Effects of Detergents on Ribosomal Precursor Subunits of *Bacillus megaterium*

BARBARA A. BODY† AND BERNARD H. BROWNSTEIN†† *

Department of Medical Microbiology and Immunology, College of Medicine, University of Kentucky,
Lexington, Kentucky 40506

Received for publication 1 August 1977

Cell extracts prepared by osmotic lysis of protoplasts were analyzed by sucrose gradient sedimentation. In the absence of detergents, ribosomal precursor particles were found in a gradient fraction which sedimented faster than mature 50S subunits and in two other fractions coincident with mature 50S and 30S ribosomal subunits. Phospholipid, an indicator of membrane, was shown to be associated with only the fastest-sedimenting ribosomal precursor particle fraction. After the extracts were treated with detergents, all phospholipid was found at the top of the gradients. Brij 58, Triton X-100, and Nonidet P-40 did not cause a change in the sedimentation values of precursors; however, the detergents deoxycholate or LOC (Amway Corp.) disrupted the fastest-sedimenting precursor and converted the ribosomal precursor subunits which sedimented at the 50S and 30S positions to five different classes of more slowly sedimenting particles. Earlier reports on the in vivo assembly of ribosomal subunits have shown that several stages of ribosomal precursor subunits exist, and, in the presence of the detergents deoxycholate and LOC, which had been used to prepare cell extracts, the precursors sedimented more slowly. Our data are consistent with the hypothesis that those detergents selectively modify the structure of ribosomal precursors and lend further support to the hypothesis that the in vivo ribosomal precursor subunits have 50S and 30S sedimentation values. In addition, these data support the idea that the ribosomal precursor particles found in the fast-sedimenting fraction may constitute a unique precursor fraction.

Precursor ribosomal subunits have been characterized as particles that sediment more slowly than mature ribosomal subunits (2, 4-6, 10, 12-14, 21). More recent studies have demonstrated the existence of ribosomal precursor subunits which cosediment with the corresponding mature 50S or 30S ribosomal subunits (1, 11, 12, 16). Lindahl suggested that these cosedimenting precursor subunits had not been observed in earlier studies because the precursor subunits were relatively unstable compared with mature subunits (12). Variations in other, more slowly sedimenting precursor particles (7, 14, 17) have been attributed to instability (7). We previously demonstrated that the sedimentation behavior of ribosomal precursor subunits depends on the method used for cell extract preparation (1) and that those precursor subunits that cosediment with mature subunits can be converted to as many as five more slowly sedimenting particles similar to those observed in other stud-

ies of ribosomal subunit assembly (1, 2, 13, 14, 22).

Although membrane fractions have been shown to contain both ribosomal precursor subunits (4, 18) and precursor ribosomal RNA (8), most other studies of ribosomal subunit assembly have used methods of cell disruption involving detergent treatments or high shear forces. These methods would disrupt membrane fragments that might be associated with precursor ribosomal subunits. In this study, the effect of ionic and nonionic detergents on a fast-sedimenting precursor particle fraction, which might be associated with membrane, was analyzed. In addition, the effect of these detergents on precursor subunits was also examined.

MATERIALS AND METHODS

Bacterium and growth of cultures. The bacterium used in this study was *Bacillus megaterium* KM. Stock cultures of the organism were maintained at -70°C in modified Wachsman medium (1, 22) containing 20% glycerol. All cultures were grown as previously described (1) in modified Wachsman medium.

Isotopic labeling of cells. Overnight cultures were grown in modified Wachsman medium containing 0.1

† Present Address: Department of Medicine, Division of Infectious Diseases, Washington University School of Medicine, St. Louis, MO 63110.

†† Present Address: Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, IL 60637.

the usual phosphate concentration. Flasks containing fresh medium with 0.1 the usual phosphate concentration were inoculated with a 1:100 dilution from an overnight culture. Cells were steady-state labeled by the addition to the culture of 10 μ Ci of [32 P]P_i (carrier-free) per ml. After four doublings, the phosphate concentration of the culture was increased to that normally used in Wachsman medium. This was accomplished by adding the appropriate amount of sterile 20 $\times$ phosphate buffer stock solution (0.344 M K₂HPO₄-0.147 M KH₂PO₄, pH 7.4) that was originally used to prepare Wachsman medium. After one more generation, cells were pulse-labeled for 2.5 min by addition of 2 μ Ci of [5,6- 3 H]uridine (specific activity, 26 Ci/mmol) per ml as previously described (1).

Preparation of protoplasts and protoplast lysates. Protoplast formation and lysis was accomplished by the procedure of Tremblay et al. (1, 20).

Detergent treatment of lysates. Portions of protoplast lysates were incubated with the detergent for 15 min at 0°C before sedimentation analysis. The final concentrations of the detergents used were sodium deoxycholate (DOC), 0.5%; LOC, 0.5%; Brij 58, 0.5%; Triton X-100, 1%; and Nonidet P-40, 1%. LOC is a nonionic detergent produced by Amway Corp., Ada, Mich.; the other detergents were obtained from the usual commercial sources.

Sucrose gradient sedimentation analysis. Ribosomal subunits and precursor particles were analyzed by sedimentation of the protoplast lysate through linear 5 to 20% (wt/vol) sucrose-TM4 gradients that had been formed over a 1.5-ml 80% sucrose-TM4 cushion, as previously described (1).

A Beckman SW27 rotor with 17-ml tubes was used for all sedimentation analyses. After 10 h of centrifugation at 4°C, the tubes were punctured at the bottom with a needle in a drop-collecting apparatus to collect fractions. Usually 40 fractions were obtained per gradient.

Phospholipid extraction for determination of membrane. Phospholipid was used as an indicator of the presence of membrane. Phospholipids were extracted by a modification of the procedure reported by Lester and Steiner (9). The modifications used were (i) to extract the phospholipids from 0.25 ml of each gradient fraction and (ii) to use 25% of the volumes specified for all other reagents in the extraction procedure. After shaking with KCl, the tubes were allowed to stand for 15 to 30 min for separation of phases rather than be centrifuged. Subsequently, 0.5 ml of the upper phase was withdrawn for determination of radioactivity. Re-extraction of the lower phase was not performed.

Determination of radioactivity. After fractionation of the gradients, the amount of radioactivity in ribosomal material and membrane in the gradient fractions was determined with separate portions of each gradient fraction. Radioactivity of ribosomal material in gradient fractions was determined by the sampling method previously described (1). The radioactivity in the phospholipid-containing upper phase from the phospholipid extractions was determined by placing the 0.5-ml sample of the upper phase in a shell vial, which was capped, and subsequently counted by Cherenkov radiation in a Packard Tri-Carb liquid scintillation spectrometer, model 3375.

RESULTS

Effect of detergents on ribosomal precursors and membrane fractions. The sedimentation profile of an untreated lysate (Fig. 1A) shows ribosomal precursor subunits cosedimenting with the mature 50S and 30S subunits. In addition, a significant amount of pulse-label was found in fast-sedimenting material that accumulated in the 80% sucrose cushion. This "heavy" fraction contained ribosomal precursor particles (1); it also contained all the phospholipid (Fig. 1A). The various detergents affected these profiles differently as follows.

After DOC treatment of the extract, no "heavy" fraction was observed, and all the phospholipid was seen at the top of the gradient (Fig. 1B). Furthermore, the ribosomal precursor subunits were now observed in positions of the gradient associated with the slower-sedimenting, "classical" ribosomal precursor particles previously described in studies of ribosome assembly with *Escherichia coli* (2, 13, 14) and *Bacillus licheniformis* (21, 22). Even mature 30S subunits sedimented more slowly when the lysate was treated with DOC (cf. Fig. 1B and 1A).

LOC treatment of the lysate resulted in a sedimentation profile similar to that observed for the DOC-treated sample (Fig. 2A). The ribosomal precursor particles were observed in slower-sedimenting, "classical" positions; the phospholipid was located at the top of the gradient; and the mature 30S subunit again sedimented more slowly. However, several differences between the DOC and LOC profiles were apparent (Fig. 1B and 2A); after LOC treatment, a small amount of pulse-labeled material was consistently seen in the "heavy" fraction (Fig. 2A), and the profile observed for the ribosomal precursor particles was not the same as that obtained with DOC treatment (Fig. 1B and 2A).

When Brij 58, Triton X-100, or Nonidet P-40 was added to the lysate (Fig. 2B, C, and D), the sedimentation profiles were similar to those of the untreated control (Fig. 1A). Neither the precursors nor mature subunits were affected by these nonionic detergents. However, treatment with Brij moved much of the phospholipid to the top of the gradient (Fig. 2B), and in those lysates treated with Triton or Nonidet P-40 all phospholipid was found at the top of the gradient (Fig. 2C and D). It is noteworthy that treatment with these nonionic detergents caused removal of phospholipid from the cushion fraction without moving the pulse-labeled material from its heavy position as did DOC or LOC.

The results of the phospholipid extractions are summarized in Table 1. All phospholipid applied to the gradients was recovered from the gradient fractions after sedimentation (compare

FIG. 1. Sucrose gradient sedimentation of protoplast lysates untreated (A) and treated with DOC at 0.5% (B). Sedimentation was from right to left. The protoplast lysates were layered onto 5 to 20% sucrose gradients in TM4 buffer formed over a 1.5-ml cushion of 80% sucrose in TM4 buffer (the arrow denotes the interface between the cushion and the 20% sucrose). Gradients were subsequently placed in 17-ml buckets of a Beckman SW27 rotor and centrifuged at 25,000 rpm for 10 h at 4°C. The fractions were collected dropwise from the bottom of the gradient. Radioactivity in ribosomal subunits and precursor particles was determined by acid precipitation of a 20- μ l sample from each gradient fraction (1). Radioactivity in phospholipid was determined by extracting the phospholipids from a 250- μ l sample of each gradient fraction and subsequently measuring Cherenkov radiation in the ether phase (9; see Materials and Methods). The ether-extracted [32 P]P_i (phospholipid) represents less than 1% of the acid-precipitable [32 P]P_i (ribosomal subunits); thus, any contribution to the ribosomal profile that was made by acid precipitation of phospholipid was negligible. Symbols: ●, [32 P]P_i (steady-state label); ○, [3 H]uridine (pulse-label); and ▲, [32 P]P_i (phospholipid).

"lysate" to others); thus, the distribution of phospholipid in the sedimentation profiles of Fig. 1 and 2 is representative of the total phospholipid content.

DISCUSSION

We previously reported (1) that various methods of cell disruption and/or the use of DOC can alter the physical properties of ribosomal precursor subunits. The data presented in this study indicated that another detergent, LOC,

which has been used by Bryant and Sypherd in studies of ribosome assembly (3, 19), also alters the sedimentation properties of ribosomal precursor subunits as well as mature 30S subunits (Fig. 2A). After completion of this work with LOC, we learned that this detergent may have more than one nonionic species in addition to coconut oil derivatives (amides) and 0.2% ethylenediaminetetraacetic acid (Amway Corp., personal communication). The final concentration of ethylenediaminetetraacetic acid used in

FIG. 2. Sucrose gradient sedimentation of protoplast lysates treated with LOC at 0.5% (A), Brij 58 at 0.5% (B), Triton X-100 at 1% (C), and Nonidet P-40 at 1% (D). Sedimentation was from right to left. See legend to Fig. 1 for procedure.

TABLE 1. Recovery of phospholipid from sucrose gradients

Detergent ^a	cpm ^b	
	Cushion	Top
DOC (34-41) ^c		1,922
LOC (34-41)		1,593
Brij 58 (1-7) + (34-40)	940	1,046
Triton X-100 (34-41)		1,982
Nonidet P-40 (34-41)		1,852
Untreated (1-7)	1,793	
Lysate ^d		

^a Indicates detergent used for treatment of lysate before sucrose gradient sedimentation.

^b ³²P in phospholipid extracted from sucrose gradient fractions.

^c Numbers in parentheses correspond to gradient fractions in Fig. 1 or 2.

^d A portion of lysate not treated and not subjected to sucrose gradient sedimentation had a total of 2,030 cpm.

this treatment would remove about one-half of the magnesium ions. This could account for its disruptive effect on the ribosomal precursor par-

ticles and mature 30S subunits, similar to the ionic detergent DOC. In contrast, nonionic detergents Brij 58, Triton X-100, and Nonidet P-40 did not alter the fast-sedimenting precursor, the 50S and 30S ribosomal precursor subunits, or the mature 50S and 30S subunits (Fig. 2B, C, and D). Subsequent work has shown that the fast-sedimenting fraction contains a 30S RNA species in addition to several forms of precursor rRNA's (B. A. Body, Ph.D. thesis, University of Kentucky, Lexington, 1977). Furthermore, kinetic studies have shown that a [³H]-uridine pulse-label chases from the 30S RNA species to the precursor rRNA's (B. A. Body and B. H. Brownstein, submitted for publication). Therefore, it appears that the fast-sedimenting fraction contains some novel form of ribosomal precursor.

Phospholipid, as an indicator of membrane, was not found associated with the 50S and 30S ribosomal precursors in untreated lysates (Fig. 1A). Therefore, the differences in sedimentation behavior of these ribosomal precursor subunits after treatment with DOC and LOC were not

due to removal of membrane fragments. DOC alters ribosomal precursor subunits directly (Fig. 1B), possibly by interacting with and/or displacing some of the ribosomal proteins. It now seems necessary to reinterpret the results of studies on ribosome assembly where the detergents DOC and LOC were used, since these have a profound effect on ribosomal precursor subunits. The data from this and previous studies lend further support to the hypothesis (1, 12, 15) that in vivo ribosomal precursor particles have 50S and 30S sedimentation values not dependent on the binding of membrane to "classical" precursor ribosomal subunits.

In untreated lysates, all membrane was found in the heavy region of the gradient, in the fast-sedimenting fraction that also contains ribosomal precursor particles (Fig. 1A). This result agrees with those of Roth and Daneo-Moore (18), who demonstrated that membranes from gently lysed, fragile forms of *Streptococcus faecalis* were associated with a fast-sedimenting fraction. It seemed possible that the membrane located in the cushion fraction was necessary to maintain the integrity of this ribosomal precursor subunit fraction. However, Triton or Nonidet P-40 treatment (Fig. 2C and D) released all phospholipid from the cushion fraction, leaving the rest of the fraction intact (Fig. 2C and D). Further support for the integrity of the fast-sedimenting precursor as such comes from the recovery of phospholipid summarized in Table 1. Equivalent amounts of phospholipid were recovered from all of the gradients; therefore, if some membrane is required for the integrity of the fast-sedimenting precursor, it must be an amount not detectable by these methods. One or more membrane proteins may be necessary for the maintenance of this precursor fraction, and these are disrupted by DOC or LOC, but not by Brij, Triton, or Nonidet P-40. Alternatively, the precursors found in the fast-sedimenting cushion fraction may constitute a unique precursor complex that is not dependent on an association of these particles with membrane.

ACKNOWLEDGMENTS

We are indebted to Robert L. Lester for his helpful suggestions on the modifications of his method for phospholipid extraction. We also thank Paul Sypherd and David Schlessinger for their critical reading of this manuscript.

This investigation was supported by a General Research Support grant to B. H. Brownstein from the College of Medicine, University of Kentucky.

LITERATURE CITED

1. Body, B. A., and A. H. Brownstein. 1976. Ribosomal precursor particles of *Bacillus megaterium*. J. Bacteriol. 126:1149-1155.
2. Britten, R. J., B. J. McCarthy, and R. B. Roberts. 1962. The synthesis of ribosomes in *E. coli*. IV. The synthesis of ribosomal protein and the assembly of ribosomes. Biophys. J. 2:83-93.
3. Bryant, R. E., and P. S. Sypherd. 1974. Genetic analysis of cold-sensitive ribosome maturation mutants of *Escherichia coli*. J. Bacteriol. 117:1082-1092.
4. DiGirolamo, M., E. Hinckley, and E. Busiello. 1968. Localization of ribosomal precursors in *Escherichia coli*. Biochim. Biophys. Acta 169:387-397.
5. Dimmitt, K., and P. S. Sypherd. 1973. Association of lysozyme with ribosomes and ribosomal precursor particles. J. Bacteriol. 116:1059-1061.
6. Guthrie, C. H., H. Nashimoto, and M. Nomura. 1969. Structure and function of *E. coli* ribosomes. VIII. Cold-sensitive mutants defective in ribosome assembly. Proc. Natl. Acad. Sci. U.S.A. 63:384-391.
7. Hayes, F., and D. H. Hayes. 1971. Biosynthesis of ribosomes in *E. coli*. I. Properties of ribosomal precursor particles and their RNA components. Biochimie 53:369-382.
8. Haywood, A. M. 1971. Cellular site of *Escherichia coli* ribosomal RNA synthesis. Proc. Natl. Acad. Sci. U.S.A. 68:435-439.
9. Lester, R. L., and M. R. Steiner. 1968. The occurrence of diphosphoinositide and triphosphoinositide in *Saccharomyces cerevisiae*. J. Biol. Chem. 243:4889-4893.
10. Lewandowski, L. J., and B. L. Brownstein. 1969. Characterization of a 43S ribo-nucleoprotein component of a mutant of *Escherichia coli*. J. Mol. Biol. 41:277-290.
11. Lindahl, L. 1973. Two new ribosomal precursor particles in *E. coli*. Nature (London) New Biol. 243:170-172.
12. Lindahl, L. 1975. Intermediate and time kinetics of the *in vivo* assembly of *Escherichia coli* ribosomes. J. Mol. Biol. 92:15-37.
13. McCarthy, B. J., R. J. Britten, and R. B. Roberts. 1962. The synthesis of ribosomes in *E. coli*. III. Synthesis of ribosomal RNA. Biophys. J. 2:57-82.
14. Mangiarotti, G., D. Apirion, D. Schlessinger, and L. Silengo. 1968. Biosynthetic precursors of 30S and 50S ribosomal particles in *Escherichia coli*. Biochemistry 7:456-472.
15. Mangiarotti, G., E. Turco, C. Perlo, and F. Altruda. 1975. Role of precursor 16S RNA in assembly of *E. coli* 30S ribosomes. Nature (London) 253:569-570.
16. Mangiarotti, G., E. Turco, A. Ponzetto, and F. Altruda. 1974. Precursor 16S RNA in active 30S ribosomes. Nature (London) 247:147-148.
17. Osawa, S., E. Otaka, T. Itoh, and T. Fukui. 1969. Biosynthesis of 50S ribosomal subunit in *Escherichia coli*. J. Mol. Biol. 40:321-351.
18. Roth, G. S., and L. Daneo-Moore. 1971. Intracellular location of ribosome biosynthesis in osmotically fragile forms of *Streptococcus faecalis*. Biochim. Biophys. Acta 240:575-587.
19. Sypherd, P. S., R. E. Bryant, K. Dimmitt, and P. Fujisawa. 1974. Genetic control of ribosome assembly. J. Supramol. Struct. 2:166-177.
20. Tremblay, G. Y., M. J. Daniels, and M. Schaefer. 1969. Isolation of a cell membrane-DNA-nascent RNA complex from bacteria. J. Mol. Biol. 40:65-76.
21. Van-Dijk-Salkinoja, M. S., and R. J. Planta. 1970. Formation and life cycle of ribosomal subunits in *Bacillus licheniformis*. Arch. Biochem. Biophys. 141:477-488.
22. Van-Dijk-Salkinoja, M. S., T. J. Stoff, and R. J. Planta. 1970. The distribution of polysomes, ribosomes, and ribosomal subunits in exponential-phase cells of *Bacillus licheniformis*. Eur. J. Biochem. 12:474-482.
23. Wachsmann, J. T., and R. Storch. 1965. Propionate-induced lysis of protoplasts of *Bacillus megaterium*. J. Bacteriol. 80:600-606.