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Ribonuclease Sensitivity of *Escherichia coli* Ribosomes

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ABSTRACT

SANTER, MELVIN (Haverford College, Haverford, Pa.), AND JOSEPHINE R. SMITH. Ribonuclease sensitivity of *Escherichia coli* ribosomes. J. Bacteriol. **92**:1099–1110. 1966.—The ribonucleic acid (RNA) contained in 70*S* ribosomes and in 50*S* and 30*S* subunits was hydrolyzed by pancreatic ribonuclease. A 7% amount of the RNA was removed from the 70*S* particle; at 10^{-4} M magnesium concentration, 50*S* and 30*S* ribosomes lost 15% of their RNA; at 10^{-5} M magnesium concentration, a maximum of 24 and 30% of the RNA in the 50*S* and the 30*S* fractions, respectively, was removed by ribonuclease. At the two lower magnesium ion concentrations, 50*S* ribosomes did not lose any protein, whereas 30*S* ribosomes lost protein as a result of ribonuclease treatment. A number of proteins were removed from the 30*S* particles by ribonuclease, and these proteins were antigenically related to proteins present in 50*S* ribosomes. The differential effect of ribonuclease on 50*S* and 30*S* ribosomes suggested that they have structural dissimilarities.

The function and chemical composition of ribosomes have been under intensive investigation. These studies have clearly demonstrated that, in all cells, ribosomes participate in protein synthesis (10). The theory (3) that ribosomes were passive participants, the "workbench," where transfer ribonucleic acid (tRNA)-amino acid compounds, messenger RNA (mRNA), and other components reacted has been recently modified to include the possibility of a more active role for the ribosome, particularly the ribosomal RNA, in protein synthesis (5, 20). The considerable evidence accumulated on the physical properties and the chemistry of *Escherichia coli* ribosomes has established that the 50*S* and 30*S* ribosomes have molecular weights of approximately 1.8×10^6 and 0.8×10^6, respectively. The 50*S* ribosome contains one molecule of 23*S* RNA (21), with an estimated molecular weight of 1.15×10^6, whereas 30*S* ribosomes have one molecule of 16*S* RNA, which has a molecular weight of 0.55×10^6 (14). The 50*S* ribosomes appear to have about 20 molecules of protein, whereas 30*S* ribosomes have about 10 molecules of protein; protein makes up about 40% of the weight of both ribosomes (30).

Spirin (7) has attempted to construct a model of a ribosome based on the chemical and physical parameters enumerated above, and on his electron microscopic observations on normal and unfolded ribosomes. In his model, a continuous thread of RNA is combined with the required number of protein molecules intermittently placed along the entire length of RNA. This RNA-protein strand assumes a more compact configuration in the presence of divalent cations. In the living cell, small molecular weight polyamines may also help maintain the compact configuration. This model brings considerable stretches of RNA in contact with the surrounding environment, a point which had already been suggested by Spirin's (27) and our (23) studies on the effect of pancreatic ribonuclease on *E. coli* ribosomes.

It was found (23) that the major portion of the 50*S* and 30*S* components was still present after ribonuclease treatment, but that intact 23*S* or 16*S* RNA molecules could not be recovered from ribonuclease-treated ribosomes. Furthermore, RNA breakdown products, apparently coming from the ribonuclease-treated ribosomes, were observed near the top of a sucrose gradient. These data indicated that ribosomal RNA was a "surface" component in contradistinction to tobacco mosaic virus RNA, for example, which is surrounded by a protein "coat" and, hence, inaccessible to ribonuclease action.

The experiments presented in this paper detail the precise amount of RNA that is removed from 50*S* and 30*S* ribosomes by treatment with ribo-

nuclease under a variety of environmental situations. In addition, it has been shown that, although a considerable amount of RNA is lost from 50*S* ribosomes, the protein complement remains intact. On the other hand, the 30*S* ribosome loses both protein and RNA after ribonuclease treatment. The protein that is lost can be quantitatively recovered near the top of the sucrose gradient. These proteins are immunologically related to the proteins present in both the 50*S* and 30*S* ribosomes.

MATERIALS AND METHODS

Large-scale growth of bacteria. E. coli K-10 (an Hfr strain obtained from C. Levinthal) was used throughout. All cells were grown aerobically. Large amounts of cells were obtained by growing the bacteria at 25 or 30 C in 15 liters of a glucose-salts medium of the following composition (in grams per liter): Na_2HPO_4, 7.0; KH_2PO_4, 2.0; NH_4Cl, 1.0; $MgSO_4$, 0.1; sodium citrate, 0.5; and glucose, 10.0. Cells were harvested during exponential growth, and washed two times with 0.02 M tris(hydroxymethyl)-aminomethane (Tris) buffer (*p*H 7.8), with 0.02 M KCl, and 0.01 M magnesium acetate (buffer A).

Growth of isotopically labeled cells (P^{32} and S^{35}). $H_3P^{32}O_4$, carrier-free, was purchased from Oak Ridge National Laboratory, Oak Ridge, Tenn. P^{32}-labeled cells were grown at 25 C in 30 ml of a medium of the following composition (in grams per liter): Tris, 12.1; NH_4Cl, 1.0; $MgSO_4$, 0.1; K_2HPO_4, 0.1; glucose, 10.0; supplemented with 0.01 mc of $P^{32}O_4^{-3}$; *p*H adjusted to 7.5 with HCl. The inoculum contained about 10^4 bacteria, and the amount of unlabeled inorganic phosphate transferred was less than 1 μg. The cells were harvested during exponential growth, and washed two times with cold phosphate buffer (0.05 M, *p*H 6.7). These cells were mixed with 300 times their weight of unlabeled carrier cells.

To label ribosomes with $S^{35}O_4^{-2}$, *E. coli* was grown on the synthetic medium described above for large-scale growth, except that $MgCl_2$ was substituted for $MgSO_4$ and the sulfur source was Na_2SO_4 mixed with 0.01 mc of $Na_2S^{35}O_4$ (purchased from the New England Nuclear Corp., Boston, Mass.). The final concentration of SO_4^{-2} was 10 μg/ml. Cells were grown in 30 ml of this medium, harvested during exponential growth, and washed once with 30 ml of buffer A. These cells were mixed with 100 times their weight of unlabeled carrier cells.

Three methods for obtaining ribosomes. When the French pressure cell was used to break open cells, cells were suspended in 3 volumes of buffer A per gram (wet weight) of cells plus 1 μg/ml of deoxyribonuclease (purchased from Worthington Biochemical Corp., Freehold, N.J.). Cells were broken in the French pressure cell at 15,000 to 20,000 psi. The extract was brought to room temperature for 10 min to allow the deoxyribonuclease to act. Unbroken cells and cell debris were removed by two cycles of centrifugation at 20,000 $\times$ *g* for 20 min in a Servall RC-2 refrigerated centrifuge.

With the lysozyme-ethylenediaminetetraacetic acid (EDTA)-sucrose method, osmotically fragile spheroplasts were prepared from cells according to the method of Neu and Heppel (17), except that the spheroplasts were shocked by rapid dilution into buffer A (plus deoxyribonuclease). The extract was cleared of cells and cell debris by two cycles of centrifugation at 20,000 $\times$ *g* for 20 min in a Servall centrifuge.

The lysozyme-freeze-thaw method was carried out according to Spirin (26), with the use of four or five freeze-thawing cycles.

The final supernatant fluid in each case was centrifuged at 4 C for 2 hr at 105,000 $\times$ *g* in the no. 40 head in a Spinco model L centrifuge. The supernatant fluid was decanted, and the centrifuge tube was drained and the inside wiped dry. The ribosomes were resuspended with a Teflon-glass homogenizer, and washed two times with buffer A.

Ribosome pellets derived from extracts prepared by use of both lysozyme procedures were transluscent, whereas ribosomes derived from pressure-cell extracts had a distinct pink color, which is probably due to the presence of cytochrome components from contaminating membrane fragments.

Dissociation of ribosomes into subunits. Samples of the various ribosome pellets were homogenized with a Teflon-glass homogenizer in buffer B (identical to buffer A, except that the Mg^{+2} ion concentration was 10^{-4} M), or in buffer C (Mg^{+2} ion concentration, 10^{-5} M). The resuspended ribosomes were then dialyzed against 200 volumes of either buffer B or C at 4 C for varying periods of time (never less than 4, or more than 7, hr). A low Mg^{+2} ion concentration (5×10^{-5} M) has been shown to maintain the stability of the 50*S* and 30*S* subunits of ribosomes (24), but it appears that all mRNA and tRNA molecules are removed under these conditions. Since we were interested in how much RNA was removed from ribosomes by ribonuclease, it was desirable that ribosomes be as free as possible from both mRNA and tRNA.

Ribonuclease treatment of ribosomes. Five times crystallized bovine pancreatic ribonuclease (protease-free) was purchased from Sigma Chemical Co., St. Louis, Mo. In some experiments, 1 μg of enzyme was used per 1.0 optical density (OD) unit (at 260 mμ) of ribosomes, which is approximately equivalent to 60 μg of ribosomes. These ratios calculate to about three enzyme molecules per ribosome. Incubation with ribonuclease was routinely carried out at 25 C for 40 min. Immediately after the incubation period terminated, equal amounts of both experimental and control ribosome suspensions were placed on top of a linear sucrose gradient and centrifuged.

Sucrose-gradient procedures, counting isotopes, and protein determinations. Linear sucrose gradients (5 to 20%) were prepared with buffer A, B, or C. The total volume of the gradient was 4 ml. Centrifugation was carried out with an SW 39 swinging-bucket rotor at 39,000 rev/min at 4 C for varying lengths of time, depending on whether the ribosomes were stabilized in high or low Mg^{+2}-containing buffers. Various

fractions were collected, and 2 ml of the appropriate buffer was added to each fraction. OD readings were taken at 250, 260, and 280 mμ in a Zeiss PMQ spectrophotometer. After OD determinations, the entire content of each tube from the P^{32}-labeled ribosome experiments was emptied into a planchet, dried, and counted in an end-window gas-flow counter connected to a Baird Atomic 1000 scaler. S^{35} counting was carried out in a Packard Tri-Carb scintillation counter, by use of 1 ml of sample mixed with 10 ml of Bray's (2) solution.

Protein determinations were carried out according to Lowry et al. (15) by use of bovine serum albumin as a standard. In experiments with ribonuclease, four-drop fractions were collected from sucrose gradients. A 2-ml amount of buffer A, modified by the addition of the appropriate concentration of Mg^{+2} ions, was added to even-numbered tubes to determine the OD, and odd-numbered tubes received distilled water and were used directly for protein determinations. In experiments where ribonuclease was not used, each fraction received 2 ml of water and was read in a spectrophotometer. Protein determinations were then carried out on 1-ml samples of each fraction.

Purification of RNA from ribosomes. Control and ribonuclease-treated ribosomes were prepared (23), and RNA was purified by the phenol method of Kirby (12), modified by Nirenberg and Matthaei (18), but without the addition of detergent. The method of Kurland (13) was used for the analytical ultracentrifuge analysis. Traces of the sedimentation diagrams were made with the Joyce-Loebl densitometer.

RESULTS

Ribonuclease effect on ribosomes. A number of investigations have shown that 70*S* ribosomes of *E. coli* and 80*S* rabbit reticulocyte ribosomes are not destroyed by ribonuclease (7, 29). However, our early experiments (23) clearly showed that, although ribosomes were still present after ribonuclease treatment, some degraded RNA was visible at the top of a sucrose gradient. Because there was some diminution in the ribosome peaks, it seemed likely that most of this material was derived from ribosomes.

To determine quantitatively the effect of ribonuclease on the 70*S* particles and the 50*S* and 30*S* subunits, a number of experiments were carried out with P^{32}-labeled ribosomes. Figure 1 illustrates the effects of ribonuclease on ribosomes suspended in buffer containing 10^{-2} M Mg^{+2}. Figure 1a contains the sedimentation profile obtained from OD readings for ribonuclease-treated and control ribosomes, and Fig. 1b shows the sedimentation profile obtained by measuring P^{32} content of ribonuclease-treated and control ribosomes. P^{32} determination served as a more precise measurement of RNA released by ribonuclease treatment, because the increased

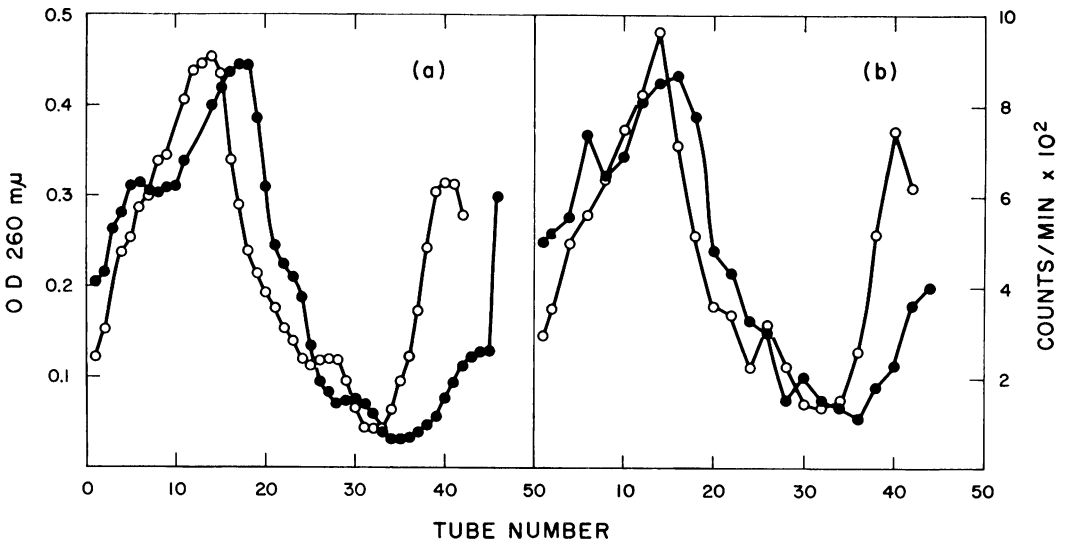

FIG. 1. *Sucrose-gradient analysis of ribonuclease-treated P^{32}-labeled 70S ribosomes (obtained from cells broken open in the French pressure cell). Each tube contained 20 OD units of ribosomes suspended in 0.1 ml of buffer A. The experimental tube was treated with 15 μg of ribonuclease, and the entire contents of both tubes were put on a sucrose gradient prepared in buffer A, and centrifuged at 39,000 rev/min for 100 min. (a) OD readings; (b) P^{32} content. Symbols: ●, control; ○, treated with ribonuclease. The dip in both P^{32} and OD readings observed in the last tube reflects the collection of (in this and some other experiments) less than the normal number of drops.*

OD measurement may reflect, in part, a hyperchromic effect. However, both measurements were in agreement and showed clearly that all of the P^{32} and all of the 260-mμ absorbing material (removed from the main 70*S* ribosome peak) was converted to a form which sedimented near the top of the gradient. The amount of RNA released was about 7% of the total in the ribosome fraction. Some of the released RNA may be mRNA and tRNA; there is, however, attack on the ribosomal RNA itself (23), but the sedimentation profile of the 70*S* ribosome does not seem to be affected by ribonuclease.

To eliminate the possibility that extraribosomal RNA was contributing to RNA released from ribosomes, and to test the effect of ribonuclease on the 50*S* and 30*S* subunits, experiments were carried out with ribosomes suspended in buffer containing 10^{-4} M Mg^{+2} and buffer containing 10^{-5} M Mg^{+2}. The data for these experiments are shown in Fig. 2 (10^{-4} M Mg^{+2} buffer) and Fig. 3 (10^{-5} M Mg^{+2} buffer). Figure 2 illustrates that the 50*S* and 30*S* ribosome components were still present after ribonuclease treatment, but that the areas of both peaks were reduced by about 15%. Under the conditions used in the experiment shown in Fig. 2, tRNA may be bound to 50*S* ribosomes (8), whereas a residue of mRNA is bound to the 30*S* component (24). Although it is likely that ribonuclease releases tRNA and mRNA, it is unlikely that these components make up 15% of the 50*S* and 30*S* particles, respectively.

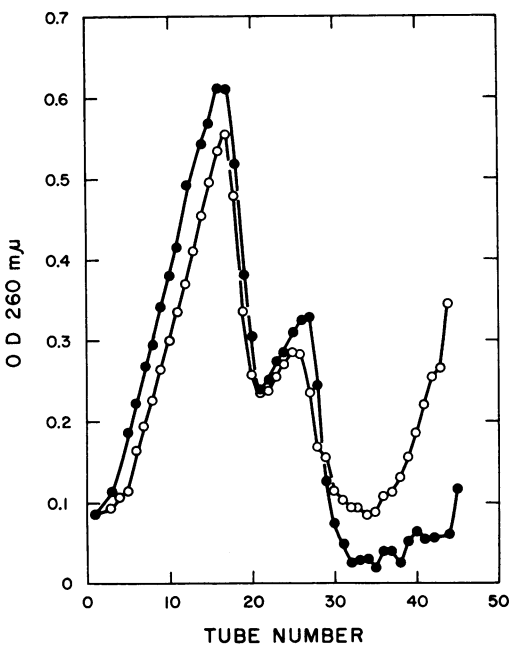

FIG. 2. *Sucrose-gradient analysis of ribonuclease-treated 50S and 30S ribosomes (obtained from cells broken open in the French pressure cell) suspended in buffer containing 10^{-4} M Mg^{+2}. Each tube contained 20 OD units of ribosomes suspended in 0.1 ml of buffer B. The experimental tube was treated with 5 μg of ribonuclease, and the entire contents were put on a sucrose gradient made in buffer B, and centrifuged at 39,000 rev/min for 100 min. Symbols: ●, control; ○, treated with ribonuclease.*

At 10^{-5} to 5×10^{-5} M Mg^{+2}, little tRNA is bound to ribosomes (8, 24), and yet Fig. 3 shows that under these conditions even more RNA was released by ribonuclease from both ribosomes. The results in Fig. 3a and b show that the area under the 50*S* component was reduced by about 20 to 23%, whereas the area under the 30*S* component was reduced by about 20 to 30%. Again, all of the P^{32} and 260-mμ absorbing material was recovered at or near the top of the sucrose gradient. These findings demonstrated conclusively that ribosomal RNA was hydrolyzed in both ribosome subunits.

It was not clear whether every molecule of ribosomal RNA was equally sensitive to the action of ribonuclease. The following experiment indicates that there was at least one available site for ribonuclease activity on every (or virtually every) molecule of 16*S* RNA, and probably more sites on 23*S* RNA (Fig. 4). Figure 4a illustrates the 16*S* and 23*S* components of control ribosomes, and Fig. 4b shows the sedimentation pattern of RNA obtained from ribosomes treated with ribonuclease. The sedimentation value ($S_{20,w}$) was about 8*S*.

Method of obtaining ribosomes and ribonuclease effect. In this first series of experiments, ribosomes were obtained from extracts prepared in the French pressure cell. Two other ways of making *E. coli* extracts were used to determine whether other preparative procedures would yield ribonuclease-sensitive ribosomes. It was found that ribosomes prepared by the lysozyme-EDTA method and the freeze-thaw lysozyme method were as sensitive to ribonuclease as ribosomes obtained after rupturing the cells with the French pressure cell. Thus, the method of preparation did not appear to select a particular sensitive class of ribosomes.

Finite susceptibility of ribosomes to ribonuclease treatment. The next experiment measured the total amount of ribosomal RNA removed by ribonuclease action. A long-term incubation period with ribonuclease was carried out. Figure 5 shows that a 3-hr incubation period with ribonuclease did not remove any more RNA from either ribosomal peak than the 40-min incubation period (see Fig. 3 for comparable conditions).

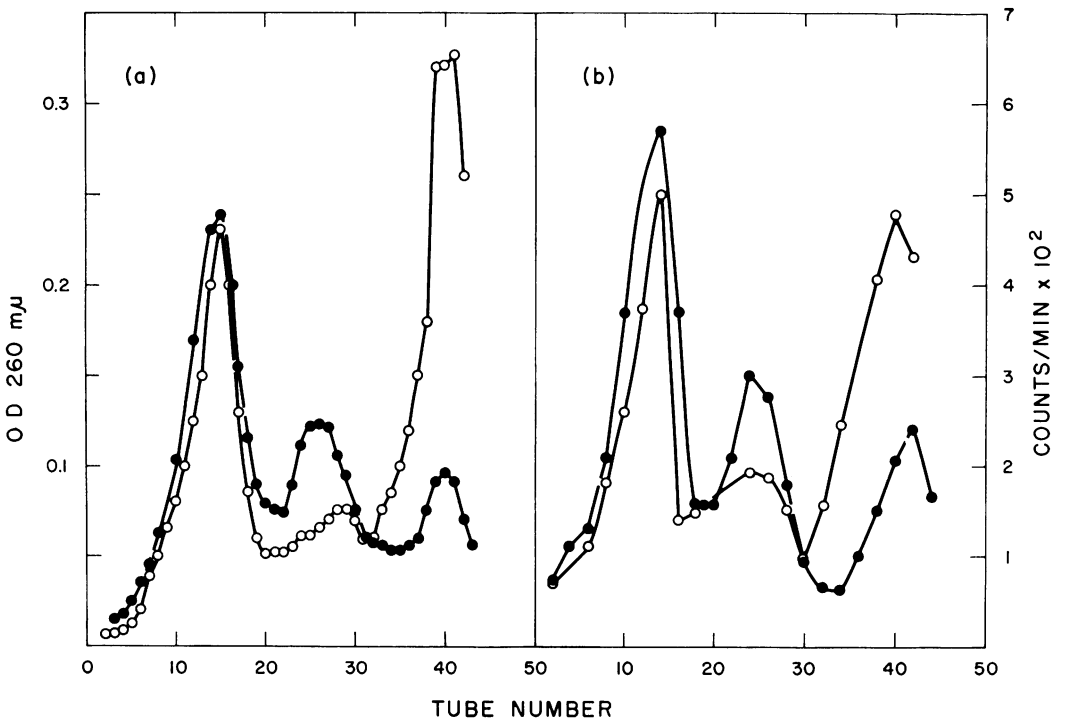

FIG. 3. *Sucrose-gradient analysis of ribonuclease-treated 50S and 30S P^{32}-labeled ribosomes (obtained from cells broken open in the French pressure cell) in buffer containing 10^{-5} M Mg^{+2}. (a) OD profiles of 50S and 30S ribosomes. Each tube contained 8 OD units of ribosomes, suspended in 0.1 ml of buffer C. The experimental tube was treated with 10 μg of ribonuclease, and the entire content was added to a sucrose gradient made in buffer C and centrifuged at 39,000 rev/min. Symbols: ●, control; ○, treated with ribonuclease. (b) P^{32} profile of 50S and 30S ribosomes. Materials, conditions, and symbols are identical to those used in part (a), except that the experiment was carried out on a separate occasion.*

Ribonuclease effect on protein content of ribosomes. The net removal of 20 to 25% of the RNA of the 50*S* peak could mean either that all ribosomes lost about one-fourth of their RNA or that approximately one out of every four ribosomes was completely destroyed with the release of both protein and RNA. It would appear that, if each 50*S* ribosome had lost 25% of its RNA, a change in sedimentation profile might have occurred. Close inspection of the curves in Fig. 5, however, illustrates that both ribonuclease-treated and control 50*S* ribosomes sedimented in exactly the same position over all parts of the peak, which indicated that there was no change in sedimentation properties of the ribonuclease-treated ribosomes. These ribosomes, however, might have undergone some conformational change which altered their sedimentation properties, so that even though they lost some of their RNA their position on the sucrose gradient remained unchanged.

To differentiate between these two alternatives, the loss of 20 to 25% of RNA from each ribosome versus the complete breakdown of the same percentage of the ribosome particles, the protein content of control and ribonuclease-treated ribosomes was determined. Figure 6 shows the S^{35} content of both ribonuclease-treated and control ribosomes, and Fig. 7 and 8 show the protein content of ribosomes after treatment with two different levels of ribonuclease. In all of these experiments, we normalized the protein data for the 50*S* peak tube of the control experiment so that it coincided with the OD readings of that peak tube. Therefore, any decrease in OD (loss of RNA due to ribonuclease treatment) without a concomitant decrease in protein was illustrated by a separation of the two curves representing both parameters at the 50*S* peak. Changes in the RNA-protein ratio of the remaining tubes can be seen by comparing parts (a) and (b) in each figure.

In Fig. 6 to 8, it is clearly shown that about 20% of the ribosomal RNA was removed from both

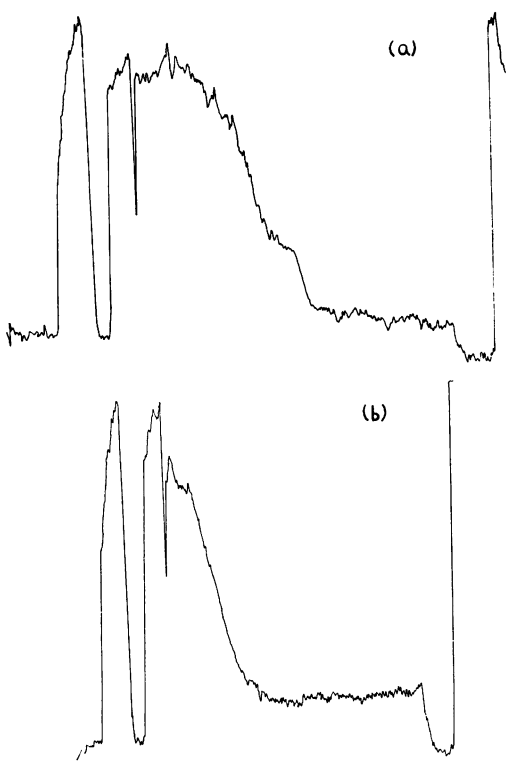

FIG. 4. *Sedimentation pattern of RNA obtained from ribonuclease-treated and control 50S and 30S ribosomes (obtained from cells broken open in the French pressure cell). RNA was dissolved in 0.1 M NaCl plus 0.01 M acetate buffer (pH 4.6) at a concentration of 15 μg/ml. Rotor speed was 39,460 rev/min. The densitometer traces shown above were obtained from pictures taken at 37 min after the rotor had attained the indicated speed. (a) Control RNA; (b) RNA from ribonuclease-treated ribosomes. Control ribosomes were treated with phenol immediately after the addition of ribonuclease, whereas the experimental ribosomes were treated with phenol after 40 min of incubation with ribonuclease at 25 C.*

particles; nevertheless, the total protein content of the 50*S* peak remained virtually unchanged. On the other hand, the 30*S* peak lost protein which now appears near the top of the sucrose gradient. This loss indicated that there may have been breakdown of some 30*S* ribosomes. The control 30*S* peak (Fig. 6 to 8) represented a narrow sedimenting zone in the gradient, but in the ribonuclease-treated ribosomes there was tailing, which suggested the presence of a ribonucleoprotein particle with a slightly lower *S* value than the 30*S* ribosome.

An attempt was made to stabilize the 30*S* fraction, and thereby prevent protein release, by carrying out the ribonuclease treatment in buffer containing 10^{-4} M Mg^{+2}. Under these conditions, there was still selective removal of RNA from the 50*S* peak, although less than at 10^{-5} M Mg^{+2} concentration. The 30*S* peak was not stabilized by the higher Mg^{+2} ion concentration, and lost both RNA and protein.

Nature of proteins released from 30S ribosomes by ribonuclease treatment. An experiment was carried out to determine whether more than one kind of protein was removed from 30*S* ribosomes, and whether that protein or proteins contained antigenic determinants found in 50*S* ribosomes. It was possible to carry out this experiment because of the availability of antiserum to proteins derived from purified and washed 50*S* ribosomes (Estrup and Santer, J. Mol. Biol., *in press*, 1966). With this antibody, antigen-antibody reactions were carried out in Preer (22) tubes with fractions derived from various portions of a sucrose gradi-

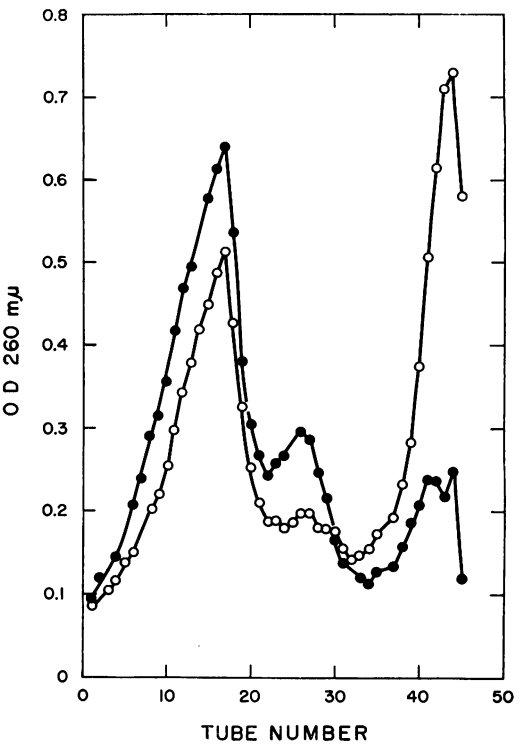

FIG. 5. *Sucrose-gradient analysis of ribosomes treated for 3 hr with ribonuclease. Each tube contained 24 OD units of ribosomes derived from shocked spheroplasts of Escherichia coli, suspended in 0.22 ml of buffer C. The experimental tube was treated with 10 μg of ribonuclease. Both tubes were held at 25 C for 3 hr. At the end of this period, the entire contents of each tube were added to sucrose gradients made in buffer C, and centrifuged at 39,000 rev/min for 100 min. Symbols:* ●, *control;* ○, *treated with ribonuclease.*

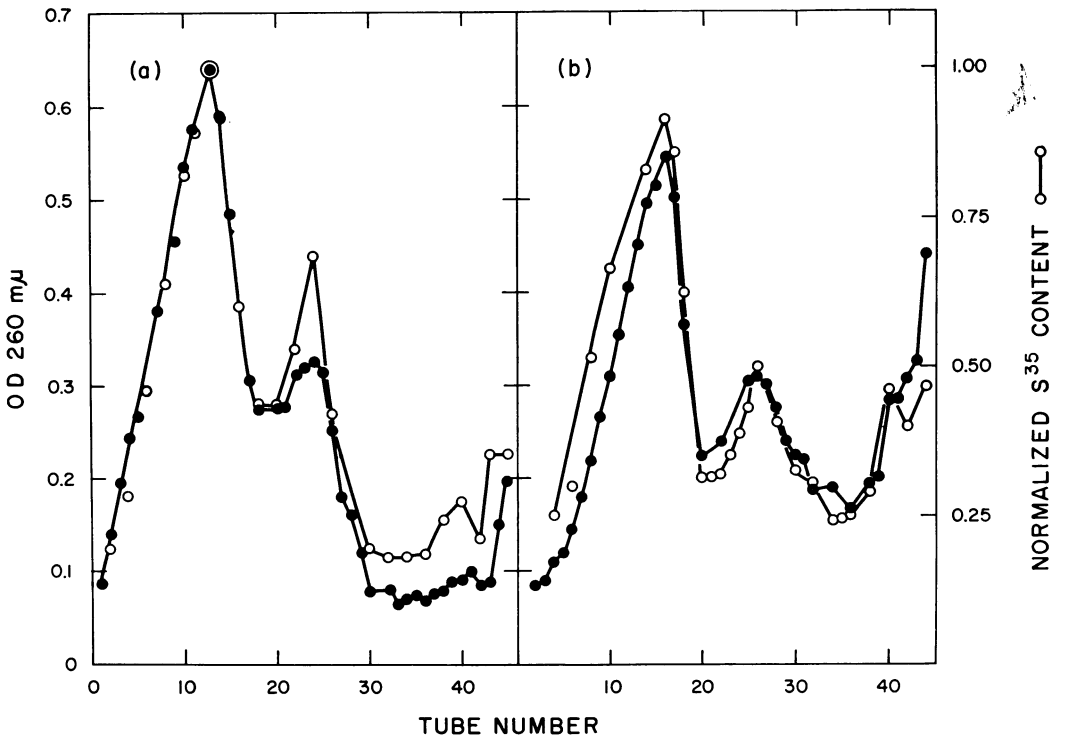

FIG. 6. *Sucrose-gradient analysis of ribonuclease-treated S^{35}-labeled 50S and 30S ribosomes. (a) Control; (b) treated with ribonuclease. Symbols: ●, OD; ○ normalized S^{35} content. Each reaction tube contained 21 OD units of S^{35} ribosomes suspended in 0.1 ml of buffer C, plus 19 μmoles of NH_4Cl. In these experiments, one tube was treated with 21 μg of ribonuclease. The entire contents were put on sucrose gradients made in buffer C, and centrifuged at 39,000 rev/min for 100 min. Fractions were collected, diluted to 2-ml volumes, and read at 260 mμ. Samples (1-ml) of various fractions were then added to 10 ml of Bray's solution, and counted three times, 15 min each time. The recorded radioactivity content of these fractions was obtained by averaging the three separate determinations. The S^{35} content of tube 13 of the control 50S ribosome peak (920 count/min) was normalized to a value of 1, and placed at the same position on the graph as the OD reading of that tube.*

ent which contained ribonuclease-treated ribosomes. The Preer tubes in Fig. 9a contained antigen from fractions 16 (50S peak), 25 (30S peak), and 34 through 40 from the control ribosome gradient; Fig. 9b shows similar fractions of ribonuclease-treated ribosomes.

In fractions 37 to 39 of the ribonuclease-treated samples, there were four precipitin bands, whereas the same fractions in the control sample contained two precipitin bands. In addition, fractions 34 to 41 in the ribonuclease-treated samples showed at least two precipitin bands. To illustrate the increased concentration of ribosomal antigens near the top of the sucrose gradient after ribonuclease treatment, another experiment was carried out in which various fractions were used at one-half the dilution used in Fig. 9a and b. These results are shown in Fig. 10. Even at this lower dilution, there were still two very prominent precipitin bands visible in fractions 35, 37, and 39. This showed that the highest concentration of antigen was present in these fractions, which correlated with the protein analysis (Fig. 6b to 8b).

DISCUSSION

The 23S and 16S RNA molecules of virtually every ribosome are attacked by pancreatic ribonuclease, resulting in the release of as much as 24 and 30% of the RNA contained in the 50S and 30S fraction, respectively. There is a selective removal of RNA from the 50S ribosomes, but the effect of ribonuclease on 30S ribosomes is more complex. Protein, as well as RNA, is removed from 30S ribosomes, and the resulting particles have a greater variation in sedimentation properties than the control 30S ribosomes. Since the protein analyses continue to follow the OD readings in the tailing region of the 30S area, it seems likely that this region of the gradient is occupied by 30S particles which have lost protein and RNA. These particles appear to have sedimenta-

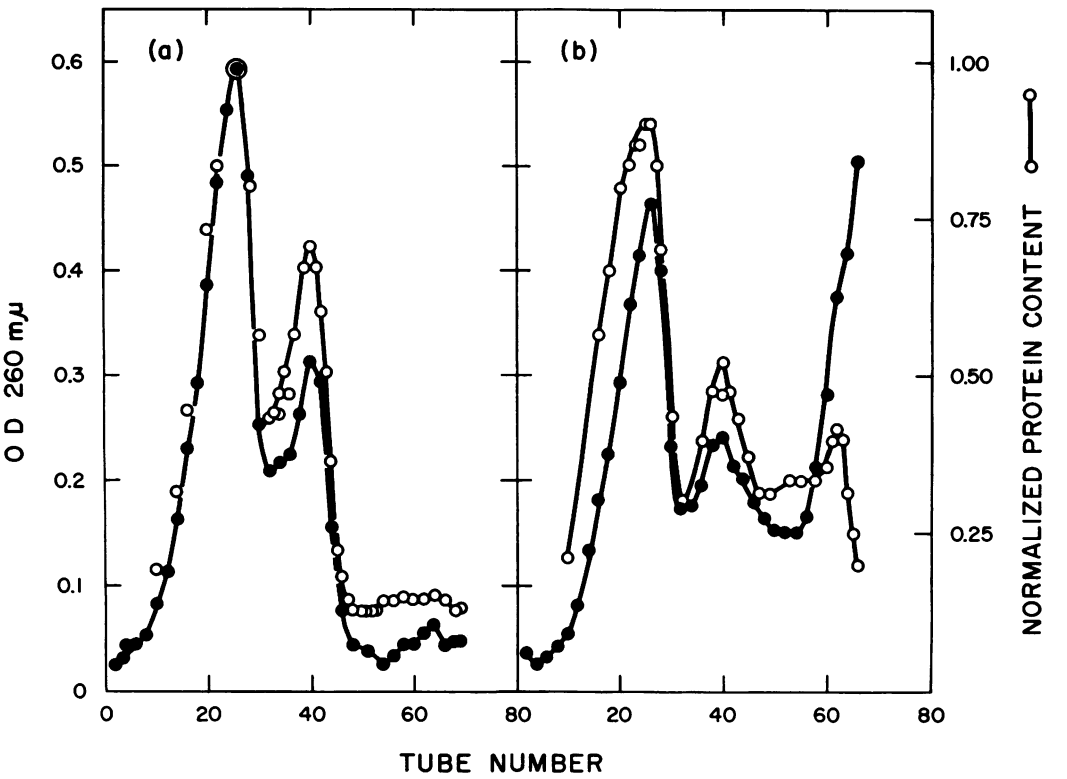

FIG. 7. *Protein analyses of 50S and 30S ribosomes after treatment with high concentration of ribonuclease. (a) control; (b) treated with ribonuclease. Symbols: ●, OD; ○, normalized protein content. Each reaction tube contained 42 OD units of ribosomes suspended in 0.242 ml of buffer C. The experimental tube contained 42 μg of ribonuclease; 0.18 ml from each reaction tube was placed on a sucrose gradient made in buffer C, and centrifuged at 39,000 rev/min for 95 min. The protein value for the 50S peak tube (a) is 15 μg/ml. This measurement has been given a value of 1 and placed at the same position on the graph as the OD of that tube.*

tion values less than 30*S*, but have not been clearly separated from the 30*S* peak by the sucrose gradient.

The disappearance of the 16*S* and 23*S* RNA molecules (Fig. 4b) indicates that the RNA in each ribosome has been attacked in at least one place, and probably more than once. The sedimentation diagram (Fig. 4b) indicates that RNA has an average $S_{20,w}$ of 8, suggesting perhaps that both 16*S* and 23*S* RNA molecules have been converted to molecules of similar size. If this turns out to be so, it indicates that there may be preferential points of attack by ribonuclease on the ribosome-contained RNA. Such sensitive points have been proposed in recent studies (9, 16, 25). Whether these sensitive points are due solely to the configuration of RNA or are influenced by ribosomal protein is unknown.

The proteins that are lost are antigenically related to the proteins present in 50*S* ribosomes, but not necessarily identical to those proteins. The loss of protein from the 30*S* and not the 50*S* ribosomes may indicate some difference in structural organization between them. For example, the 30*S* ribosome might possess a section of RNA (an "end") which, when cleaved by ribonuclease, separates from the ribosome with a number of proteins attached to it. On the other hand, the 50*S* ribosome may lack any RNA "ends," and may lose only RNA that is exposed between protein molecules.

Although considerable RNA was removed from ribonuclease-treated 50*S* ribosomes, they had sedimentation properties similar to untreated ribosomes. This may result from the contraction of the particle size after RNA is lost. It is possible that a small decrease in *S* value occurred which was not detected by the experiments carried out so far.

Although it is now quite clear that ribosomes

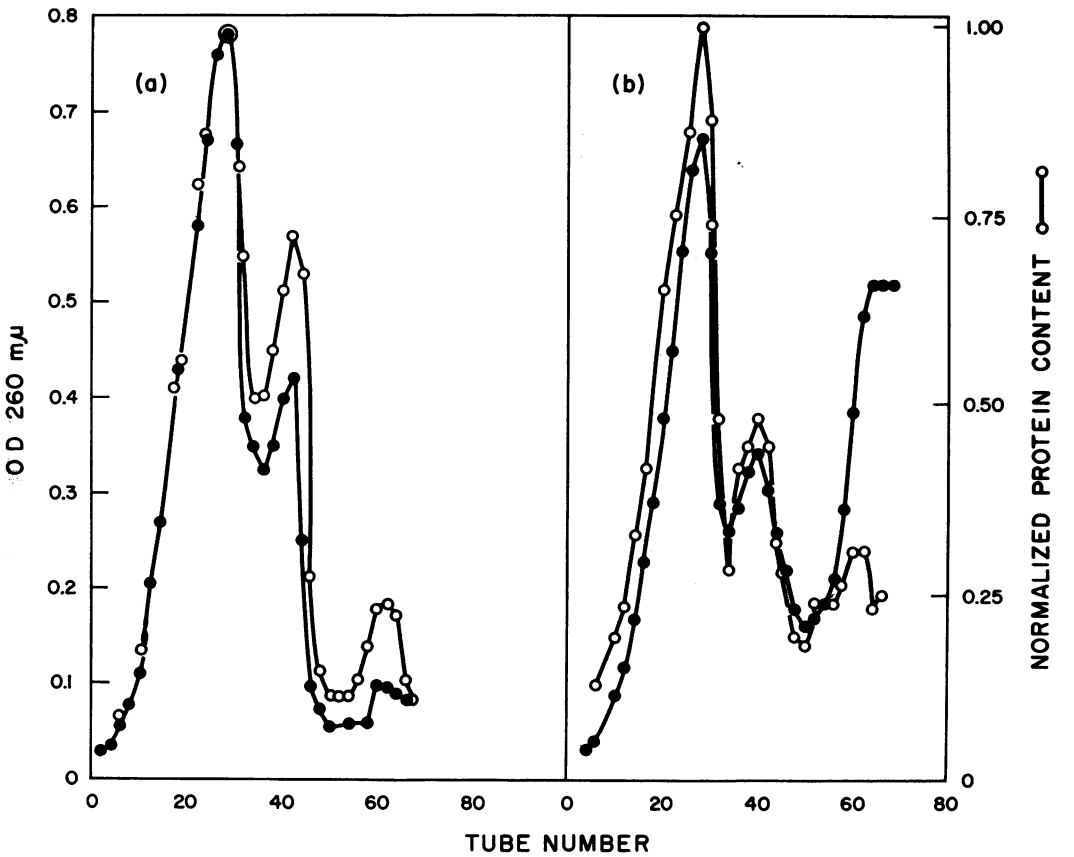

FIG. 8. *Protein analyses of 50S and 30S ribosomes after treatment with a low concentration of ribonuclease. (a) Control; (b) treated with ribonuclease. Symbols: ● OD; ○, normalized protein content. Each reaction tube contained 70 units of ribosomes suspended in 0.267 ml of buffer C. The experimental tube contained 14 μg of ribonuclease. The entire contents of each reaction tube was put on a sucrose gradient made in buffer C, and centrifuged at 39,000 rev/min for 95 min. The protein value for the 50S peak tube (a) is 20.5 μg/ml. This measurement was assigned a value of 1, and placed at the same position on the graph as the OD of that tube.*

are sensitive to ribonuclease, they resist complete breakdown. Any speculation on why ribosomes resist complete breakdown must be based on some ideas concerning ribosomal structure.

The nucleoprotein of intact ribosomes may exist in two configurations: an unfolded strand whose sedimentation value is about 25*S*, and the normal folded particle with an *S* value of 50. The unfolded ribonucleoprotein strand may be converted to a 50*S* ribosome by the addition of magnesium ions (27). The former particle is wholly sensitive to ribonuclease, that is, no residue of a particle remains after ribonuclease treatment; however, the folded configuration retains its sedimentation properties while losing a portion of its RNA.

There are certain ribosome-like particles, however, which do not have the full complement of protein, and have sedimentation values of about 25*S* and 18*S*. These particles are completely destroyed by ribonuclease (19, 6, 28). This complete ribonuclease sensitivity suggests that these particles are partially unfolded ribonucleoprotein strands like the 25*S* particles described by Spirin (27). The sedimentation properties of these ribosome-like particles are not, however, influenced by magnesium ions. Thus, the combination of about 20 molecules of protein with a 23*S* molecule of RNA in the presence of magnesium ions forms a three-dimensional structure with a high *S* value. Simultaneously, 76% of the RNA becomes inaccessible to ribonuclease action. Selective loss of RNA from this particles does not significantly lower the *S* value. The basic shape of

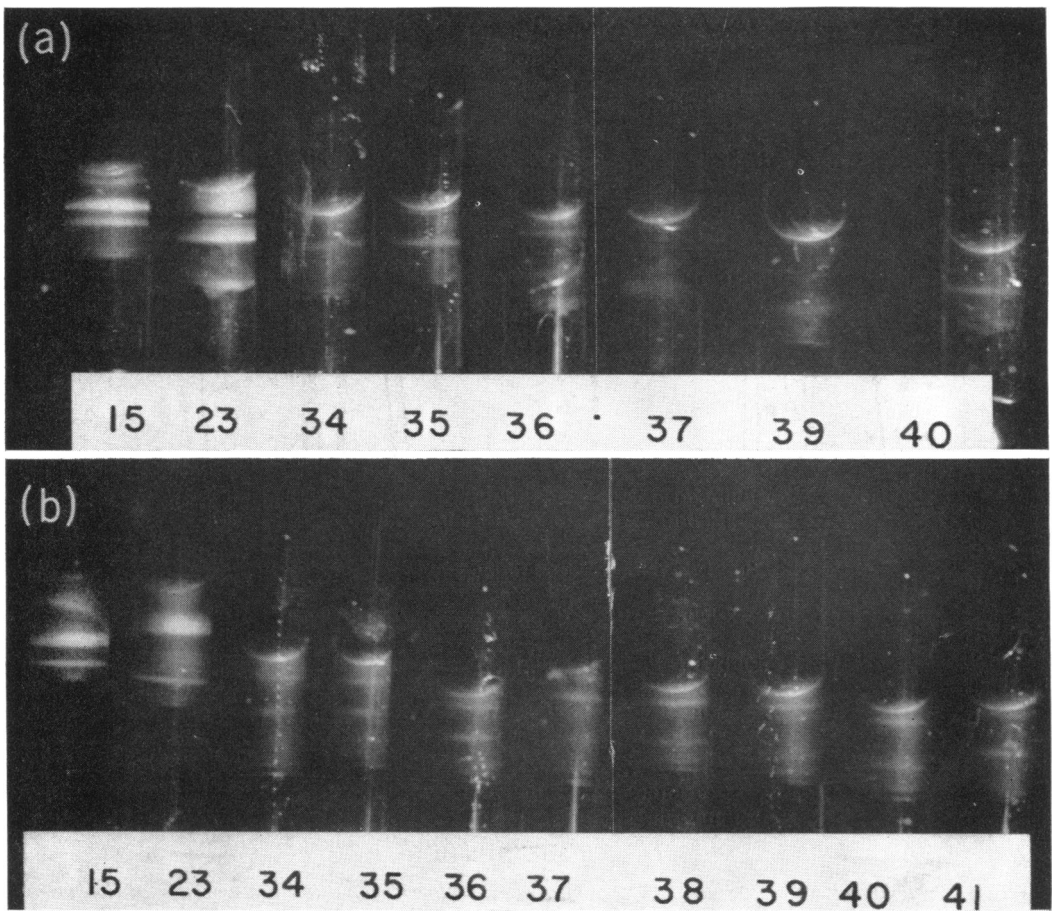

FIG. 9. *Immunological detection of proteins released from 30S ribosomes by ribonuclease. In (a) and (b), 86 OD units of ribosomes in 0.26 ml of buffer C was treated with 60 μg of ribonuclease. Both control and ribonuclease-treated ribosomes were put on a sucrose gradient made in buffer C, and centrifuged for 95 min at 39,000 rev/min. Six drops per fraction were collected, diluted 1:1 with buffer C, and used as antigen. The bottom layer in each Preer tube contained antibody prepared against 50S ribosome proteins. (a) Preer tubes containing antigens obtained from various fractions of a sucrose gradient which contained untreated 50S and 30S ribosomes. (b) Preer tubes containing antigens obtained from fractions of sucrose gradient which had separated ribonuclease-treated ribosomes. In each case, tube 15 is the 50S peak fraction, and tube 23 is the 30S peak fraction. Tubes 34 to 41 represent fractions from the top 16% of the sucrose gradient.*

50*S* ribosome is maintained, presumably, because most of the RNA remains in the interior of the ribosome, and all the protein is still firmly bound.

The tRNA is bound to 50*S* and 30*S* ribosomes (4, 11). Davies et al. (5) and Old and Gorini (20) demonstrated that streptomycin, which binds to 30*S* ribosomes, alters the coding properties of the mRNA-ribosome complex. Allen and Zamecnik (1) showed that T1 ribonuclease inhibits polyuridylic acid-stimulated phenylalanine incorporation by rabbit reticulocyte ribosomes, although T1 ribonuclease has no effect on polyuridylic acid. These important findings illustrate the "passive" and "active" role the ribosome plays in protein synthesis. The ribosome may be important not only for simple binding of the molecules required for protein synthesis, but also for participating actively in the translating process by interacting with mRNA. If it is the ribosomal RNA which plays these apparently dual functions, then it becomes quite clear why a portion is on the "surface" of the ribosome. There it can be easily accessible to all extraribosomal RNA molecules and, incidentally, to ribonuclease.

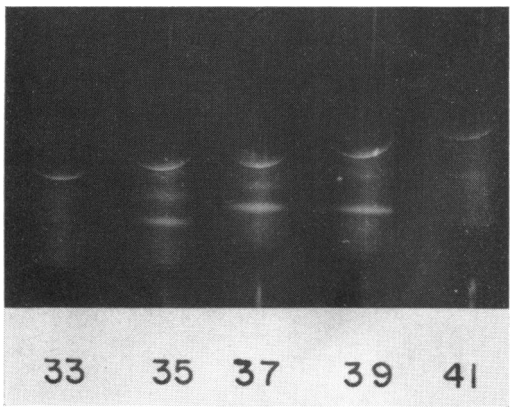

FIG. 10. *Immunological detection of proteins released from 30S ribosomes by ribonuclease. All conditions and materials were identical to those in Fig. 9, except that antigens from odd-numbered tubes 33 to 41 were used at one-half the concentration used in Fig. 9b. Antiserum did not react with pancreatic ribonuclease over a wide range of ribonuclease concentrations.*

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