding of Phe-tRNA to 30S subunits becomes independent of SI. If $K \times [U] \leq I$, we observe a mutual stimulation of Phe-tRNA and poly(U) binding to 30S subunits (Fig. IB, curves I and 2) or respectively a SI and tRNA stimulation, as was observed by Lind et al. (24). At saturating poly(U) concentrations every 30S subunit binds one poly(U) molecule independently of Phe-tRNA concentration (Fig.I2).

The binding of poly(U) is very dependent upon the ionic strength. The logarithm of the equilibrium constant is proportional to the logarithm of ammonium concentration (Fig.7). Another effect of ionic strength reduction is the increase in the fraction of stable complexes (from 0.5 to 0.9, Fig.6). This effect of ionic strength is equivalent to SI addition. The large reduction of K with increasing ionic strength indicates a very substantial contribution of electrostatic forces to the binding energy. Record et al. (25) studied the effect of ionic strength on DNA and RNA-protein interactions and showed that $\delta \log K / \delta \log [M^+] = -m' \psi$, where K is the observed association constant, $[M^+]$ is the concentration of monovalent cations, ψ accounts for the screening of the phosphate groups in poly(U) by counterions (for poly(U) $\psi = 0.68$ (25)), m' is the number of ionic bonds involved in the binding of the

Fig.12. Binding isotherm of ^{14}C -Phe-tRNA to 30S. 3H -poly(U) complexes. 1 - binding of ^{14}C -Phe-tRNA, 2 - 3H -poly(U) binding. The incubation mixture contained, in 400 μ l of buffer I: 10 pmole of 30S subunits, 150 pmole 3H -poly(U) and 4 - 30 pmole ^{14}C -Phe-tRNA.

ligand to the nucleic acid. Application of this equation to our data yields a value of approximately 2.6 ± 0.4 ionic bonds participating in the binding of a poly(U) molecule to a ribosome. Comparison of ΔG^0 values of higher and lower concentrations of ammonium ions enables us to estimate separately the electrostatic and non-electrostatic components of the free energy of ribosome-poly(U) complex formation. The non-electrostatic interaction is more essential :

$$-\Delta G_I^0 \text{ } M \text{ } NH_4^+ = 9.5 \pm 0.3 \frac{\text{kcal}}{\text{mole}}, -\Delta G_{0.2}^0 \text{ } M \text{ } NH_4^+ = 11.3 \pm 0.5 \frac{\text{kcal}}{\text{mole}}.$$

The interaction of poly(U) with ribosomes does not depend on pH in the pH range 6.6 - 8.1. Thus it is possible that lysine amino groups are specifically involved in poly(U) binding (26). Participation of lysine amino groups in mRNA binding is confirmed by data on chemical modification of ribosomal proteins (4).

The effect of blocking of ribosomal SH-groups is various - from inhibition to stimulation of overall polypeptide synthesis (27,28,30,31,32). The same is true in respect to 30S subunits.

In the presence of elongation factors the 30S subunit has been identified as being responsible for inhibitory (27,30), but in the case of factor-free system- for stimulative effect of PCMB (31,32). In our work high concentration of PCMB ($2 \times 10^{-4} M$) was used, so we assumed that the saturation number of reacted SH-groups was reached (27), in spite of rather short incubation time. We observed strong inhibition of poly(U) binding to the pretreated by PCMB 30S subunits (Fig.10). Further, PCMB decreases also the association constant of Phe-tRNA^{Phe} with 30S. poly(U) complex in accordance with earlier observation (33), but without changing of the fraction of active subunits (our unpublished data). The relationship between these results and stimulative or inhibitory effects of PCMB on the overall process of polypeptide synthesis (see above) is unclear at the moment and is a matter for further investigations.

Isolated 50S subunits do not bind to mRNA (34) and the latter does not affect tRNA binding to 50S subunits. The observed identity of thermodynamic values for poly(U) interaction with 30S subunits and 70S ribosomes implies that the binding occurs to an active centre in the 30S subunit with no specific interaction with the 50S subunit.

Many other proteins, not only S1, take part in the mRNA binding centres of ribosomes - S4, S11, S12, S13, S18, S21 (3,4). Hence, these proteins must contribute to K_1 and K_2 values. All these proteins (except S4) are "fractional" and their content depends on the method of subunit isolation (15, 16). Moreover, the affinity of poly(U) to ribosomes and the number of interacting S - proteins increases with increasing molecular weight of poly(U) (3,35). All these facts must be taken into account when comparing different data on the binding of poly(U) to ribosomes, as we found earlier for poly(U) with a molecular weight of 30,000 daltons $K_1 \sim 10^7 M^{-1}$ (21). This is almost an order of magnitude higher than reported in this paper. The same situation is true for more stable complexes (S1 containing). K_2 for different preparations of 30S subunits varied between $2.7 \times 10^8 M^{-1}$ and $5.5 \times 10^9 M^{-1}$ due to above mentioned circumstances.

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