AN IMMUNOCHEMICAL APPROACH TO THE STRUCTURE OF THE RIBOSOMAL PARTICLES

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An important aspect of the ribosomal system is related to the topological relationship between the constituents of the system itself. The present work, concerned with certain features of the molecular arrangement between the ribosomal RNA's and proteins, was rendered possible by the use of purified anti-RNA antibodies (Panijel 1963 a,b ; Panijel et al. 1966 a,b; Souleil et al. 1966). The latter, which precipitate RNA's and polyribonucleotides, also precipitate the bacterial and animal ribosomes (Cayeux et al. 1965). We show here that the number of specific antigenic sites accessible to the antibodies differ for the 30 S, 50 S and 70 S particles.

Material and Methods.- The experiments are carried out on the particles and the ribosomal RNA's of E.Coli RNAse I^-_{10} . Crude ribosomes are prepared by conventional procedures and contain 37% protein and 63% RNA. Washed ribosomes are obtained from the crude ribosomes as described by Shin & Moldave (1966) and contain 33% protein and 67% RNA. Crude and washed ribosomes, suspended in K_1 buffer(♦) are purified on a 5-20% linear sucrose gradient. A part of this material is used for preparing 30 and 50 S sub-units (Pestka & Nirenberg 1966). Reassociated ribosomes are obtained by dialysis of a mixture of sub-units in a 1:1 ratio against Tris buffer containing 10 mM Mg^{++} and purified by centrifugation through a sucrose density gradient.

Ribosomal RNA's are extracted from the bacterial homogenate by the phenol-SDS method and separated on sucrose gradient (Midgley 1965 a,b,c).

The antibodies are the NG I antibodies previously used in the immunochemical study of the polynucleotides. The $S_{w,20}$ values are 9.4 ± 0.2 in K_2 buffer(♦), 9.6 ± 0.2 in K_3 buffer(♦), the NG I concentration being 2-3 mg/ml. The immunochemical reactions (reaction time: 30-45 min) and the analysis of the specific precipitates are performed as previously described. In the

(♦) Buffers: Tris-HCl 5mM, pH 7.4, KCl 0.1 M, Mg acetate 10 mM (K_1 buffer) or 1mM (K_2 buffer) or 4 mM (K_3 buffer)

K_2 or K_3 buffers used for the immunochemical reaction, the profiles obtained on sucrose gradient both of the particles and the RNA's are not changed.

Results.- Preliminary experiments show that all the particles are precipitated at 100% when they react with a sufficiently large quantity of NG I antibodies : thus, the sub-units, like the ribosomes themselves, present a certain portion of their RNA accessible to the antibodies. Furthermore, this result eliminates the hypothesis that possible differences in the reactivity of different particles could be due to the presence of antibodies with distinct specificities directed against one of the particles and not the other.

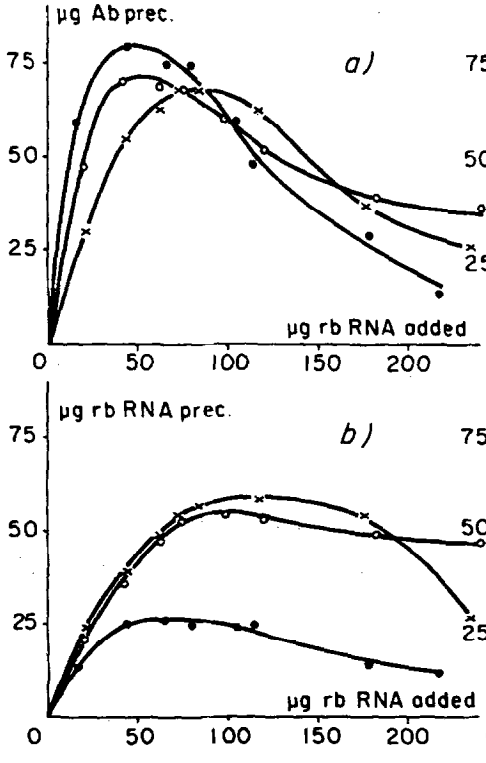

Fig. 1

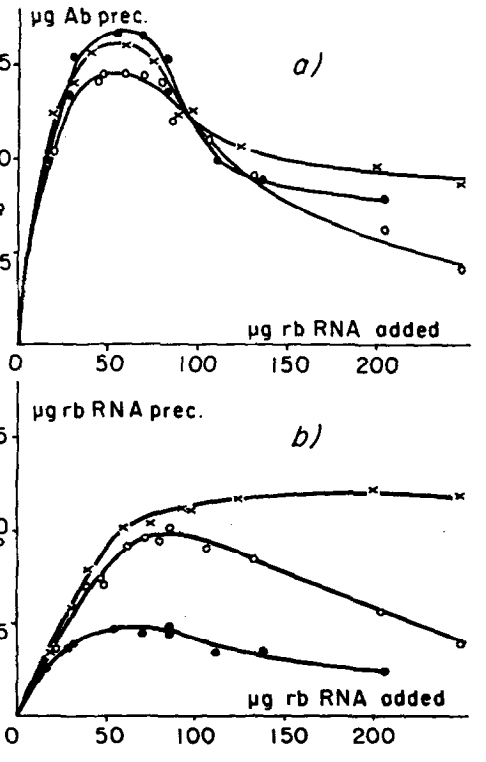

Fig. 2

Fig. 1 - Amounts of antibody (a) and antigen (b) precipitated by crude ribosomal particles in K_2 medium. The particles, estimated in terms of their RNA content (rb RNA) were added in ever-increasing amounts, the amount of antibody being kept constant. All the reactions were carried out in duplicate or triplicate. Symbols: •——• 30 S sub-units ; o——o 50 S sub-units ; x——x 70 S ribosomes.

Fig. 2 - Amounts of antibody (a) and antigen (b) precipitated by washed particles in the K_2 medium. Methods and symbols as in the Fig. 1.

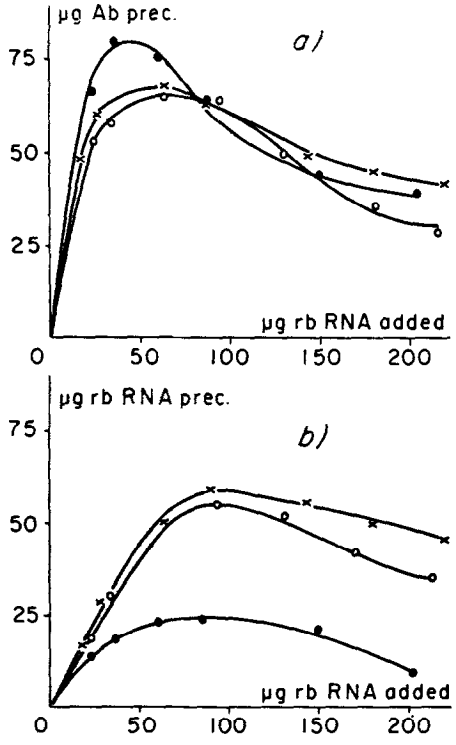

Fig. 3 - Amounts of antibody (a) and antigen (b) precipitated by washed particles in the K_3 medium. Methods and symbols as in the Fig. 1.

TABLE 1

Ratios of precipitated antibody to precipitated antigen (estimated in terms of RNA precipitated) at the equivalence zone ($(Ab/Ag)_{eq}$) for the different ribosomal particles and ribosomal RNA's.

Antigen	30 S	50 S	70 S	30 S + 50 S (non associated)	16 S	23 S
Crude particles	3.07	1.2	1.17	1.9		
Washed particles						
in K_2	3.5	1.4	1.4	2		
in K_3	3.3	1.2	1.2	1.9		
RNA's						
in K_2					3.5	3
in K_3					3.4	3

The curves of Fig. 1 (crude particles),2 and 3 (washed particles) show clearly that differences in reactivity do in fact exist. It appears that: (1) The results are identical in K_2 (Mg^{++} 1 mM) or K_3 (Mg^{++} 4 mM) buffers. (2) For the same amount of antibodies precipitated,the amount of 70 S and 50 S particles is greater than that of 30 S particles: so,the Ab/Ag values at the equivalence zone ($(Ab/Ag)_{eq}$) are 2 to 2.5 times greater for the 30 than for the 50 or 70 S particles (Table 1). The fact that the Ab/Ag values for the washed particles are superior by 15-20% to those of the corresponding crude particles is predictable if one admits that the non structural proteins,stripped by 0.5 M NH_4Cl,are associated with a part of the "accessible" RNA and hinder the fixation of a certain quantity of antibodies. (3) The $(Ab/Ag)_{eq}$ ratios for 70 S ribosomes are less than the theoretical

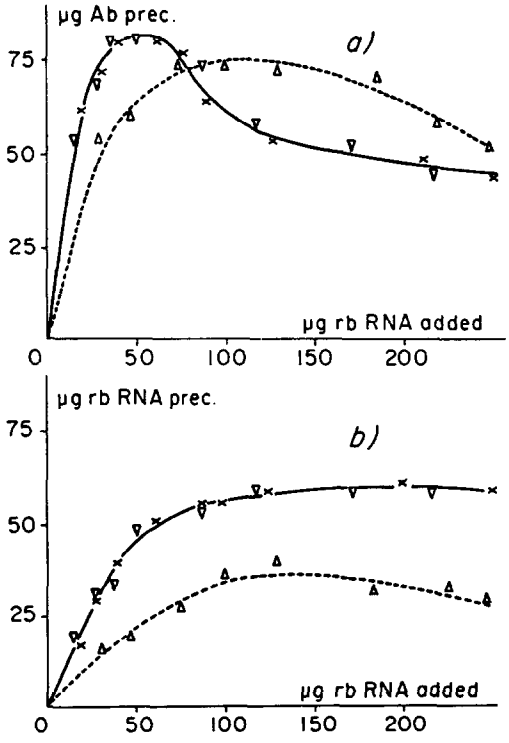

Fig. 4 - Comparison between the amounts of antibody (a) and antigen (b) precipitated in K_2 medium by 70 S washed ribosomes (native or reassociated) and a mixture in a 1:1 ratio of 30 and 50 S non associated sub-units. The theoretical curves were calculated from the individual precipitation of each sub-unit in K_2 medium. The washed sub-units no showed any sign of reassociation in K_2 medium. Symbols: x——x native washed 70 S ribosomes ; ▽——▽ reassociated 70 S ribosomes ; △——△ 30 S sub-units + 50 S sub-units mixed in a 1:1 ratio ; ------ theoretical curve for the mixture 30 S + 50 S calculated from the individual precipitation of each sub-unit.

values calculated adding up the values obtained with the sub-units. However this calculated value is in fact the one obtained when a mixture of equal parts of 30 and 50 S is reacted under conditions preventing any reassociation (Fig. 4). In contrast, reassociated ribosomes behave like native ribosomes; the picture is the same for ribosomes preincubated (15 min at 37°, pH 7.8, in 0.1 mM Mg^{++}) in order to eliminate any residual messenger RNA.

The significance of the immunochemical reactivity of the ribosomal particles can only be appreciated by comparison with the ribosomal RNA's. Fig. 5 and 6 show the precipitation curves in K_2 or K_3 medium. The $(Ab/Ag)_{eq}$ ratios differ little, but the slope of the curves shows a dissolution in excess antigen less sharp for the 16 S RNA than for the 23 S : the phenomenon is probably due to the difference in size, as shown in the case of fragments produced by hydrolysis of 23 S RNA (3 min at 90°).

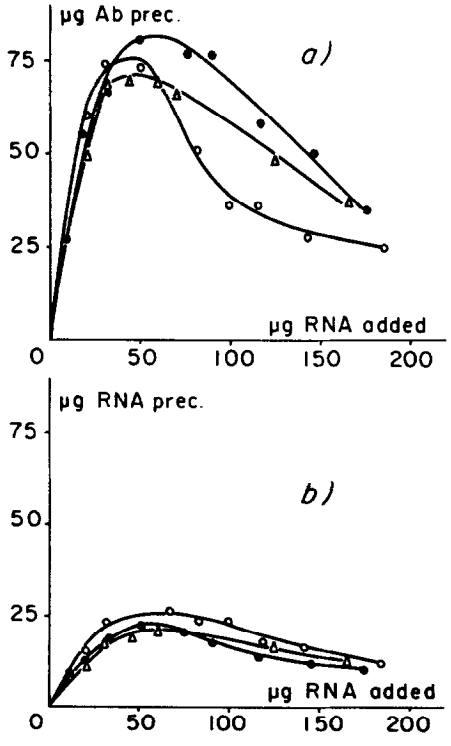

Fig. 5 - Amounts of antibody (a) and antigen (b) precipitated in the K_2 medium by isolated RNA's. Symbols: •——• 16 S RNA ; o——o 23 S RNA ; Δ——Δ 23 S RNA after hydrolysis in fragments approximating in size to 16 S RNA: these fragments were collected from the corresponding tubes obtained by centrifugation through a sucrose gradient.

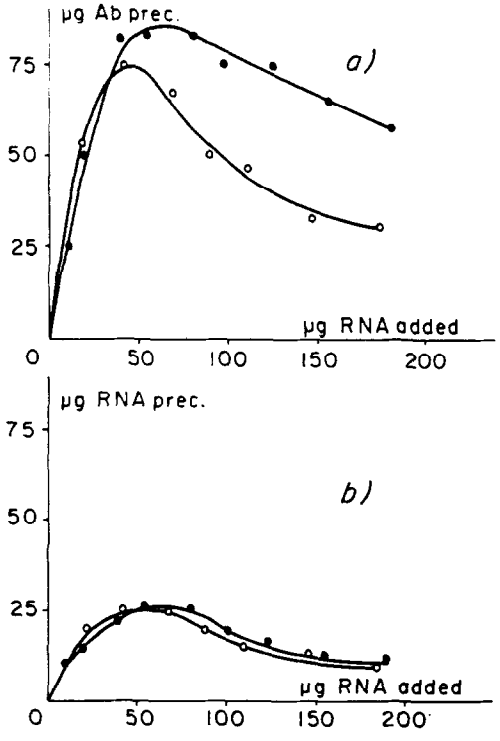

Fig. 6 - Amounts of antibody (a) and antigen (b) precipitated by the ribosomal RNA's in the K_3 medium. Methods and symbols as in the Fig. 5.

Discussion.- Several observations can be made from these experiments:

(1) The structures of the 30 and 50 S particles seem to be quite different, since the $(Ab/Ag)_{eq}$ ratios, -which are a measure of the maximum number of specific sites present on the different antigens-, are identical for the 16 S RNA and the 30 S sub-units, but not for the 23 S and the 50S RNA.

(2) Thus, the totality of the 16 S RNA remains accessible to the antibodies when it forms part of the 30 S sub-unit. Conversely, the inclusion of the 23 S RNA in the 50 S provokes a loss of 55-60% of the antigenic sites. From the $S_{w,20}$ of the NG I, a M.W. of 2.5-3 x 10^5 would be indicated by comparison with other IgG. Taking for the M.W. of ribosomal RNA's of E.Coli the values established by Kurland (1960), and taking into account the admitted bivalence of the antibodies eliciting a network of precipitation, the number N of specific sites would be: $N = 2 \times (M.W.RNA/M.W.NG\ I) \times (Ab/Ag)_{eq}$. This gives values of 25-31 for the number of sites of the 23 S RNA and 14-19 for the sites of 16 S RNA. The latter all remain accessible to the antibodies in the 30 S sub-unit, but only 10-12 in the 50 S. The same calcula-

tion gives in the case of the crude particles: 12-15 sites for the 30 S sub-unit, 9-11 for the 50 S. The non-structural proteins stripped by 0.5 M NH_4Cl would mask 2-3 accessible sites on each ribosomal RNA.

(3) The association of a 50 and 30 S sub-units to a 70 S ribosome gives rise to a value $(Ab/Ag)_{eq}$ which is much smaller than that obtained with a mixture of non associated sub-units in a 1:1 ratio. Thus, association is accompanied by the loss of a certain number of antigenic sites. The most reasonable explanation is the pairing between the accessible fractions of the respective RNA's of each sub-unit, as supported by the fact that 16 S and 23 S RNA are able to yield specific association products (Marcot-Queiroz and Monier 1965). Such an interaction would be accompanied by a masking of a certain number of specific sites, analogous to that which occurs with a polynucleotide complex (Panijel et al. 1966 b ; Souleil et al. 1966), translated by a significant diminution of the ratio $(Ab/Ag)_{eq}$.

Such a pairing between the accessible fractions of ribosomal RNA's must be related to the role of Mg^{++}. When the dissociation of the 70 S ribosomes take place in the presence of 0.1 M KCl by lowering the Mg^{++} concentration from about 7-8 mM to 1-1.5 mM, the value of the ratio $Mg/P^{(*)}$ passes from 0.5 to about 0.2 (Goldberg 1966 ; Choi & Carr 1967) : hence, about 60% of the RNA no longer binds Mg^{++}. This percentage is precisely that which would result from the pairing of the two ribosomal RNA's, since this pairing should take place between nucleotidic sequences of equal length; it should therefore correspond, at a maximum, to the length of the accessible fraction of the 23 S RNA, and should thus form a double-stranded segment corresponding to a M.W. of about 1×10^6, i.e. 60% of the sum of the M.W. of the two ribosomal RNA's (1.66×10^6).

One consequence of this situation is the following: single stranded sequence which remains accessible in the 70 S ribosome should correspond to a maximum M.W. of $5-6 \times 10^4$ .The apparent paradox whereby messengers RNA's associate specifically with 30 S sub-units, but interact both with the 16 S or 23 S RNA (Watson 1964 ; Hayes et al. 1966 a,b) could be explained by such structural considerations.

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(*) Moles bound Mg / Moles ribosomal Phosphate

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