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Involvement of Protein and Ribonucleic Acid in the Association of Deoxyribonucleic Acid with Other Cell Components of *Escherichia coli*

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Cells of *Escherichia coli* were labeled with precursors of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein, lysed with detergent, and examined by starch-block electrophoresis and CsCl density gradient centrifugation. A large amount of the DNA was seen to remain at positions of low electrophoretic mobility and light density along with tryptophan and arginine-containing proteins and some RNA. Addition of labeled, phenol-extracted DNA to unlabeled cells prior to lysis and electrophoresis showed that only a small amount of the DNA became associated during or after lysis. Sonic treatment of a lysate removed most of the DNA to a position of electrophoretic mobility and density similar to that of free DNA, whereas pronase and ribonuclease released only a part of the DNA. We concluded that binding of DNA to cell membranes or other cell components occurs in the cell prior to lysis and involves protein and probably a specific type of RNA.

Jacob, Brenner, and Cuzin (8) proposed that the contact of deoxyribonucleic acid (DNA) with the cell membrane is important in the replication of bacterial DNA, and Goldstein and Brown (7) found evidence for the preferential location of replicating DNA in contact with large aggregates of material in lysed cells of *Escherichia coli*. Ganesan and Lederberg (5) later showed that a large amount of the replicating DNA of *Bacillus subtilis* was linked to cell membranes, an association which could be reduced by pronase treatment.

The manner in which DNA is bound to other cellular components is obscure, but there is some evidence that the binding may involve ribonucleic acid (RNA) or protein or both. Godson, Hunter, and Butler (6) found that the membrane fraction of *B. megaterium* protoplasts lysed by osmotic treatment contained up to 50% of the total RNA, mainly in the ribosomal form. The action of pronase in releasing DNA from the interphase pellet during phenol extraction is now common knowledge, and Davern (4) has shown that pronase releases DNA from lighter fractions during CsCl density gradient sedimentation of *Escherichia coli* spheroplasts lysed by detergent.

In this study, we investigated the role of RNA and protein in the binding of DNA to other cell components within a single bacterial system. Cells of *E. coli* grown in medium containing radioactive precursors of DNA, RNA, or protein were lysed with detergent, then sonic-treated or treated with pronase or ribonuclease, and subsequently studied by starch block electrophoresis and CsCl density gradient sedimentation.

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MATERIALS AND METHODS

Bacterial growth and labeling. *E. coli* strains K-12 CR34 and TAU bar were used in these experiments. The growth medium contained 0.08 M NaCl, 0.02 M KCl, 2×10^{-4} M $CaCl_2$, 2×10^{-6} M $FeCl_3 \cdot 6H_2O$, 2.5×10^{-3} M Na_2SO_4, 6.4×10^{-3} M KH_2PO_4, 0.2% glucose, and 0.12 M tris(hydroxymethyl)aminomethane (Tris) at *p*H 7.0. For growth of unlabeled TAU bar, thymine (2 μg/ml), uracil (10 μg/ml), arginine, methionine, proline, and tryptophan (20 μg/ml each), and Vitamin Free Casamino Acids (1 mg/ml) were added to the medium. For growth of unlabeled CR34, only thymine and Casamino Acids were added.

In labeling of either strain with thymidine-methyl-^3H or labeling TAU bar with L-arginine-^{14}C (uniformly labeled) or DL-tryptophan-2,3-^3H, 10 μc was added to each milliliter of growth medium and the final concentration of the substance was adjusted to the level given above for unlabeled compounds. When labeling with arginine, Casamino Acids were omitted. For growth with thymine-2-^{14}C (4.2 mg/mc) or uracil-2-^{14}C (3.74 mg/mc), 10 μc of either compound was added per milliliter of growth medium with no additional thymine or uracil. All isotopes were

purchased from New England Nuclear Corp., Boston, Mass.

Ten ml of labeled bacteria was grown in 50-ml Erlenmeyer flasks and 50 ml of unlabeled bacteria in 250-ml flasks at 37 C on a shaker. After 5 to 5.5 hr of growth, 10 ml of labeled cells and 30 ml of unlabeled cells were combined, harvested in a centrifuge, and washed three times with lysis buffer (0.01 M Tris, 0.15 M NaCl, 0.015 M ethylenediaminetetraacetic acid, *p*H 8.0). Growth curves showed that at the time of harvest cultures of either strain were in the late logarithmic stage of growth and contained about 5×10^8 viable cells/ml.

Lysis and sample preparation. Bacteria were resuspended in lysis buffer to 10^9 cells/ml for lysis with 1% sodium lauryl sulfate (SLS) or 5×10^8 cells/ml for lysis with 1% sarkosyl 97 (sodium lauryl sarcosinate, Geigy Industrial Chemicals). Cells were then incubated at 60 C for 20 min.

Lysates were sonically treated with a Sonifier converter model S75 and attached power supply (Branson Instruments, Inc., Stamford, Conn.), using six 10-sec exposures, with several minutes of cooling in an ice bath between exposures. For enzyme treatment, lysates to be used in electrophoresis were incubated for 6 to 8 hr at 37 C with pronase (B grade; Calbiochem, Los Angeles, Calif.) or with ribonuclease (ribonuclease A; Sigma Chemical Co., St. Louis, Mo.), both at a concentration of 1 mg/ml. Lysates used in CsCl density gradient sedimentation were incubated with either enzyme for 12 hr. ^{14}C- or ^{3}H-labeled DNA was prepared by phenol extraction from *E. coli* strain K-12 CR34 grown for 5.5 hr in media containing ^{14}C-thymine or ^{3}H-thymidine.

Electrophoresis. Starch block electrophoresis was performed with a method similar to that described by Matsubara and Takagi (12). Two samples, experimental and control, were run at one time in adjacent plastic trays (36 × 5 × 1 cm) containing washed potato starch suspended in buffer. Each well of the electrophoretic chamber contained 1,600 ml of the same buffer which consisted of 0.05 M boric acid and 0.05 M NaCl, and had a pH of 8.6. A constant voltage of 200 v was applied for 12 hr at 4 C; the current varied between 15 and 20 ma. After a run, the starch was cut into 1-cm sections and was eluted with 5 ml of 0.9% NaCl; 0.5 ml of each fraction was used in counting radioactivity.

CsCl density gradient sedimentation. CsCl density gradient sedimentation was carried out with a sample-CsCl mixture at an overall density of about 1.7 g/ml. The samples were centrifuged at 10 C in the SW-39 head (3 ml plus 2 ml of mineral oil) or SW-50 head (5 ml) of a model L-2 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) for 60 hr at 99,972 × *g* or for 50 hr at 73,449 × *g*. Two-drop fractions were collected from the 3-ml samples and three-drop fractions from the 5-ml samples by puncturing the bottom of the tubes with an insect pin. Fractions were collected directly into counting vials or into small tubes containing 2 ml of lysis buffer. In the latter case, 0.2 ml from each tube was pipetted into vials for counting.

The drained tubes, which retained the floating cellular debris, were cut into sections and placed in a test tube with 10 ml of 0.1 N NaOH; then they were shaken until all visible material dissolved. A 0.5-ml amount of the solution was then removed for counting. Removal of the top pellet with a tube slicer gave similar results, showing that material adhering to the sides or the bottom of the tube is negligible.

Counting procedure. Radioactivity was counted in a liquid scintillation counter (Packard Instrument Co. Inc., Downers Grove, Ill.) with 10 ml of dioxane scintillation fluid, which has been shown to be very resistant to quenching when volumes of aqueous sample up to 10% are added (2).

RESULTS

At the beginning of this investigation, attempts were made to estimate the amount of cellular material in each fraction after electrophoresis by measurement of 260-mμ absorption and by colorimetric tests for DNA, RNA, and protein. We found that absorption at 260 mμ was not an accurate method for estimation of cellular material in electrophoretic fractions after lysis at 10^9 cells/ml, since the 260-mμ absorbing substances contributed by other sources, presumably SLS and materials in the starch, were as great as those contributed by the cell lysate. When cell lysates were used at higher concentrations, the electrophoretic mobility of the cellular materials was reduced and inconsistent results were obtained, probably due to incomplete lysis of the bacteria at these higher concentrations. Determination of DNA, RNA, and protein in the electrophoretic fractions by colorimetric tests was also found to be unworkable, because of the presence of interfering substances in some cases and because of the low concentrations of DNA, RNA, and protein in cells lysed at a concentration suitable for reproducible electrophoretic separation. For these reasons, amounts of RNA and protein relative to DNA in each fraction were estimated in a qualitative way; we observed the degree of radioactive DNA, RNA, and protein in the various fractions after the cells were grown in the presence of radioactive precursors of DNA (thymidine), RNA (uracil), and protein (tryptophan and arginine), lysed, and subjected to electrophoresis.

Sonic-treated and untreated SLS lysates of *E. coli* strain CR34, labeled for 5 to 5.5 hr with ^{3}H-thymidine, were placed in adjacent trays and were subjected to starch-block electrophoresis. We found (Fig. 1) that sonic treatment markedly increased the electrophoretic mobility of ^{3}H-labeled material in these lysates. This suggested some association of DNA with electrophoretically less mobile materials from which it could be released by sonic treatment. The addition of ^{3}H-DNA to unlabeled cells prior to lysis had

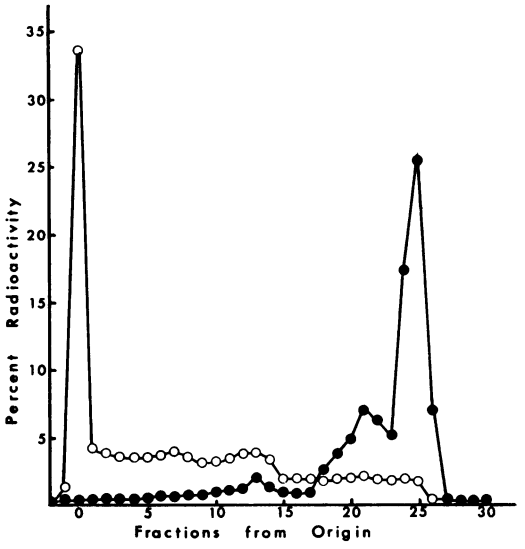

FIG. 1. *Electrophoresis of sonic-treated and untreated SLS lysates of Escherichia coli strain CR34 grown in media containing ^{3}H-thymidine. Symbols:* (○) *untreated, total counts/min = 19,554;* (●) *sonic-treated, total counts/min = 20,167.*

little effect on the electrophoretic mobility of the DNA (Fig. 2). Thus, the association of DNA with other cell materials existed in the cells prior to lysis and did not occur during or after lysis. The fact that relatively whole, non-sonic-treated, phenol-extracted DNA moved rapidly during electrophoresis, even in the presence of lysed cells, also indicated that the increased electrophoretic mobility following sonic treatment could not be explained only by the smaller size of sonic-treated pieces of DNA. Similar effects of sonic treatment were observed by Goldstein and Brown (7), who reported that sonic treatment of *E. coli* cells or lysates released a great deal of the DNA from the particulate fraction after centrifugation.

CsCl density-gradient sediment of an untreated SLS lysate labeled with ^{3}H-thymidine showed that 89% of the label appeared in association with material of lighter density which was found floating on the surface of the gradient (Fig. 3). If the lysate was sonic-treated before centrifugation, only 6% of the label was found in the floating pellet. These results confirm those of the electrophoresis experiments, and agree with those of Davern (4), who found most of the DNA from *E. coli* spheroplasts lysed with detergent to be at or near the meniscus in CsCl density gradients. In the present study, some of this association was due to the insolubility of SLS in CsCl. However, when the CsCl-soluble detergent sarkosyl was used to lyse cells, 69% of the radioactivity of a ^{3}H-thymidine-labeled lysate was still found at the top of the gradient and sonic treatment reduced this to 3% (Table 1).

The material associated with DNA probably includes cell membranes and debris from cell

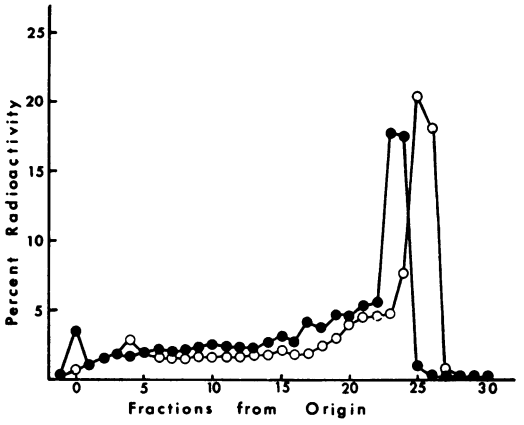

FIG. 2. *Electrophoresis of ^{3}H-labeled DNA extracted with phenol from Escherichia coli strain CR34 and similar DNA added to unlabeled Escherichia coli strain CR34 prior to lysis and electrophoresis. Symbols:* (○) *^{3}H-labeled DNA, total counts/min = 133,862;* (●) *^{3}H-labeled DNA added to unlabeled cells followed by lysis and electrophoresis, total counts/min = 159,855.*

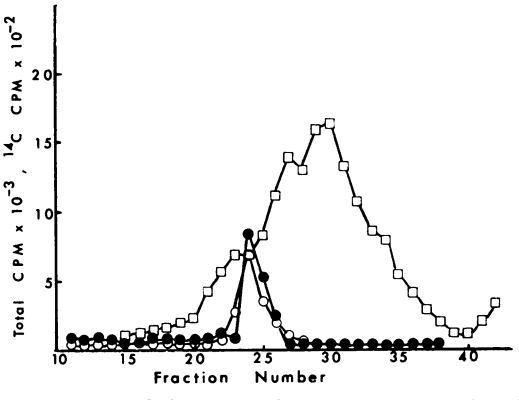

FIG. 3. *CsCl density gradient of sonic-treated and untreated SLS lysates of Escherichia coli strain CR34 grown in medium containing ^{3}H-thymidine and ^{14}C-labeled DNA extracted with phenol from Escherichia coli strain CR34. The ^{14}C-DNA and the lysate which was not sonic-treated were run simultaneously in one tube and the sonic-treated lysate was prepared and run at a different time. Symbols:* (○) *untreated lysate (floating pellet = 89% of total radioactivity);* (●) *^{14}C-labeled DNA (floating pellet = 16% of ^{14}C-radioactivity);* (□) *sonic-treated lysate (floating pellet = 6% of radioactivity).*

TABLE 1. *Percentage of total radioactivity found in the floating pellet after CsCl density gradient centrifugation of sonic-treated and untreated sarkosyl lysates of Escherichi coli strain TAU bar grown in media containing various labeled compounds*[a]

Labeled compound	Percentage of radioactivity in floating pellet	
	Untreated	Sonic-treated
^{3}H-Thymidine	69	3
^{14}C-Uracil	54	40
^{3}H-Tryptophan[b]	99	96
^{14}C-Arginine[b]	92	93

[a] Total counts/min measured for untreated and sonic-treated preparations are, respectively: ^{3}H-thymidine, 75,798 and 56,141; ^{14}C-uracil, 300,315 and 260,746; ^{3}H-tryptophan, 59,513 and 61,663; ^{14}C-arginine, 140,925 and 181,600.

[b] Includes top two fractions as well.

walls, in conjunction with protein and RNA of various types. Ganesan and Lederberg (5) demonstrated cell membrane-DNA complexes, from which DNA was susceptible to release by pronase, in *B. subtilis* lysozyme lysates. Complexes of this type in *E. coli*, which yield DNA of uniform molecular size after pronase, phenol, and ribonuclease treatment, have been reported by Massie and Zimm (11).

We found that the enzyme pronase did have a definite effect on the electrophoretic mobility of DNA in SLS lysates (Fig. 4). Compared to an untreated, ^{3}H-thymidine-labeled lysate, electrophoresis of a pronase-treated lysate gave, on the average, 10% less radioactivity in the first 20 fractions, and 10% more radioactivity in the faster migrating fractions (21 to 28). CsCl density gradient analysis of a pronase-treated lysate showed 10% less radioactivity in the floating pellet than in a similar untreated lysate. Thus, it appeared that at least part of the DNA which was released by sonic treatment from an association with other cell materials of lower electrophoretic mobility or lighter density was bound there by protein.

We felt it would be of interest to determine if DNA which migrates more rapidly after sonic treatment of a lysate still retains protein attached to it. Basic and acidic proteins, or both, have been found to be attached to DNA isolated in different ways from higher organisms and bacteria. Of particular interest is the recent work of Yoshikawa (Abstr. Biophys. Soc., 10th Ann. Meeting, Boston, p. 103, 1966), who found protein complexed to DNA at a density of 1.63 to 1.65 after CsCl density gradient sedimentation of phenol-extracted *B. subtilis* DNA. In our investigation, the amino acid tryptophan, which has been found to be absent in histone-like protein (1), and the amino acid arginine, a common constituent of basic protein (1), were employed with a strain of *E. coli* requiring these amino acids.

The results of electrophoresis with sonic-treated and untreated lysates of ^{3}H-tryptophan- and ^{14}C-arginine-labeled bacteria (Fig. 5 and 6) did not show any significant effect of sonic treatment on the electrophoretic mobility of tryptophan- or arginine-containing proteins. CsCl density gradients were run with ^{3}H-tryptophan- and ^{14}C-arginine-labeled lysates using a CsCl concentration at which DNA would band near the center of the gradient. Virtually 100% of the radioactivity was found in the floating pellet or in the top few fractions and sonic treatment was not observed to affect this distribution (Table 1). Therefore, it appears that DNA released from other cell debris by sonic treatment is not associated with any protein which remains attached during electrophoresis or density gradient treatment, although the experiments do not rule out the possible presence of proteins lacking both of these amino acids.

We also attempted to determine whether RNA was present in fractions containing DNA associated with other cellular material of low electrophoretic mobility and light density. We found (Fig. 7) that ribonuclease treatment of SLS lysates of ^{3}H-thymidine-labeled cells was even

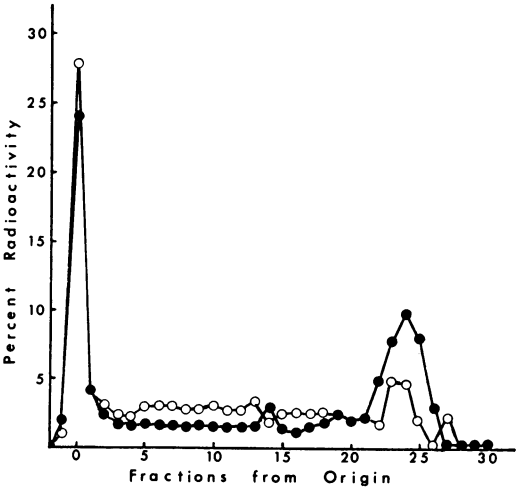

FIG. 4. *Electrophoresis of pronase-treated and untreated SLS lysates of Escherichia coli strain CR34 grown in media containing ^{3}H-thymidine. Symbols:* (○) *untreated, total counts/min = 21,218;* (●) *pronase-treated, total counts/min = 30,512.*

more effective than pronase treatment in increasing the electrophoretic mobility of radioactive material. However, CsCl density gradient centrifugation of the ribonuclease-treated lysate showed that the percentage of total radioactivity found in the floating pellet was only 3% lower than in that of an untreated lysate, a factor which could be accounted for by experimental error. Perhaps ribonuclease breaks an RNA linkage between DNA and cell membranes or other structures in a manner similar to that proposed by Byrne et al. (3). DNA may be bound more loosely to these structures after ribonuclease treatment and could be separable by electrophoresis but not by density gradient sedimentation.

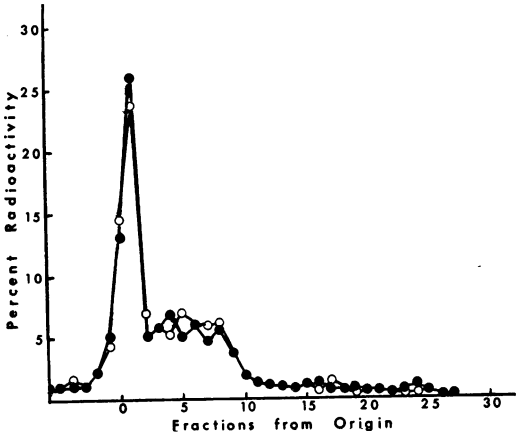

FIG. 5. *Electrophoresis of sonic-treated and untreated SLS lysates of Escherichia coli strain TAU bar grown in media containing ^{3}H-tryptophan. Symbols: (○) untreated, total counts (5 min) = 14,572; (●) sonic-treated, total counts (5 min) = 10,329.*

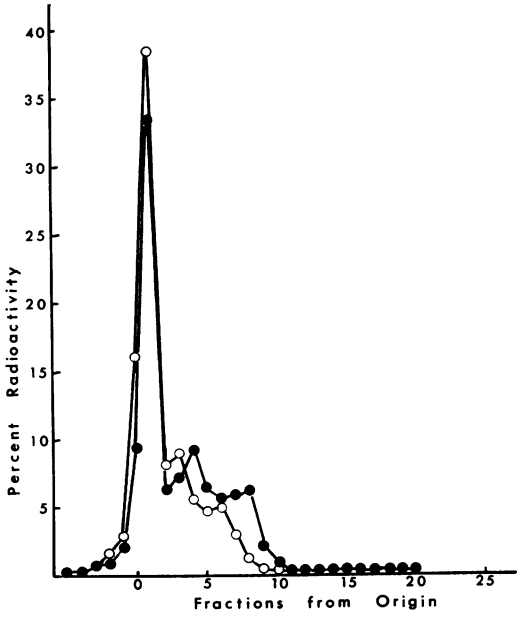

FIG. 6. *Electrophoresis of sonic-treated and untreated SLS lysates of Escherichia coli strain TAU bar grown in media containing ^{14}C-arginine. Symbols: (○) untreated, total counts (5 min) = 45,449; (●) sonic-treated, total counts (5 min) = 38,150.*

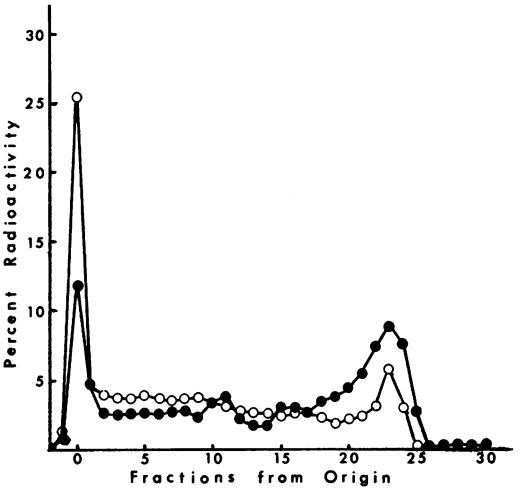

FIG. 7. *Electrophoresis of ribonuclease-treated and untreated SLS lysates of Escherichia coli strain TAU bar grown in media containing ^{3}H-thymidine. Symbols: (○) untreated, total counts/min = 11,513; (●) ribonuclease-treated, total counts/min = 10,397.*

To find out whether sonic treatment affected the distribution of the cellular RNA in electrophoretic and density gradient fractions containing DNA, ^{14}C-uracil-labeled lysates of *E. coli* strain TAU bar were prepared. Electrophoresis of these lysates showed that a relatively small amount of the radioactivity remained at the origin with the untreated lysate, and almost none with the sonic-treated lysate (Fig. 8). Thus, both RNA and DNA do occur in the same electrophoretic fractions. Very little RNA of an untreated lysate appears at the origin after electrophoresis, but treatment with ribonuclease releases a substantial amount of DNA to positions of greater electrophoretic mobility. This seems to indicate that a small amount of the cell's total RNA, possibly RNA of a specific type, is involved in the association of DNA with materials of lower electrophoretic mobility.

After CsCl density gradient centrifugation of a sonic-treated ^{14}C-uracil-labeled sarkosyl lysate, 40% of the total radioactivity was found in the floating pellet, as compared to 53% in an un-

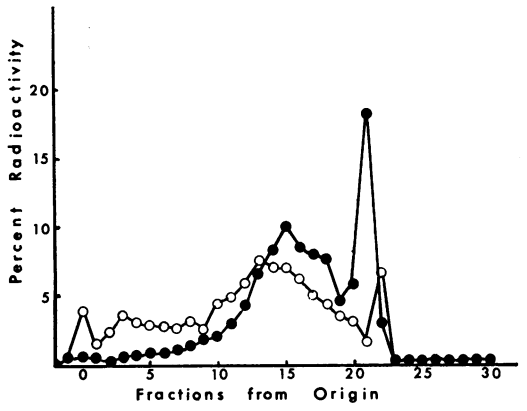

FIG. 8. *Electrophoresis of sonic-treated and untreated SLS lysates of Escherichia coli strain TAU bar grown in media containing ^{14}C-uracil. Symbols: untreated, total counts/min = 65,306; (●) sonic-treated, total counts/min = 55,972.*

treated lysate (Table 1). Although a significant amount, this was less than the decrease from 69 to 3% in the case of a ^{3}H-thymidine-labeled lysate. Thus, sonic treatment seems to release less RNA than DNA from association with other cell materials of lighter density or slower electrophoretic mobility. Since a large amount of the RNA is found in ribosomes, which contain about 40% protein and may be membrane bound (6, 13), this would account for the lighter density of much of the RNA even after sonic treatment.

DISCUSSION

Sonic treatment released most of the DNA from an association with other cellular components which were of low electrophoretic mobility and light density. This finding and evidence reported by Goldstein and Brown (7) and by Ganesan and Lederberg (5) indicate that this association probably includes the cell membrane. The small amount of labeled DNA which does remain complexed with materials of lower electrophoretic mobility and lighter density after sonic treatment may reflect a more stable membrane-bound portion of the DNA involved in replication. In *E. coli*, this interpretation is supported by the work of Kozinski and Lin (9) and by the current theory of genetic replication (8, 10).

Since labeled protein was found in positions of low electrophoretic mobility and light density after detergent lysis and since pronase partially released DNA from these positions, protein is probably active in the binding of DNA to other components such as the cell membrane. Whether pronase primarily affects replicative or nonreplicative DNA cannot be determined from the experiments reported here. DNA which was released by sonic treatment was not associated with tryptophan or arginine-containing proteins to any great extent. Thus, if this type of protein functions in *E. coli* as a genetic repressor, as a structural component of the bacterial genome, or as linker between DNA and other cell components, it must be largely separable by electrophoresis or by CsCl density gradient sedimentation. However, the protein-DNA association is not due to random adsorption which occurs after lysis. This is indicated by the finding that the addition of ^{3}H-labeled DNA to unlabeled cells prior to lysis resulted in very little adsorption of the DNA to material of low electrophoretic mobility. The most probable association would thus seem to be one in which the genome has a close functional attachment to the membrane at one or more points. After lysis, whole nuclei or large nuclear fragments may remain attached to or entangled with fragments of membrane. Sonic treatment removes DNA from this association, perhaps by breaking the DNA strands themselves or by breaking other macromolecular strands which connect DNA to the cell membrane.

Ribonuclease can release a definite fraction of the DNA from positions of low electrophoretic mobility, indicating that some DNA must be attached to the membrane or other slow migrating cellular components through RNA structure. This ribonuclease-releasable fraction may be DNA sections being transcribed into messenger RNA, perhaps also attached to ribosomes. It would be of interest to determine whether the DNA which seems to be released by ribonuclease is different in any way from that released by pronase. The relatively small amount of the total RNA found at the origin after electrophoresis of an untreated lysate seems to indicate that, if RNA links DNA to other cell components, it probably involves a specific complementary type of association, similar to that proposed by Byrne et al. (3).

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