THE IMMUNOLOGICAL REACTIVITY OF 30S RIBOSOMAL PROTEINS IN 70S RIBOSOMES FROM ESCHERICHIA COLI[1]

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SUMMARY: Mouse antisera to purified E. coli 30S ribosomal proteins S2, S3, S4, S5, S7, S8, S12, and S20 were produced in mice and found to react with these proteins in the intact 30S ribosome. They were also reactive with the same proteins in the 70S particle, suggesting that at least some of their antigenic determinants remain exposed after formation of a complete ribosome. Some implications of these findings for models of ribosome structure are briefly discussed.

INTRODUCTION

Although the association-dissociation behavior of Escherichia coli ribosomal subparticles has been studied extensively (1-4) little is known about the disposition of ribosomal proteins and rRNA in the intact (70S) ribosome. Stöffler and his co-workers have found that every one of the 21 30S ribosomal proteins in a 30S particle is immunologically unique (5) and has at least some of its antigenic sites accessible for interaction with corresponding rabbit antibodies (6). In the experiments reported here we have confirmed the immunological accessibility of proteins in the 30S subparticle to antisera to eight of the 21 30S ribosomal proteins. In addition we have examined the immunological reactivity of these 30S proteins in the intact 70S ribosome and have found all of them to be reactive. The implication of these findings for models of ribosome structure is discussed.

MATERIALS AND METHODS

Preparation of 30S Ribosomal Proteins: Proteins were prepared from mid-log phase Escherichia coli Q-13 cells (obtained from General Biochemicals) according to the method of Hardy et al. (7). In addition, proteins S2, S4 and S6[2] were subjected to preparative gel electrophoresis after phosphocellulose

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[2]The nomenclature of Hardy et al. (7) was converted into International nomenclature (8).

chromatography using an Ortec #4100 gel electrophoresis system. Details of this procedure will appear elsewhere. All proteins were judged >95% pure by analytical gel electrophoresis using the one dimensional soft and hard gel system described by Voynow and Kurland (9).

Production of Antisera to 30S Ribosomal Proteins: Purified proteins (50 µg) in 0.2 ml of a mixture of equal parts Complete Freunds Adjuvant and 0.15 M NaCl, 0.01 M potassium phosphate buffer, pH 7.4 containing approximately 1 M urea, were injected into outbred mice, 4-8 weeks of age. Two weeks later, four injections of 25 µg each (without adjuvant) were made over a one-week period. Mice were bled every other day for one week from the retro-orbital sinus, commencing two days after the last injection. Pooled pre-immune and antisera from the animals were stored at -20° until used.

Preparation of Ribosomes: 30S ribosomal subparticles were purified according to Traub et al. (10) except that the starting 70S ribosomes were washed twice with 1 M NH_4Cl prior to separation of the subunits. Cold run-off 70S ribosomes were prepared from freshly grown E. coli Q13 using the procedure of Tai and Davis (11). The 70S particles were purified by two successive separations on 10-30% sucrose gradients in Buffer I (10 mM Tris, pH 7.5, 100 mM NH_4Cl, 20 mM $Mg(OAc)_2$) using the SW 27 rotor. The purified 70S ribosomes contained less than 1 per cent 30S subparticle (see Figure 2). They were stored in Buffer I at 0°. No detectable breakdown to subunits occurred in 15 days.

Reaction of Specific Antisera with 30S or 70S Ribosomes: Antiserum (50 µl) was combined with 1.0 M magnesium acetate solution (1 µl) to bring the Mg^{2+} concentration to 20 mM. 70S (300 µg) or 30S (100 µg) ribosomes in 10 µl Buffer I was added and the tubes were mixed and incubated 10 minutes at room temperature. In the case of the 30S reactions, the mixtures were layered in 9 ml centrifuge tubes over 2.0 ml of 20 percent sucrose solution in Buffer I containing 250 mM KCl. In addition the sucrose solutions contained 0.5 mg of 30S ribosomes per ml. The 70S antiserum reaction mixtures were layered over plugs composed of 1.0 ml 20 percent sucrose in Buffer I containing 0.5 mg 70S ribosomes layered over 1.0 ml 25 percent sucrose in Buffer I containing an additional 250 mM KCl and 0.5 mg 70S ribosomes. The tubes were centrifuged at 40,000 RPM in a No. 40 rotor for 4 hours at 4°. The pellets were rinsed two times with Buffer I and suspended in 50 µl of the same buffer.

Ouchterlony Plates: 1.0% Agarose (Sigma) in sodium barbital buffer, pH 8.6, was poured between plastic Ouchterlony templates and glass slides coated with agarose. The outside wells were 2 mm from the center well. The wells were cleared by aspiration after the agarose had hardened. The antibody treated ribosome solution (10 µl, see above) was put in one of the outside wells and goat anti-mouse IgG (10 µl, Miles-Yeda Ltd.) was put in the center well. The plates were kept in a humidity chamber for 24 hours at room temperature after which the templates were removed and the slides were photographed with a Polaroid MP-3 camera. The plates were washed 12 hours in saline and dried.

Sucrose Gradients: Antibody-ribosome complexes were analyzed in sucrose gradients as described in the legend to Figure 2.

RESULTS

The ribosomal proteins used in this study are listed in Table 1 together with some information on their stoichiometry in 30S ribosomes and their role in ribosome function. In general it was found that mice are capable of producing high-titer antisera to ribosomal proteins using a total of only 150 µg

TABLE 1

30S Ribosomal Proteins Used for Antibody Production

Protein	Stoichiometry	Site Specific RNA (16S) Complex	Function and Mutant Phenotype
S2	Fractional	-	A site
S3	Marginal	-	A site
S4	Unit	+	ram suppressors
S5	Marginal	-	Spectinomycin resistance
S7	Unit	+	--
S8	Unit	+	--
S12	--	-	Streptomycin resistance and dependence
S20	Fractional	+	--

30S ribosomal proteins were purified as described in Materials and Methods. The data on stoichiometry, site specific 16S rRNA complex formation and functional properties were taken from recent reviews by Kurland (8) and Nomura (13).

of purified ribosomal protein per mouse. All sera used except anti S7 gave a positive ring test with whole 30S ribosomal proteins at a dilution of 1 to 4 or greater. The one weaker anti-serum was concentrated 10-fold by ammonium sulfate precipitation prior to use.

All of the anti-30S protein antisera examined gave a positive reaction with the 30S subunit (Figure 1), as judged by the capacity of ribosomes treated with mouse anti-ribosomal protein antisera to react with anti-mouse IgG. No binding of pre-immune mouse IgG to ribosome was detected by the procedure. We next sought information on the immunological accessibility of the same 30S proteins in a 70S ribosome. Accordingly, cold run-off 70S ribosomes were reacted with the different antisera and examined for bound antibody (see Materials and Methods). All the antisera were still found to be capable of reacting with the corresponding 30S proteins in a 70S ribosome (Figure 1).

To conclude from these observations that the 30S proteins in question remain immunologically accessible in 70S ribosomes, it was necessary to eliminate

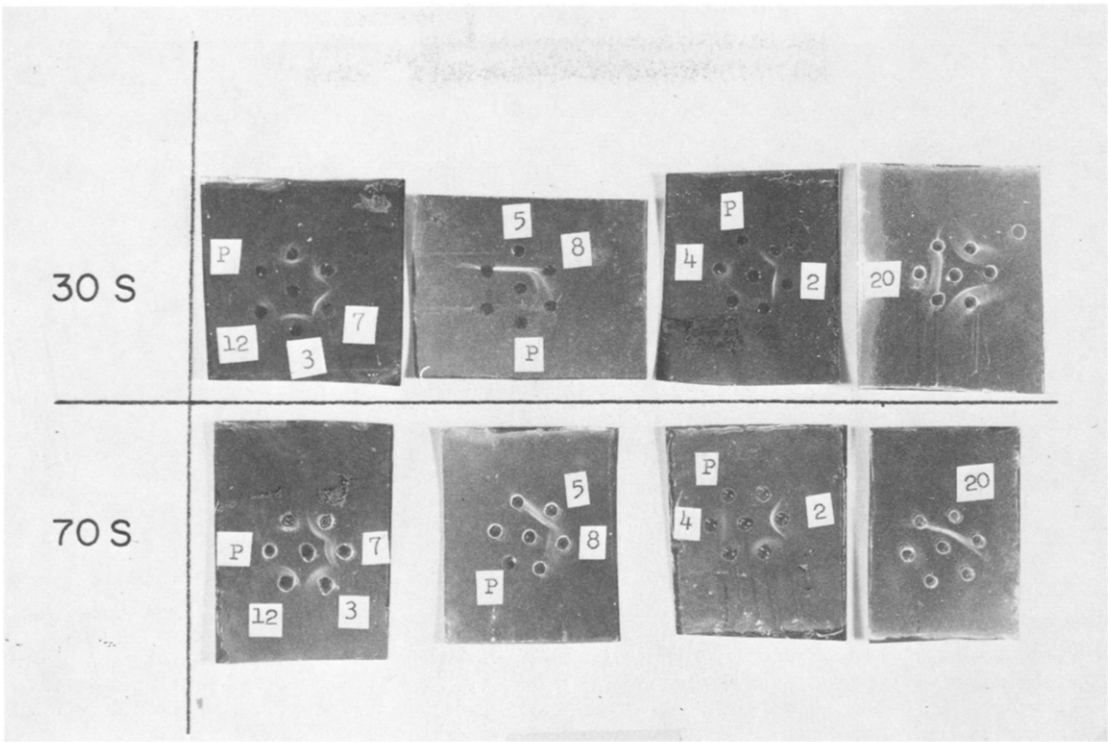

Figure 1: Ouchterlony plates of ribosomes-antibody complexes and antimouse IgG. P = pre-immune serum. 2, 3, etc. = Anti S2, S3, etc.

the possibility that the antisera used were capable of dissociating 70S ribosomes into subparticles, by shifting the equilibrium between subparticles and whole ribosomes. If such were the case, then it might be expected that incubation of 70S ribosomes with the different antisera would produce a significant increase in the 30S ribosome content and a corresponding reduction in the amount of starting 70S particles. We have examined the 70S preparation after incubation with the different antisera. The information obtained from the sucrose density gradient profiles, presented in Figure 2, can be summarized as follows:

(1) No observable dissociation into subparticles was found after treatment of 70S ribosomes with antisera to the 30S proteins.

(2) With the exception of antiserum to S8, all the antisera examined caused the aggregation of 70S particles, usually into 100S dimers.

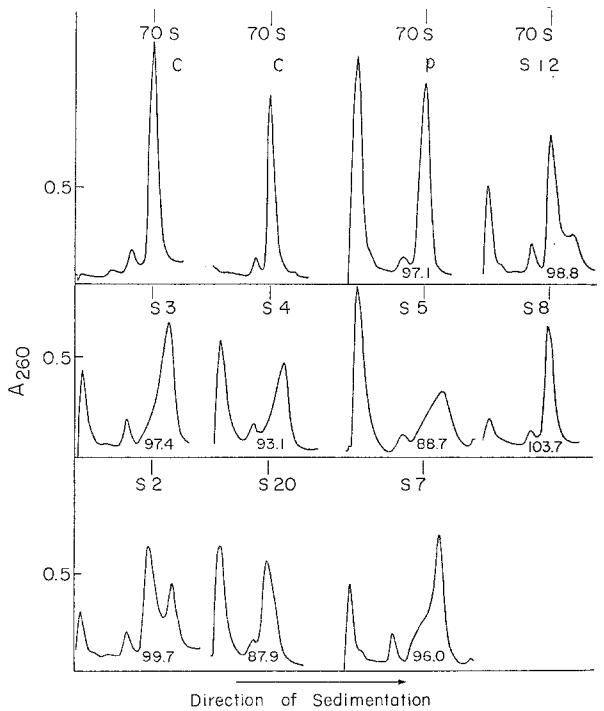

Figure 2: Sucrose density gradients of run-off 70S ribosomes reacted with antisera to 30S ribosomal proteins. Antiserum (50 µl, containing 20 mM Mg^{+2}) and 70S ribosome solution (50 µl, 40 A_{260} units/ml) were combined and incubated for 10 minutes at room temperature. 95 µl aliquots of the incubation mixtures were layered over 17 ml 5-25 percent sucrose gradients in Buffer I. They were centrifuged at 27,000 RPM for 5 hours in an SW 27 rotor. The gradients were analyzed from the top using an Autodensiflow (Buchler) and a Gilford Model 240 Spectrophotometer equipped with a flow cell (10 mm light path) and 10 inch chart recorder. The 70S-100S peaks were integrated using a compensating polar planimeter, and the areas were expressed as a percent of a standard 70S peak indicated by the number under each curve. P = pre-immune serum, C = starting ribosomes.

(3) In all cases, 90 percent or more of the A_{260} units applied to the gradient were recovered in the form of 70S particles or a dimer thereof.

From these experiments we conclude that 30S ribosomal proteins S2, S3, S4, S5, S7, S8, S12 and S20 have at least some of their antigenic determinants accessible in intact 70S ribosomes.

DISCUSSION

In agreement with the findings of Stöffler and his colleagues on the 21 different 30S proteins, we have observed that 8 of these proteins are immuno-

logically reactive in the intact 30S particle (Figure 1). These results further substantiate the conclusion drawn by Kurland (8) that most if not all of the 30S proteins are located at or near the surface of particle[3].

Most models of the ribosome either predict or postulate rather extensive interaction between ribosomal subparticles. Indeed the strong affinity of the subparticles for one another (4) suggests a rather extended contact region. Therefore we were somewhat surprised to find that all of the antisera tested could react with their corresponding 30S proteins in the intact 70S particle (Figure 1). That this is not due to an antibody mediated dissociation of the ribosome into subparticles is demonstrated by sucrose gradient analysis of the ribosomes after reaction with the different antisera (Figure 2). Indeed, the ability of all but one of the different antisera to aggregate 70S ribosomes implies that at least one antigenic determinant of each of these proteins is exposed. The inability of anti-S8 to produce 70S dimers may be due to steric hindrance of a second antibody combining site by the ribosome.

Since the proteins examined in this study represent a fairly balanced selection among the different 30S proteins (Table 1) we cannot resist making the extrapolation that most if not all of the 30S ribosomal proteins will be at least partly exposed in the 70S ribosome.

Some of the results presented here are at apparent odds with the work of Huang and Cantor (12) who studied the reaction of fluorescein isothiocyanate with 30S ribosomes. They found protein S8 unreactive in both the 30S and 70S particles and protein S12 protected from reaction in the 70S ribosome. If studies with 30S ribosomes are used as example (8), it may be difficult to reach valid conclusions about buried and exposed proteins in 70S ribosomes using chemical modification techniques. In any event, if one pictures the 30S ribosome as an oblate ellipsoid of revolution consisting of a protein monolayer

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[3]The well known inaccessibility of viral and cell surface antigens to their corresponding antibodies clearly demonstrates that immunological accessibility is not a universal feature of highly organized structures.

held together by RNA (8), it is quite conceivable that parts of many or all of the 30S proteins are in contact with 50S particles on one side while the back side of the ellipsoid contains immunologically active regions which can still bind antibody. Such a model could well explain lack of reactivity toward some modifying reagents of certain 30S (or 50S) proteins in 70S ribosomes. Immunological analysis of the remaining ribosomal proteins in 70S particles should provide useful information on this subject.

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