ROLE OF 5S RIBOSOMAL RNA IN POLYPEPTIDE SYNTHESIS. II. DISSOCIATION OF 5S RIBOSOMAL RNA FROM 50S RIBOSOMES IN ESCHERICHIA COLI.*

M.A.Q. Siddiqui and Keiichi Hosokawa

Space Sciences Laboratory
University of California
Berkeley, California 94720

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50S ribosomes obtained from E. coli were dissociated into 5S rRNA and 44S particles in which at least one of the protein components was missing. Dissociation was produced by dialysis vs. 0.5 M NH_4Cl in Mg^{++}-free medium and the resulting 44S particles were isolated by centrifugation through Mg^{++}-free sucrose solution. A pronounced sensitivity of the 44S particles to RNase was noted, suggesting that the particles had a partially uncoiled structure.

MATERIALS AND METHODS

E. coli Q13 was obtained from Dr. W. Gilbert, Harvard University, through Dr. M. Nomura, University of Wisconsin and used in most of the experiments. Methods for sucrose gradients, CsCl centrifugation and preparation of ^{32}P-labeled and ^{35}S-labeled (E. coli B) ribosome were described previously (1,17), Unlabeled and ^{32}P-labeled 5S rRNA were separated (3) from the RNA phenol-extracted from total cells by Sephadex columns (3,9). MAK columns were made according to Mandell and Hershey (4) and the chromatography was described previously (5). The method of polyacrylamide gel electrophoresis was modified (6) from that reported by Reisfield, Lewis and Williams (7).

RESULTS AND DISCUSSION

In E. coli 50S ribosomes contain approximately one molecule of 5S rRNA per mole particle (Fig. 1), but 5S rRNA

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is absent from 30S ribosomes (3,8,10). To determine whether 5S rRNA is retained in the 40S CsCl core particles (ρ = 1.65)(2), RNA was extracted from these particles and analyzed by MAK column chromatography. 1.2 mole 5S rRNA was found per mole 40S core particles (23S rRNA) which was approximately the same proportion as in intact 50S ribosomes (Table I). More protein was removed by subjecting 50S ribosomes to CsCl centrifugation (1) in the presence of 0.12 M sodium citrate and 0.04 M Mg^{++} (11). The resultant particles had a density of 1.71 (11). We found by MAK column chromatography that 5S rRNA was still retained at 1.2 mole per mole 23S rRNA (Table I). When the Mg^{++} was reduced to 0.02 M in CsCl solution, a partial loss of 5S rRNA from the denser core (ρ = 1.71) was observed; 0.7 mole remained per mole 23S rRNA. Simultaneously, the yield of denser particles decreased, presumably from breakage

TABLE I

Presence of 5S rRNA in various ribonucleoprotein particles derived from 50S ribosomes of E. coli.

50S ribosomes or its subparticles	Concentration of Mg^{++} treated	Ratio: 5S rRNA/23S rRNA	
		% in A_{260}	molar * ratio
50S ribosomes	3×10^{-4} - 10^{-2} M	5.1	1.4
CsCl core particles (ρ = 1.65)	4×10^{-2} M	4.2	1.2
Denser CsCl core particles (ρ = 1.71)	4×10^{-2} M	4.3	1.2
"	2×10^{-2} M	2.5	0.7
50S-A (44S particles)	0	1.1	0.3

RNA extracted from 50S ribosomes or their derivatives was subjected to MAK column chromatography as described in the legend to Fig. 1. Total A_{260} units for 5S rRNA and 23S rRNA were calculated from the optical pattern of elution.

*Calculations were made assuming that molecular weights for 23S rRNA and 5S rRNA were 1.1×10^{-6} (20) and 3.9×10^{4} (21) respectively.

of some of the particles, as judged from the optical pattern of CsCl centrifugation. The ribosomal structure was completely broken down when 50S ribosomes were centrifuged in CsCl with citrate or EDTA (0.01 M) (12) but without added Mg^{++}; 5S and 23S rRNAs both sedimented on the bottom, as shown by MAK column chromatography, and a disc of proteins formed on top of the CsCl gradient.

Milder conditions for dissociating 5S rRNA were produced by treating 50S ribosomes with either 1 M LiCl, 1 M NH_4Cl or 0.5% sodium deoxycholate in TMA (-2) for 30 min. at 4°C. However, 5S rRNA could not be found in the supernatant above these particles after centrifuging as shown by MAK column

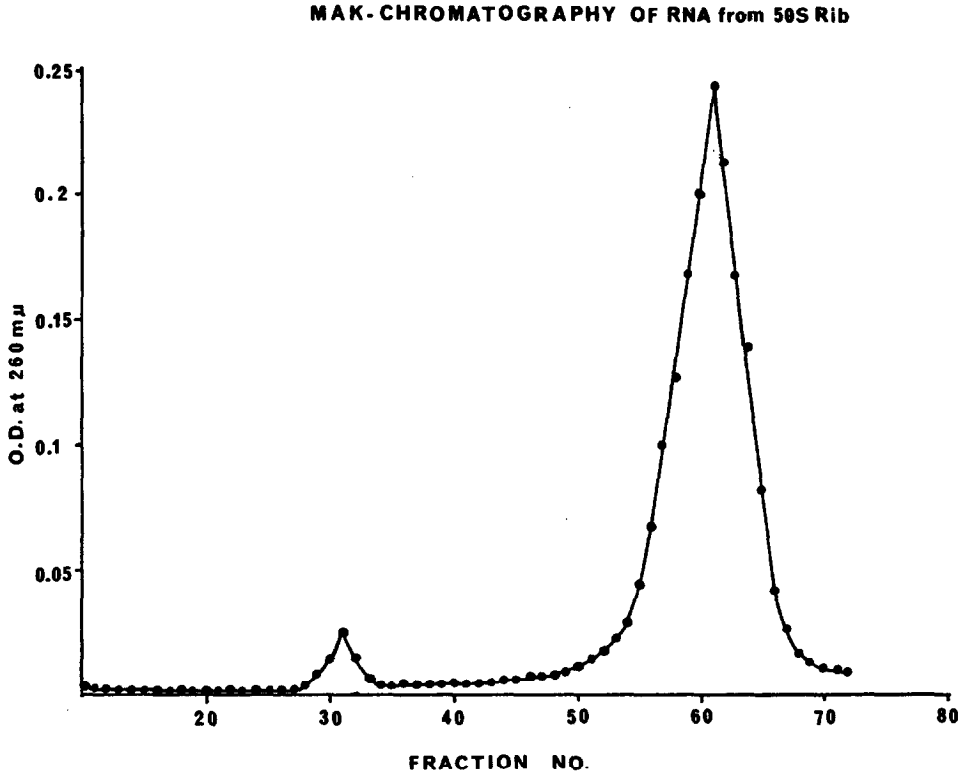

Fig. 1 MAK column chromatography of RNA from 50S ribosomes. 0.5 mg of RNA in 5 ml TMS was applied to the column and eluted with a linear gradient of 0.3 to 1.3 M NaCl in 0.05 M potassium phosphate buffer, pH 6.7 in a total volume of 400 ml. 4 ml volume fractions were collected and analyzed for A_{260}.

chromatography of RNA extracted with 30S ribosomes added. Dialysis of 50S ribosomes vs. TMA (-5) also failed to dissociate 5S rRNA.

A suspension of 50S ribosomes (200 A_{260} units in 2 ml) was dialyzed overnight vs. 2 l of 10^{-2}M Tris Cl - 0.5 M NH_4Cl-6 x 10^{-3}M mercaptoethanol, pH 7.8. One ml of the dialyzed ribosome suspension was placed on 3 ml of 10% sucrose in 10^{-2}M Tris Cl - 0.5 M NH_4Cl- 6 x 10^{-3}M mercaptoethanol layered on 2 ml of 30% sucrose in TMA (-4). The tube was filled with the Mg^{++}-free buffer solution and centrifuged at 140,000 xg for 6 hrs; 5 ml of upper solution was removed by suction, and the next 4 ml and the pellet with 1 ml of lower supernatant were pooled separately. As deduced from an experiment with a duplicate tube, free 5S rRNA should remain in the upper sucrose solution.

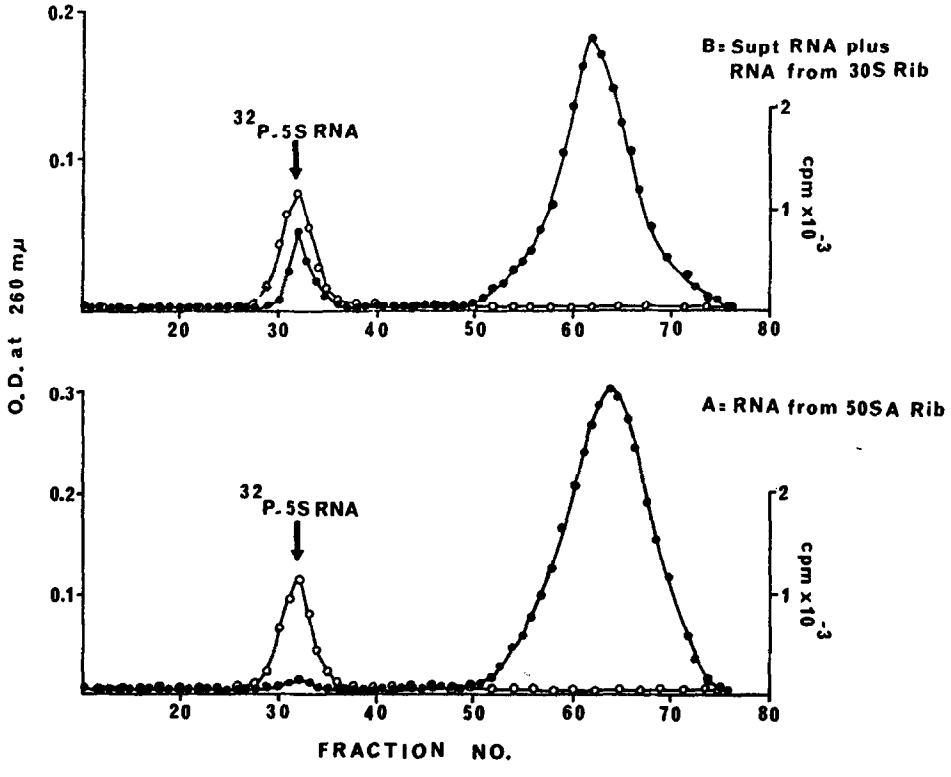

Fig. 2. MAK column chromatography of RNA from NH_4Cl-treated 50S ribosomes and ribosome-free supernatant. Each sample contained 1 mg RNA in 5 ml TMS and ^{32}P-labeled 5S rRNA (2.7 x 10^3 cpm in 0.003 A_{260} unit) as a marker. Chromatography was done as described in Fig. 1.

RNA was extracted from the supernatant, using 30S ribosomes as a carrier, and from the pellet. MAK column chromatography indicated that ribonucleoprotein particles recovered from the pellet retained only 20% of 5S rRNA (Table I), and that 5S rRNA removed from pelleted particles was completely recovered in the supernatant RNA fraction (Fig.1,2). The remaining preparation of ammonium-chloride-treated 50S ribosomes (50S-A) was mixed with ^{32}P-labeled 50S as a marker and subjected to sucrose gradient centrifugation. The sedimentation constant was calculated from Fig. 3 to be 44S (13). Treatment of 50S-A with 0.5 µg per ml pancreatic RNase for 10 min. at 4°C resulted in complete breakdown of RNA as shown by MAK column chromatography after the extraction of RNA, but the RNA of control 50S ribosomes was intact after the same treatment.

The proteins from 50S-A were analyzed by polyacrylamide gel electrophoresis to find whether protein is split from 50S ribosomes during treatment with 0.5 M NH_4Cl in the absence of Mg^{++}. Only the band closest to the origin

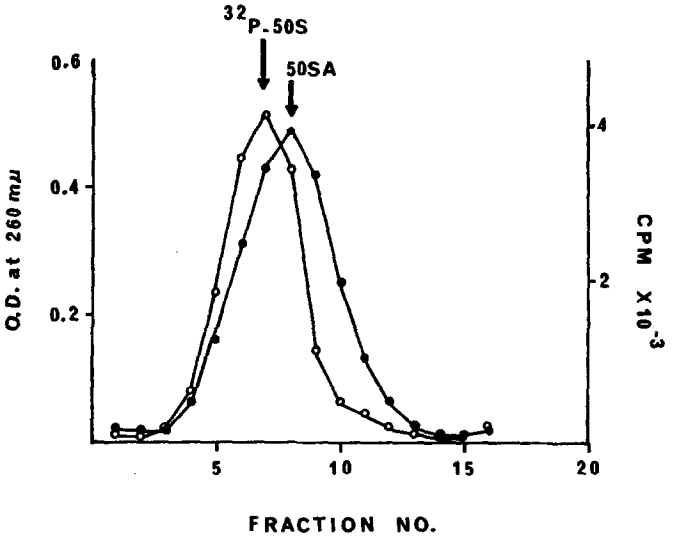

Fig. 3. Sedimentation behavior of NH_4Cl-treated 50S ribosomes. 10 A_{260} units of NH_4Cl-treated 50S ribosomes in 0.3 ml TMA (-4) were mixed with ^{32}P-labeled 50S ribosome-marker (25 x 10^5 cpm in 0.3 A_{260} unit) and layered on 30-10% sucrose gradient in TMA (-4). Conditions of centrifugation and the method of analysis are described in the text.

was missing in 50S-A as compared with the electrophoretic pattern of control 50S ribosomal proteins, (Fig. 4). When ^{35}S-labeled 50S ribosomes prepared from E. coli B were treated with 0.5 M NH_4Cl in the absence of Mg^{++} and subjected to sucrose gradient analysis, 5.9% of the label separated on top. This may well explain the removal of at least one protein component.

We conclude that in the presence of Mg^{++}, 5S rRNA is tightly associated with 50S ribosomal particles. Treatment with LiCl, NH_4Cl, CsCl or deoxycholate in the presence of Mg^{++} thus failed to remove 5S rRNA from 50S ribosomes. Dialyzing 50S ribosome vs. Mg^{++}-free buffer containing 0.5 M NH_4Cl resulted in removal of up to 80% 5S rRNA from the particles (Table I). By this treatment, 50S ribosomes were changed to 44S particles and all but one of the protein components were retained. The remarkable RNase-sensitivity of 50S-A as compared with the original particles suggests a partially uncoiled

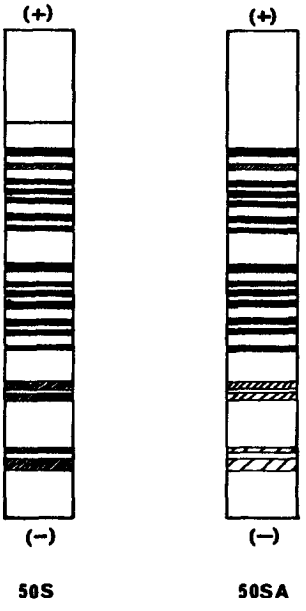

Fig. 4. Polyacrylamide gel electrophoresis of proteins from NH_4Cl-treated 50S ribosomes (50S-A) and the control 50S ribosomes. Ribosomal protein was extracted by 2 M LiCl-4 M urea (23), and 50 µl of sample containing proteins equivalent to 2 A_{260} unit ribosome was analyzed by polyacrylamide gel electrophoresis.

structure which was not reconverted to 50S particles when excess 5S rRNA was added. Release of 5S rRNA and splitting of proteins from 50S ribosomes seem to proceed independently. Lowering of the concentration of Mg^{++} or treatment with chelating reagents such as citrate (11) and EDTA (14,16) caused unfolding of the structure of ribosomes or the subparticles and release of 5S rRNA. On the other hand, treatment with high concentrations of salts such as CsCl resulted in preferential dissociation of proteins from 50S ribosomes. While these results were being prepared for publication, Morell and Marmur (10) reported the removal of 5S rRNA and the production of A' particles from 50S ribosomes of B. subtilis and E. coli, using the procedures of Lerman et al (18). The particles were deficient in protein. The absence of 5S rRNA in CsCl A' particles may be explained by the low Mg^{++} level by Morell and Marmur. The particles were less stable than those described in this communication.

In experiments in collaboration with Dr. M. Nomura (12) it was found that a similar treatment (11) of 30S ribosomes also produced a dense core (density 1.71). Active 30S ribosomes were efficiently reconstituted from the core and proteins. A streptomycin resistant site exists in the dense core of 30S ribosomes. In the biosynthesis of 50S ribosomes, 5S rRNA appears not to be associated (15) with 25S precursor (17), but to be added at the stage of either 32S (20) or 40S particles (10). The removal of 5S rRNA in vitro did not appear to proceed in an order reversing that of biosynthesis (19).

Attempts are being made to reconstitute 50S ribosomes from 50S-A, 5S rRNA and proteins; and the functional properties of 50S-A particles are being studied.

ABBREVIATIONS

rRNA, ribosomal RNA; RNase, ribonuclease; MAK, methylated albumin Kieselguhr; A_{260}, absorbance at 260 mμ; TMA (-2) consists of 10^{-2} M Tris HCl + 5 x 10^{-2} M NH_4Cl + 3 x 10^{-6} M mercaptoethanol + 10^{-2} M Mg acetate, pH 7.8. TMA (-4) consists of the same mixture but with 3 x 10^{-4} M Mg acetate instead of 10^{-2} M Mg acetate. TMA (-5) consists of the same mixture but with 10^{-5} M Mg acetate.

TMS consists of 10^{-2} M Tris HCl - 10^{-3} M $MgCl_2$ - 10^{-1} M NaCl, pH 7.3; 30S and 50S ribosomes refer to 30S and 50S ribosomal subunits.

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REFERENCES

1. Hosokawa, K., Fujimura, R. and Nomura, M., Proc. Natl. Acad. Sci., 55, 198 (1966).
2. Meselsom, M., Nomura, M., Brenner, S., Davern, C. and Schlessinger, D., J. Mol. Biol., 9, 696 (1964).
3. Siddiqui, M.A.Q. and Hosokawa, K., Fed. Proc., 27, 460 (1968) and in preparation.
4. Mandell, J.D. and Hershey, A.D., Anal. Biochem., 1, 66 (1960).
5. Hosokawa, K. and Fraser, M.J., Canadian J. Biochem., 46, 155 (1968).
6. Traub, P., Hosokawa, K., Craven, G.R. and Nomura, J., Proc. Natl. Acad. Sci., 58, 2430 (1967).
7. Reisfield, R.A., Lewis, U.J. and Williams, D.E., Nature, 195, 281 (1962).
8. Rosset, R., Monier, R. and Julien, J., Bul. Soc. Chim., 46, 87 (1964).
9. Galibert, F., Lelong, J.C., Larsen, C.J. and Boiron, M., Biochim. Biophys. Acta, 142, 89 (1967).
10. Morell, P. and Marmur, J., Biochemistry, 7, 1141 (1968).
11. Hosokawa, K., to be published.
12. Traub, P., Nomura, M. and Tu, L., J. Mol. Biol., 19, 215 (1966) and Hosokawa, K. and Nomura, M., unpublished results.
13. Martin, R.G. and Ames, B.N., J. Biol. Chem., 236, 1372 (1961).
14. Gestland, R.F., J. Mol. Biol., 18, 356 (1966).
15. Galibert, F., Lelong, J. and Larsen, C., C.R. Acad. Sci., Paris, 265, 279 (1967).
16. Gavrilova, L.P., Ivanov, D.A. and Spirin, A., J. Mol. Biol., 16, 473 (1966).
17. Hosokawa, K. and Nomura, M., J. Mol. Biol., 12, 225 (1965).
18. Lerman, M.I., Spirin, A.S., Gavrilova, L.P. and Golov, V.F., J. Mol. Biol., 15, 268 (1966).
19. Mangiarotti, G., Apirion, D., Schlessinger, D. and Silengo, L., Biochemistry, 7, 456 (1968).
20. Galibert, F., personal communication.
21. Kurland, C.G., J. Mol. Biol., 2, 83 (1960).
22. Brownlee, G.G., Sanger, F. and Barrell, B.G., Nature, 215, 735 (1967).
23. Cox, E.C., White, J.R. and Flaks, J.G., Proc. Natl. Acad. Sci., 51, 703 (1964).