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Unique Protein Moieties for 30S and 50S Ribosomes of *Escherichia coli*

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Each major component of the proteins of 30S ribosomes from *Escherichia coli* was compared with the proteins of 50S ribosomes. The comparisons were done by using polyacrylamide gel electrophoresis in urea with differentially labeled proteins. The data show that no major protein is common to both ribosomes.

Protein extracted from the 30S ribosomal particles and electrophoresed on polyacrylamide gels shows a complex band pattern. Recent experiments have shown that each band represents one or more proteins whose primary structure is different from that of all others (6, 11, 20). Experiments involving reconstitution of the 30S subunit from "core" particles and "split" proteins have indicated that these proteins are functionally heterogeneous (16-18). A preliminary comparison of the electrophoretic mobilities of proteins from the 30S subunit with those from the 50S subunit, both in this laboratory and others (19), suggested the existence of proteins which were common to both subunits. However, mixing experiments involving the combination of "core" particles from one subunit with "split" protein from the other suggest that the "split" proteins from the 30S particle are not functionally equivalent to those from the 50S particle (9, 17). In this report, we present results obtained by use of electrophoresis on acrylamide gel as a criterion of physical identity. The data show that none of the major structural proteins is common to both the 30S and 50S ribosomes.

MATERIALS AND METHODS

Preparation of radioactive ribosomal proteins. Ribosomes were isolated from a ribonucleaseless strain of *Escherichia coli* K-12 (8). Two 500-ml cultures were grown in minimal-salts medium (15) containing 0.5% glucose. The culture to be labeled with ^{14}C contained in addition 30 μg of L-methionine per ml, 4 μg of L-arginine hydrochloride per ml, and 20 μc of ^{14}C-L-arginine (1.44 μc per μg). The culture to be labeled with ^{3}H contained in addition 17.5 μg of L-methionine per ml, 4 μg of L-arginine hydrochloride per ml, and 200 μc of ^{3}H-L-arginine (6.3 μc per μg). The level of cold arginine was exhausted from the medium at 30 to 40 Klett units of growth (100 Klett units at 540 nm is approximately 7×10^8 cells/ml). The cultures were harvested by centrifugation when they reached 150 Klett units.

The ribosomes from each culture were purified separately. The cells were washed with 0.01 M tris-(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.4) containing 0.01 M magnesium acetate (TM/2). They were resuspended in TM/2 containing 0.5% Brij 58 and 2 μg of deoxyribonuclease per ml. The cells were broken in a French pressure cell at 9,000 psi, and the debris was removed by centrifugation at 25,000 $\times$ *g* for 30 min. Ribosomes were prepared by the first ammonium sulfate precipitation step of the previously described procedure (7). The pellet was resuspended in TM/2 containing 0.6 M $(NH_4)_2SO_4$ and centrifuged at 130,000 $\times$ *g* for 3 hr. The ribosome pellet was resuspended in TM/2, dialyzed against TM/2, and then dialyzed against 0.01 M Tris buffer (*p*H 7.4) containing 0.0001 M magnesium acetate (TM/4).

The 30S and 50S ribosomal subunits were separated on linear 5 to 20% sucrose gradients in TM/4. Sedimentation of the particles was carried out at 5 C in an SW 25.1 rotor for 9 hr at 24,000 rev/min. The optical density profile at 260 nm was determined, and the fractions in the 30S peak were pooled. In the case of the 50S peak, the fractions from the heavy side were pooled to minimize contamination by 30S particles. It was estimated that 30S particles accounted for 5 to 10% of the material under the heavy side of the 50S peak. Sucrose was removed by dialysis, the volume of the subunit solutions was reduced by flash evaporation, and the protein was extracted with 2-chloroethanol as previously described (7).

Analytical acrylamide gel electrophoresis. Analytical gel electrophoresis was carried out at *p*H 4.5, 7.5, and 8.9 with gels of various compositions. The compositions of the upper and lower buffers and the running gel of the *p*H 4.5 cationic system have previously been described (7). In addition, a stacking gel was used on the gels shown in Fig. 1 and 2. The composition of the stock solutions for the stacking gel was as follows. Solution D contained 10.0 g of acrylamide, 0.8 g of bis-acrylamide, and 8 M urea to 100 ml. Solution E contained 48.0 ml of 1 N KOH, 2.9 ml of glacial acetic acid, 0.1 ml of *N,N,N',N'*-tetramethylethylenediamine (TEMED), and 8 M urea to 100 ml. Solution F contained 2 mg of riboflavine and 8 M urea to 100 ml. The stacking gel was formed by mixing one part of D

with one part of E and two parts of F; 0.3 ml of this mixture was then layered on top of the running gel.

The compositions of the solutions for the pH 7.5 cationic system were as follows. The upper buffer contained 3.4 g of imidazole per liter of water, and the *p*H was made to 8.0 by the addition of glycine. The lower buffer contained 13.68 g of $K_2HPO_3 \cdot 3H_2O$ per liter of water, and the *p*H was brought to 7.5 by the addition of concentrated phosphoric acid. Gel solution A contained 2.75 g of $K_2HPO_4 \cdot 3H_2O$, 0.08 ml of TEMED, concentrated phosphoric acid to *p*H 7.5, and 8 M urea to 100 ml. Gel solution B contained acrylamide and bis-acrylamide at concentrations that upon dilution would yield gels of the desired composition. It was made up in 8 M urea. Gel solution C contained 0.070 g of ammonium persulfate and 8 M urea to 100 ml. The gel was formed by mixing one part of A and one part of B with two parts of C.

The anionic systems used were those described by Davis (4) at *p*H 8.9 and by Williams and Reisfeld (21) at *p*H 7.5. In both anionic systems, the stacking gel was omitted.

The running gels were formed in tubes 0.6 cm in diameter by the addition of either 3.4 ml (Fig. 1, 2, and 6–11) or 2.2 ml (Fig. 3–5) of gel solution. All analytical electrophoresis was carried out at constant voltage toward the bottom in Fig. 1 and 6–11 and toward the right in Fig. 2–5.

Isolation of individual protein bands from either the total 30S or the total 50S protein. For the isolation of individual radioactive protein bands from small quantities of total 30*S* or total 50*S* ribosomal protein, the normal analytical gel in the *p*H 4.5 cationic system was employed in a preparative capacity. A number of gels were prepared by use of 3.4 ml of running gel solution plus 0.3 ml of stacking gel solution. Samples (0.2 ml) containing either ^{3}H-labeled total 30*S* ribosomal protein or ^{14}C-labeled total 50*S* ribosomal protein in 8 M urea were layered on each gel. Electrophoresis was carried out at 15 v per cm for 9 hr. The gels were removed from the tubes, stained for 5 sec in 0.25% amido black in 7% acetic acid, and then destained by shaking in 7% acetic acid for 0.5 hr. The rapid staining procedure fixes the protein around the outer circumference of the gel, and after 0.5 hr a faint band pattern is visible which makes it possible to slice out individual bands. At the same time, the protein in the center of the gel is not fixed and can therefore be eluted. Several slices of each band were placed in a vial containing 1 M propionic acid. The bands were eluted for 3 days at 4 C, after which the eluates of the bands to be compared were pooled, flash-evaporated to dryness, and taken up in a small volume of 6 M urea.

The preparation of individual nonradioactive protein bands from either total 30*S* or total 50*S* ribosomal protein was performed according to procedures previously described (7).

Slicing and counting of gels containing radioactive protein. Gels containing radioactive protein were sliced on a CO_2-cooled microtome. Since the microtome would not accommodate a length of gel longer than 2 cm, the acrylamide gels were first cut into longitudinal segments 1 to 2 cm in length after freezing the gel on dry ice. It was found that embedding the gel in a cylinder of 20% sucrose, 1.6 cm in diameter, facilitated slicing. The sucrose surrounding the gel slice melts readily as the slice slides up on the microtome knife, and virtually no sucrose is transferred to the scintillation vial. Either 0.5-mm (Fig. 2) or 0.25-mm (Figs. 3–5) slices were made. Each slice was placed in a separate vial, and 0.5 ml of 30% H_2O_2 was added to each vial. The caps were screwed on tight, and the vials were heated at 100 C in an Arnold sterilizer for 30 min. The vials were cooled to room temperature, and 20 ml of toluene-ethyl alcohol scintillation fluid was added to each vial. Toluene-ethyl alcohol scintillation fluid was made up as follows: 4.0 g of 2,5-diphenyloxazole and 0.05 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene in 1 liter of toluene plus 500 ml of absolute ethyl alcohol. The gel slices were counted for either 4 min (Fig. 2) or 10 min (Fig. 3–5). Background on the ^{14}C and ^{3}H channels was 11 and 9 counts/min, respectively; 19% of the counts per minute on the ^{14}C channel were on the ^{3}H channel, and 0.3% of the counts per minute on the ^{3}H channel were on the ^{14}C channel.

RESULTS

A preliminary examination of the band patterns obtained on polyacrylamide gel with either total protein from the 50*S* ribosomal subunit or total protein from the 30*S* subunit (Fig. 1)

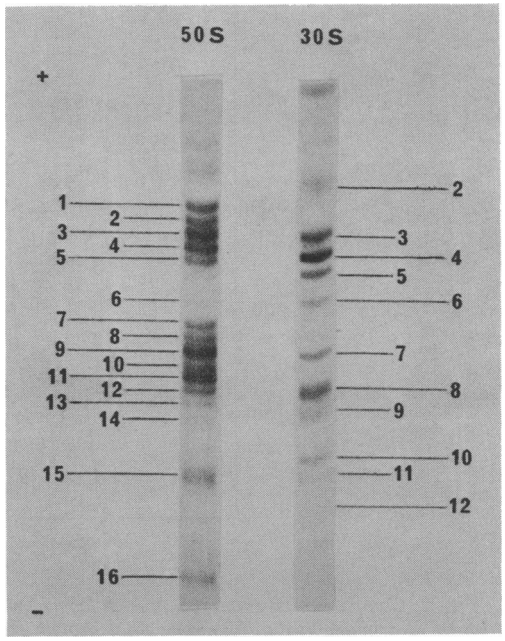

FIG. 1. *Comparison of the total protein extracted from the 50S ribosomal subunit (left gel) with the total protein from the 30S subunit (right gel). The running gel consisted of 7.5% acrylamide, 0.2% bis-acrylamide. Electrophoresis was carried out at pH 4.5 for 6.5 hr at 18 v per cm.*

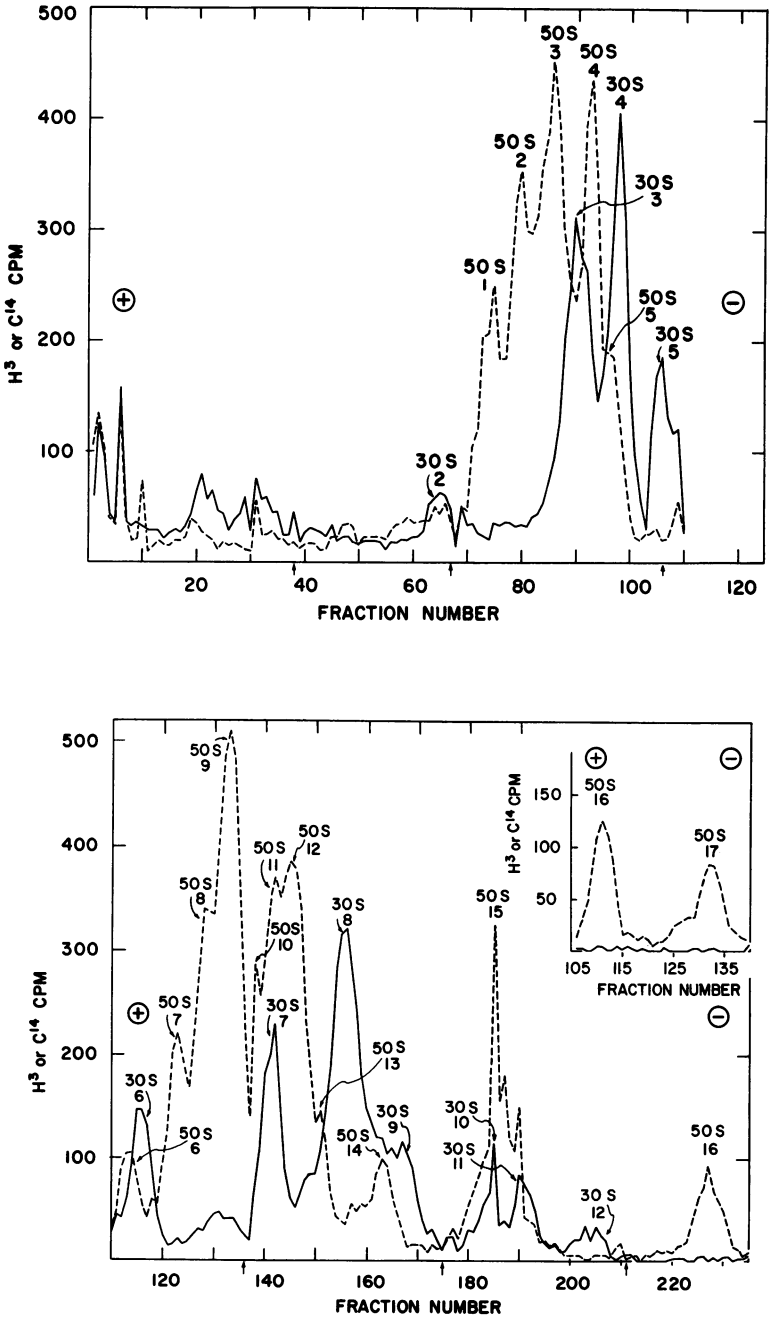

FIG. 2. *Comparison of total 50S protein labeled with* ^{3}*H-L-arginine (broken line) with total 30S protein labeled with* ^{14}*C-L-arginine (solid line). The total protein from both subunits was electrophoresed on the same gel. The running gel consisted of 7.5% acrylamide, 0.2% bis-acrylamide. Electrophoresis was carried out at pH 4.5 for 7 hr at 18 v per cm. The arrows on the abscissa indicate the points at which the gel was cut into segments for slicing. The insert shows a segment cut from a gel in which the protein mixture was electrophoresed for only 3 hr. Band 50S-17 has migrated off the gel by 7 hr.*

indicates that there are a number of bands which appear to be common to both subunits. A more sensitive way to compare the mobilities of these proteins is by differentially labeling the 50*S* protein and 30*S* protein and subjecting the mixture to electrophoresis. Figure 2 shows the results of such an experiment. Although the results are not shown on the graph, the stacking gel was found to contain 1.5% (370 counts/min) of the total ^{3}H counts and 0.8% (125 counts/min) of the total ^{14}C counts. An examination of the graph indicates that 30*S*-7 and 30*S*-10 had the same mobilities as 50*S*-11 and 50*S*-15, respectively. Bands 30*S*-3, -4, -8, and -9 all contained two or more components (6, 7). Therefore, it was conceivable that some of the 50*S* bands, like the 30*S* bands, might contain more than one component, and that some of these components might be common to both subunits. To test more critically for the existence of common proteins, individual bands were isolated, and comparisons were made on gels of varying composition and *p*H. Figures 3, 4, and 5 show results of comparisons made with individual radioactive bands. Only those gel segments which contained stainable quantities of protein are represented in these figures. In every case, it was possible to separate the components comprising the 30*S* subunits from those comprising the 50*S* subunit by varying the composition of the gel.

The remaining electrophoretic comparisons were made with nonlabeled protein. This was

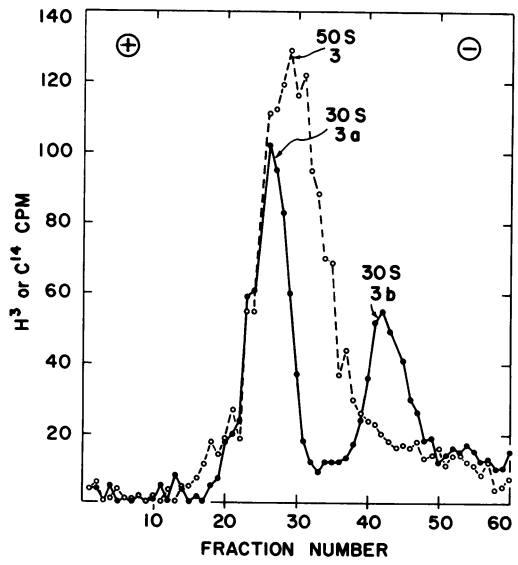

FIG. 3. *Comparison of ^{3}H-L-arginine 30S-3 (solid line) with ^{14}C-L-arginine 50S-3 (broken line). The gel consisted of 12% acrylamide, 0.3% bis-acrylamide. Electrophoresis was carried out at pH 4.5 for 11.5 hr at 14 v per cm.*

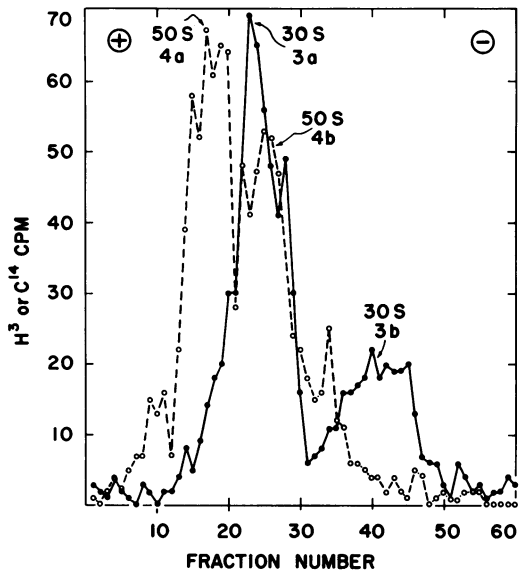

FIG. 4. *Comparison of ^{3}H-L-arginine 30S-3 (solid line) with ^{14}C-L-arginine 50S-4 (broken line). The gel consisted of 7.5% acrylamide, 0.8% bis-acrylamide. Electrophoresis was carried out at pH 4.5 for 5 hr at 21 v per cm.*

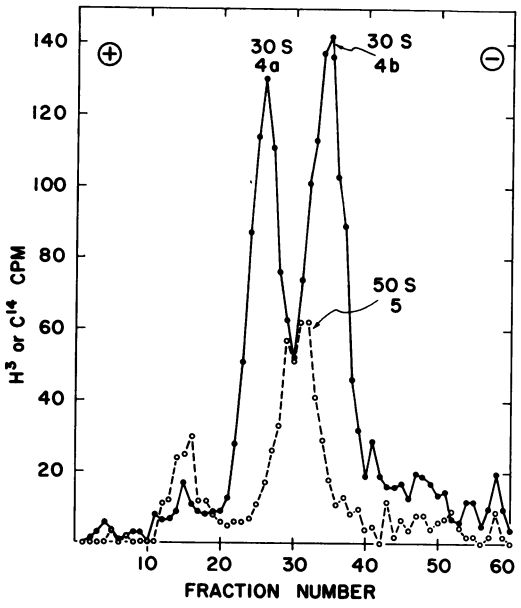

FIG. 5. *Comparison of ^{3}H-L-arginine 30S-4 (solid line) with ^{14}C-L-arginine 50S-5 (broken line). The gel consisted of 12% acrylamide, 0.3% bis-acrylamide. Electrophoresis was carried out at pH 4.5 for 11.5 hr at 14 v per cm.*

done by running each of the isolated bands to be compared on separate gels and at the same time running a mixture of the two bands on another gel. Therefore, for each comparison, a

total of three gels were run. Figure 6 shows the results of such a comparison between 30S-10 and 50S-15.

Preliminary attempts to separate the remaining bands with the *p*H 4.5 system met with failure. For this reason, separation was attempted at a higher *p*H. Since virtually all of the proteins in either of the subunits migrate towards the cathode even at *p*H 8.9, a cationic system had to be developed to carry out electrophoresis at a higher *p*H. Such a system was developed at *p*H 7.5 and is described in Materials and Methods. Although the bands are more diffuse in this system than in the *p*H 4.5 system, three of the remaining pairs could be separated. In Fig. 7, the separation between the components of 30S-9 and 50S-14 is clearly demonstrated.

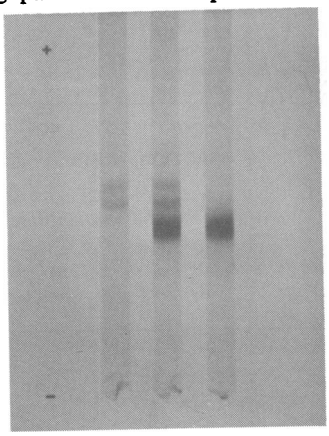

FIG. 6. *Comparison of 30S-10 (left gel) with 50S-15 (right gel). The mixture was run on the center gel. The gels consisted of 7.5% acrylamide, 0.8% bis-acrylamide. Electrophoresis was carried out at pH 4.5 for 7.5 hr at 18 v per cm.*

A problem arose in the purification of 50S-11 and 50S-13 for comparison with the appropriate bands from the 30S subunit. The purification of individual bands by preparative acrylamide gel electrophoresis depends on there being sufficient separation between bands so that they can be cut out pure. This is rarely a problem with the bands from the 30S subunit, but some of the bands from the 50S subunit were too close together. This was the case with 50S-11 and 50S-13. Band 50S-11 was shown to be contaminated with 50S-10 and -12, and 50S-13 was contaminated with 50S-12 and -14. Nevertheless, Fig. 8 and 9 clearly show that 30S-7 is different from each of the components in the mixture of 50S-10, -11, and -12, and that the three components of 30S-8 are different from each of the components in the mixture of 50S-12, -13, and -14.

As mentioned above, when electrophoresis is carried out in an anionic system at either *p*H 8.9 or 7.5, virtually none of the proteins migrate into the gel (i.e., toward the anode). However, there are two proteins in both the total 30S protein and the total 50S protein that do migrate as anions at these *p*H levels. Figure 10 shows that the bands closest to the cathode can be separated. It was not possible to separate the bands closest to the anode either at *p*H 8.9 or 7.5 by varying the composition of the gel. This band was purified from the total 30S protein by preparative gel electrophoresis with the use of the anionic *p*H 8.9 system. The band was then electrophoresed in the *p*H 4.5 cationic system. Figure 11 shows that this band migrates in the

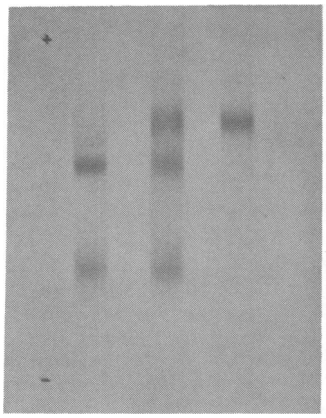

FIG. 7. *Comparison of 30S-9 (left gel) with 50S-14 (right gel). The mixture was run on the center gel. The gels consisted of 7.5% acrylamide, 0.2% bis-acrylamide. Electrophoresis was carried out at pH 7.5 for 7 hr at 18 v per cm.*

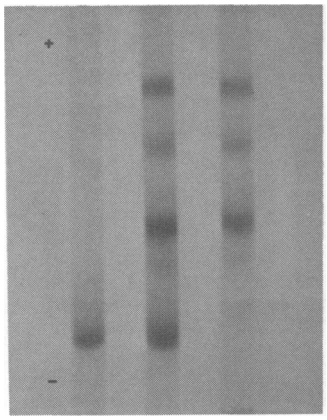

FIG. 8. *Comparison of 30S-7 (left gel) with 50S-10, -11, and -12 (right gel). The mixture was run on the center gel. The gels consisted of 7.5% acrylamide, 0.2% bis-acrylamide. Electrophoresis was carried out at pH 7.5 for 12.5 hr at 18 v per cm.*

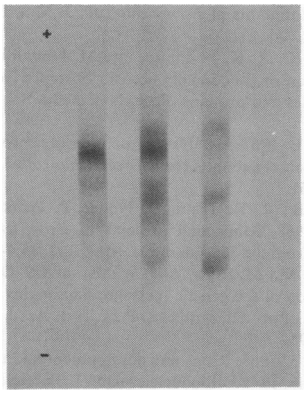

FIG. 9. *Comparison of 30S-8 (left gel) with 50S-12, -13, and -14 (right gel). The mixture was run on the center gel. The gels consisted of 12% acrylamide, 0.6% bis-acrylamide. Electrophorese was carried out at pH 7.5 for 26 hr at 18 v per cm.*

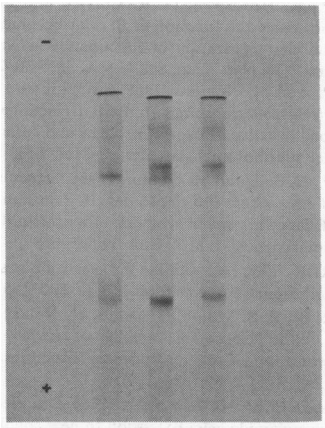

FIG. 10. *Comparison of total 30S protein (left gel) with total 50S protein (right gel). The mixture was run on the center gel. The gels consisted of 7.5% acrylamide, 0.2% bis-acrylamide. Electrophoresis was carried out at pH 8.9 for 7 hr at 27 v per cm.*

region between 30S-1 and 30S-2. Since the radioactive data do not indicate the existence of any major peak in this area, it is probable that this protein is a minor contaminant from the supernatant fluid which is adsorbed to both subunits. The appearance of the two weakly staining components seen in the 30S protein pattern between bands 1 and 2 is quite variable between preparations.

DISCUSSION

It has been shown that none of the major ribosomal proteins is common to both the 30S and 50S subunits, when electrophoretic mobility on polyacrylamide gels is used as the criterion.

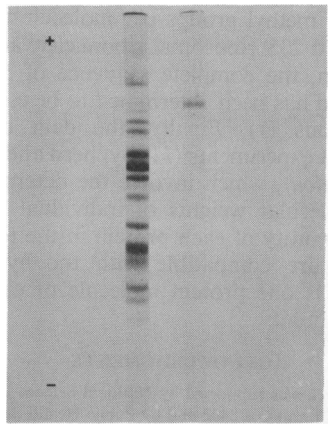

FIG. 11. *Comparison of total 30S protein (left gel) with the band closest to the anode in Fig. 10 (right gel). The gels consist of 7.5% acrylamide, 0.2% bis-acrylamide. Electrophoresis was carried out at pH 4.5 for 5.5 hr at 16 v per cm.*

Since there is a minimum of 18 proteins in the 30S subunit and a minimum of 17 proteins in the 50S subunit, a question arises concerning the function of all of these proteins. Three possible functions will be discussed.

The first possibility is that these proteins act directly by performing some enzymatic function during protein synthesis or by participating in the binding of messenger and transfer ribonucleic acids. Evidence has recently been obtained that the enzyme responsible for peptide bond formation, the peptidyl transferase, is indeed located on the 50S subunit (10). On the other hand, a number of initiation, translocation (3, 12), and release (2) factors involved in translation have been isolated from the supernatant fraction obtained upon pelleting ribosomes from extracts in the presence of high concentrations of salt.

The second possibility is that these proteins act indirectly by determining the secondary and tertiary configurations of the ribosomal ribonucleic acids. Evidence that the ribosomal ribonucleic acids do undergo conformational changes during ribosome maturation has been obtained from studies involving a comparison of relaxed particle ribonucleic acid with ribosomal by methylated albumin-kieselguhr column chromatography (13, 14).

The third possibility is that the ribosome population is heterogeneous. While there is no compelling evidence to suggest that it is the case, there is some evidence to the contrary. Quantitative determination of the number of individual sequences containing methyl groups has been shown to be in good agreement with the total

number of methyl groups per molecule for both the 16*S* and 23*S* ribosomal ribonucleic acids (5). In addition, the complete sequence of 5*S* ribonucleic acid has been determined to be essentially homogeneous (1). Finally, the data obtained from recent experiments (11; Sypherd and Strnad, *in preparation*), which involve the determination of the molecular weights of individual proteins and the quantity of each protein in the total 30*S* ribosome, are compatible with the hypothesis that there is one protein molecule of each type per 30*S* subunit.

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