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Solubilization of the Cytoplasmic Membrane of *Escherichia coli* by the Ionic Detergent Sodium-Lauryl Sarcosinate

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The sensitivity of the outer and cytoplasmic membranes of *Escherichia coli* to detergent was examined by isopycnic sucrose density gradient centrifugation. Sodium lauryl sarcosinate (Sarkosyl) was found to disrupt the cytoplasmic membrane selectively under conditions in which Triton X-100 and dodecyl sodium sulfate solubilized all membrane protein. These results were verified by gel electrophoresis; membrane proteins solubilized by Sarkosyl were identical to those of the cytoplasmic membrane. The presence of Mg^{2+} during treatment with Sarkosyl was found to afford partial protection of the cytoplasmic membrane from dissolution.

The envelope of gram-negative bacteria is a complex structure which consists of an outer membrane, an intermediate layer composed of peptidoglycan, and an inner, cytoplasmic membrane. Each of these surface layers is morphologically and chemically distinct (5, 9). The outer and inner membrane contain the usual constituents of membrane, protein and phospholipid, and the outer membrane contains essentially all of the lipopolysaccharides of the cell envelope as well. For this reason, the outer membrane is also known as the lipopolysaccharide or L membrane. The density of the outer membrane is 1.22 g/cm^3, and that of the cytoplasmic membrane is approximately 1.16 g/cm^3 (10). The difference in densities of the two membrane species has led to the development of techniques, applicable to *Salmonella typhimurium* (10) and *Escherichia coli* (12) cells, in which isopycnic sucrose density gradient centrifugation is used for separation of the outer from the inner membrane.

The L and cytoplasmic membrane of *E. coli* differ also in sensitivity to detergent; Triton X-100, a nonionic detergent, has been shown to solubilize only the cytoplasmic membrane when Mg^{2+} is present (3, 13). In this report, we compare the effects of several ionic and nonionic detergents on the outer and inner membrane of *E. coli*, in the presence and absence of Mg^{2+}. A second procedure for selectively solubilizing cytoplasmic membrane is described. The results also help to provide a rationale at the membrane level for the Mg^{2+}-sodium lauryl sarcosinate (Sarkosyl) crystals technique (14), which is used to detect deoxyribonucleic acid (DNA) associated with membrane.

MATERIALS AND METHODS

E. coli B was employed in all experiments. Broth, top agar, plates, and dilution fluid have been described elsewhere (4).

All radiochemicals were purchased from New England Nuclear Corp. Sarkosyl and Brij 58 were gifts of Geigy Chemical Corp., Ardsley, N.Y., and Atlas Chemical Industries, Inc., Wilmington, Del, respectively. Triton X-100 was obtained from Sigma Chemical Co., St. Louis, Mo., and sodium dodecyl sulfate (SDS) was purchased from Matheson, Coleman and Bell, East Rutherford, N.J.

Separation of cytoplasmic from outer membrane. The procedure of Osborn et al. (10) was used with a few modifications. Cultures (25 to 100 ml) growing exponentially in broth at 37 C were harvested at a concentration of 5×10^8 cells/ml. Subsequent operations differed from those described previously (10) only in that the ethylenediaminetetraacetate (EDTA) employed to aid in the conversion of cells to spheroplasts was added through a slow siphon and the spheroplasts were invariably lysed by sonic oscillation.

Samples from the isopycnic sucrose density gradient were collected by taking 8-drop fractions into 10% trichloroacetic acid. These fractions were counted on Whatman GF/A glass-fiber filters after being washed twice with 5% trichloroacetic acid and once with 1% acetic acid. After drying, 8 ml of toluene-Permafluor

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(Packard Instrument Co.) scintillation fluid was added, and a Beckman LS-250 liquid scintillation spectrometer was used to determine radioactivity.

Isolation of outer or cytoplasmic membrane. Fractions containing inner or L membrane were identified by several means. When membranes obtained from greater than 2.5×10^{10} cells were applied to the sucrose gradient, outer and inner membrane bands were clearly visible and could be collected by observation. When smaller quantities of membrane were involved, odd-numbered fractions were precipitated with trichloroacetic acid, collected on filters, and washed; the radioactivity was then determined as described above. Alternatively, two gradients were run. One was acid-precipitated, and the radioactivity in each fraction was determined; the appropriate fractions of the remaining gradient were then pooled. Pooled samples of inner or outer membrane were diluted to 0.25 M sucrose and 1.0 mM EDTA $\cdot Na_2$ and were pelleted by centrifugation for 2 h at 60,000 rpm in a Beckman 60Ti rotor.

Polyacrylamide gel electrophoresis. Acrylamide gels (7.5%) containing SDS and cross-linked with ethylene diacrylate were prepared according to the procedure of Inouye and Guthrie (6). Samples to be run were digested (6) after being pelleted by high-speed centrifugation as described above for the isolation of outer and inner membrane, or, in the case of proteins solubilized by Sarkosyl treatment, after concentration by pervaporation. The samples were applied to the gels and run at 3 mA/gel for 15 h. After electrophoresis, the gels were divided into 100 fractions of 1.3 mm each with a Savant autogeldivider, and each sample was then mixed with 0.65 ml of Biosolv (Beckman Instrument Co.). After shaking at 37 C for 2 h, 10 ml of toluene-Permafluor scintillation fluid was added. Samples were dark-adapted for 12 h, and the radioactivity in each was determined. The data were processed by means of the computer program described by Yund, Yund, and Kafatos (18) modified for use with a Control Data Corp. 6600 computer.

RESULTS

Effects of detergents on total membrane preparations. Figure 1A illustrates a typical result obtained when membrane from cells which have been converted to spheroplasts by lysozyme-EDTA treatment and then lysed by sonic treatment is centrifuged in an isopycnic sucrose density gradient. The peak at position 0.2 from the bottom of the gradient corresponds to the outer membrane, and that at 0.6 to 0.65 contains cytoplasmic membrane. The buoyant densities of the two bands of membrane, 1.22 and 1.16 g/cm^3 for the outer and inner membrane, respectively, are in good agreement with previously reported data (10). Further evidence that the bands do represent the two types of membrane is given below. The effects of treatment with the detergents Sarkosyl, SDS, Brij 58, or Triton X-100 are shown in Fig. 1B–E, respectively. In each case, a total membrane preparation was incubated with detergent for 20 min at 23 C immediately before sucrose gradient centrifugation. The detergent concentrations employed were chosen as being typical of those used to lyse spheroplast preparations. The results of detergent treatment suggest that the outer membrane is resistant to solubilization by 0.5% Sarkosyl (Fig. 1B). Subsequent experiments demonstrated that concentrations of Sarkosyl ranging from 0.25 to 2% gave identical results. The temperature at which 0.5% Sarkosyl and membrane are incubated does not

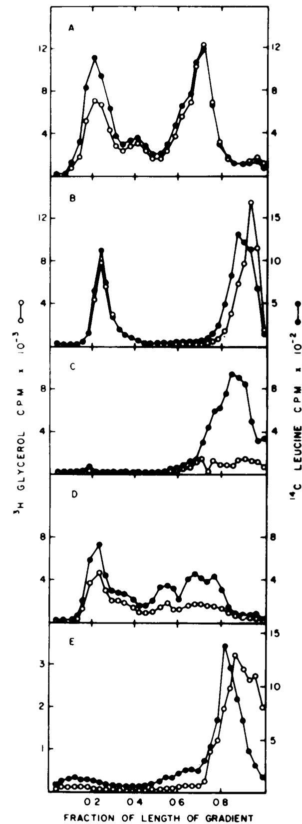

FIG. 1. *Separation of membranes after detergent treatment. Overnight cultures of E. coli were diluted 1:200 and grown in broth supplemented with 0.075 µCi/ml of L-[^{14}C]leucine and [2 – ^{3}C]glycerol (0.15 µCi/ml; 6 µg/ml). Cultures (200 ml) were harvested at a concentration of 5.6×10^8 cells/ml, and the membrane fraction was then isolated. Treatment with detergent was carried out for 20 min at 23 C, and samples were then applied to isopycnic sucrose density gradients and centrifuged for 16 h at 38,000 rpm in an SW41 rotor. Symbols:* O, *[2-^{3}C]glycerol;* ●, *L-[^{14}C]leucine. (A) Control; (B) 0.5% Sarkosyl (vol/vol); (C) 0.5% SDS (wt/vol); (D) 2% Brij 58 (wt/wt); (E) 2% Triton X-100 (vol/vol).*

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appear to be critical; 20-min exposure at 4 C solubilized cytoplasmic membrane as effectively as did incubation for a similar period of time at 23 C. Treatment with Sarkosyl immediately after lysis of the spheroplasts and before purification of the total membrane preparation yielded a final gradient containing only an outer membrane band. This demonstrates that the protein label which is affected by Sarkosyl is not pelleted during 2 h of centrifugation at 360,000 $\times$ g (R_{max}) in buffer containing 0.25 M sucrose. This is the only information available regarding the state of the solubilized material. Schnaitman (13) found that "Triton-soluble" protein is excluded from Sephadex G-150; protein solubilized by Sarkosyl may also be present in some type of aggregated form.

In contrast to the action of Sarkosyl, another ionic detergent, SDS, resulted in complete solubilization of all membrane (Fig. 1C). Brij 58, a nonionic detergent, appeared to have a slight effect on inner membrane (Fig. 1D). Triton X-100, at concentrations ranging from 0.5 to 2%, disrupted both species of membrane (Fig. 1E).

Effect of Sarkosyl on isolated membrane fractions. The sensitivity to Sarkosyl of the several bands of membrane present in the initial fractionation gradient was examined (Fig. 2). Three fractions of membrane, the inner membrane, the outer membrane, and a middle, intermediate-density fraction (Fig. 2A), were isolated and concentrated, and half of each fraction was treated with Sarkosyl prior to rebanding on density gradients. Sarkosyl had essentially no effect on the outer membrane fraction (Fig. 2B). A middle band, observed when separation was less than optimal, was converted by Sarkosyl to outer membrane and solubilized protein, which presumably had been present in inner membrane (Fig. 2C). The cytoplasmic membrane was totally sensitive to disruption by detergent (Fig. 2D).

Polyacrylamide gel profiles of membrane protein. The gel profiles presented in Fig. 3 provide additional evidence that the inner and outer membranes of *E. coli* are being isolated and that Sarkosyl selectively disrupts inner membrane. Shown in Fig. 3A are the results obtained when outer membrane, labeled with ^{14}C-leucine, was subjected to co-electrophoresis with ^{3}H-leucine-labeled inner membrane. The results agree well with previous studies of inner and outer membrane protein from *E. coli* (11, 12) and *S. typhimurium* (10). Of particular significance is the large region of outer membrane protein in fractions 36 to 45. This region is composed of several proteins which are the major components of the outer membrane (12). The resolution of these proteins by SDS gel electrophoresis is dependent on the conditions

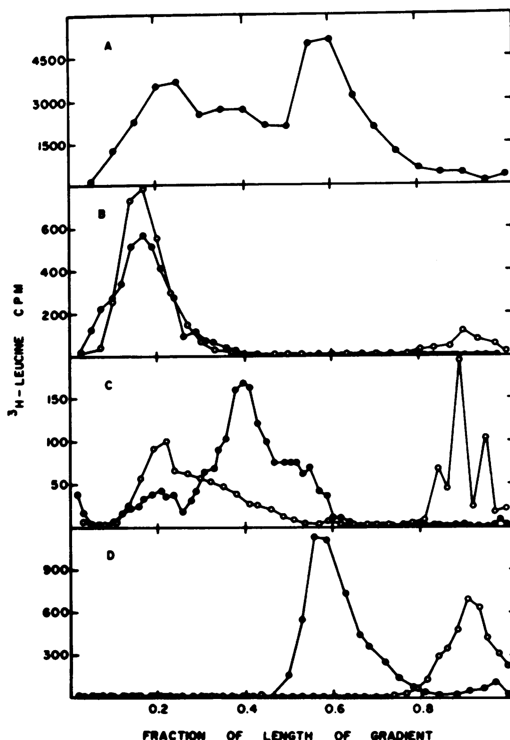

FIG. 2. *Effect of Sarkosyl on isolated inner and outer membrane. A 200-ml culture of E. coli was grown in broth containing 0.5 μCi/ml of L-[³H]leucine to a concentration of 4.8 × 10⁸ cells/ml. Membranes were isolated and separated. Three fractions of membrane, inner, outer, and a "middle" fraction banding between inner and outer membrane, were identified and isolated. The three fractions were centrifuged as described in Materials and Methods, and each pellet was then resuspended in 2.0 ml of cold 25% sucrose (wt/wt) containing 5 mM EDTA (pH 7.5). Each membrane fraction was split into two 1.0-ml fractions, one of which then received 0.1 ml of a 5% Sarkosyl solution. Both samples of each membrane fraction were incubated for 20 min at 23 C, layered onto sucrose gradients, and centrifuged. Symbols: ●, not exposed to Sarkosyl; ○, treated with Sarkosyl. Panels B, C, and D each represent a composite of two isopycnic sucrose density gradients. In each case, the effect of Sarkosyl on an isolated membrane fraction is compared to a nontreated control preparation. (A) Initial separation of L-[³H]leucine-labeled preparation of total membrane. Outer, "middle," and inner membranes were isolated by pooling material in fractions 0.05–0.31, 0.36–0.46, and 0.51–0.77 of the fraction of the length of the gradient, respectively. (B) Effect of Sarkosyl on outer membrane. (C) Effect of Sarkosyl on a mixture of outer and inner membrane. (D) Effect of Sarkosyl on cytoplasmic membrane.*

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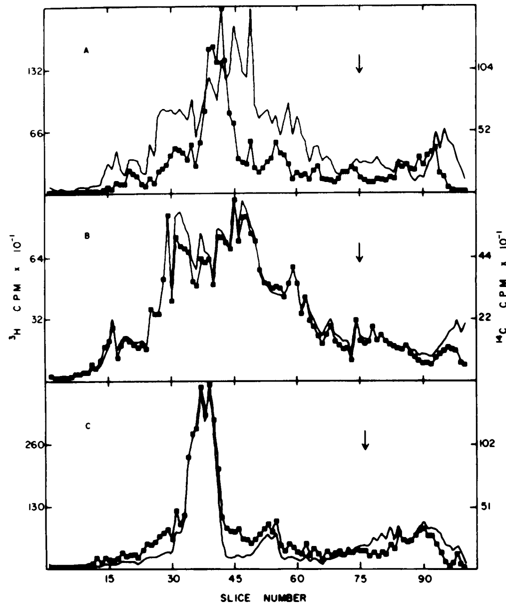

FIG. 3. *Gel electrophoresis of membrane protein. The various membrane fractions were isolated, digested, and subjected to electrophoresis as described in Materials and Methods. The vertical line in each panel indicates the position to which the dye marker (bromophenol blue) moved in the gel. Symbols:* ■, *L-[^{14}C]leucine;* —, *L-[^{3}H]leucine. (A) L-[^{3}H]leucine-labeled cytoplasmic membrane subjected to co-electrophoresis with L-[^{14}C]leucine-labeled outer membrane. (B) Co-electrophoresis of L-[^{3}H]leucine-labeled protein solubilized by Sarkosyl treatment with L-[^{14}C]-leucine-labeled inner membrane. (C) L-[^{3}H]leucine-labeled outer membrane recovered after Sarkosyl treatment of a total membrane preparation subjected to co-electrophoresis with L-[^{14}C]leucine-labeled outer membrane.*

used to solubilize the envelope proteins (7, 8, 18); under our conditions, several proteins, having apparent molecular weights ranging from 39,000 to 53,000, were found.

An electropherogram in which proteins solubilized by Sarkosyl treatment were subjected to co-electrophoresis with the protein of isolated inner membrane is shown in Fig. 3B. In Fig. 3C, protein from the membrane fraction which bands at a density of 1.22 g/cm^3 after Sarkosyl treatment has been subjected to co-electrophoresis with protein from purified outer membrane. A comparison of the membrane protein profiles in the several panels of Fig. 3 indicates that the proteins which are present in material banding at a density of 1.22 g/cm^3 after detergent treatment are primarily those found in outer membrane. Conversely, proteins of the cytoplasmic membrane were solubilized by Sarkosyl. This work, in conjunction with the results obtained from Sarkosyl treatment of isolated inner and outer membrane fractions, demonstrates that the protein in membrane which has a density of 1.16 g/cm^3 is specifically solubilized by Sarkosyl.

Effect of Mg^{2+} on membrane solubilization and separations. Mg^{2+} has been reported to protect outer membrane from solubilization by Triton X-100 (3, 13) and is frequently also present when cells which have been rendered fragile are lysed with detergent. It was therefore of interest to conduct experiments in which Mg^{2+} was present in the total membrane preparation during detergent treatment. The results are shown in Fig. 4. (Panels in Fig. 4 can be compared with the corresponding panels in Fig. 1 to note the specific effects of Mg^{2+} in altering

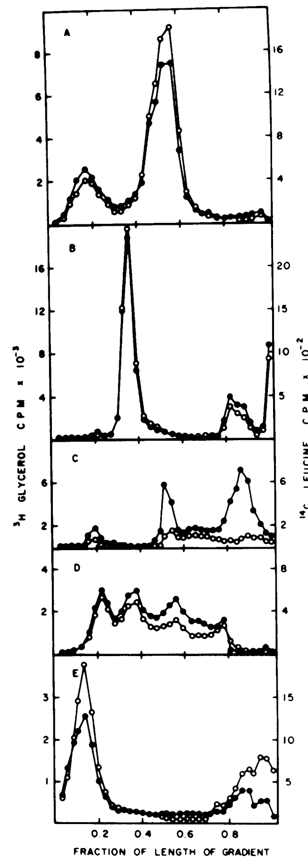

FIG. 4. *Effect of Mg^{2+} on membrane solubilization and separation. Growth conditions and membrane isolation and fractionation procedures were identical to those described in the legend for Fig. 1. $MgCl_2$ (0.03 M) was present during the 20-min incubation prior to sucrose density gradient centrifugation. Symbols and identification of panels are the same as those used in Fig. 1.*

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disruption by detergent of membrane.)

As reported by Osborn et al. (10), good separation of outer and inner membrane requires maintenance of low ionic strength during the preparation of the membrane fraction. The results shown in Fig. 4A indicate that exposure to 0.03 M $MgCl_2$ immediately prior to isopycnic sucrose density gradient centrifugation prevents good fractionation of the two membrane species; the major peak is present at the density expected for unseparated envelope material (10).

Mg^{2+} prevented complete disruption of the cytoplasmic membrane by Sarkosyl (Fig. 4B). The major peak is less dense than outer membrane and thus appears to contain some inner membrane as well. Crystals of Mg^{2+} and Sarkosyl formed under the treatment conditions used; these banded at a density of approximately 1.13 g/cm^3. In different experiments, various amounts of membrane material were found, either associated or trapped, with these crystals. In Fig. 4B, the small peak at 0.8 of the length of the gradient represents such membrane.

SDS disrupted membrane regardless of the presence of Mg^{2+} (Fig. 4C), and Mg^{2+} had little effect on the ability of Brij 58 to solubilize membrane (Fig. 4D). In Fig. 4E, Mg^{2+} is shown to protect outer membrane from solubilization by Triton X-100; this effect has been previously demonstrated by use of other membrane separation procedures (3, 13).

DISCUSSION

The results presented indicate that the L membrane of *E. coli* is resistant to solubilization by the detergent Sarkosyl. The basis for this resistance is not known; under conditions in which Sarkosyl appears to solubilize protein of the inner membrane selectively, a second ionic detergent, SDS, solubilizes all membrane protein, as does the neutral detergent Triton X-100. Previous studies (3, 13) which demonstrated that the inner membrane of spheroplasts or total membrane preparations is specifically solubilized by Triton X-100 were explainable, at least in part, on the basis that Mg^{2+} acts to stabilize L membrane. Mg^{2+} is unlikely to be involved in the present case. The membrane separation procedure employed removed Mg^{2+} with EDTA during the step in which cells are converted to spheroplasts, and subsequent operations are carried out in low ionic strength solutions containing 1.5 to 5 mM EDTA.

The Mg^{2+}-Sarkosyl crystals (M-band) technique is used for the detection of membrane-bound DNA (14). In this procedure, fragile cells are gently lysed with Sarkosyl in the presence of Mg^{2+}. After low-speed centrifugation, a visible band, which contains crystals of Mg^{2+}-sarcosinate, membrane, and DNA, is present. Membrane has been shown to have an affinity for Mg^{2+}-sarcosinate crystals (1, 2, 14); the cosedimentation of DNA with complexes containing crystals and membrane has been attributed to the association of DNA with membrane. That Mg^{2+} protects cytoplasmic membrane from total dissolution by Sarkosyl, and that separation of outer from inner membrane does not occur possibly provides additional insight into the molecular basis of this technique.

The relationship of these results to the effect of detergent on the cell envelope of intact cells is unclear. There is evidence which suggests that the results presented in this paper are relevant to studies carried out with cells rendered fragile by treatment with lysozyme and EDTA. In both the membrane fractionation procedure used throughout this work and in the M-band procedure, cells are broken after incubation with lysozyme and EDTA. Analysis by gel electrophoresis of the proteins present in M-bands indicates they are very similar to those present in preparations of total membrane (Wulff and Earhart, unpublished data). This would be predicted on the basis of results obtained concerning the action of Sarkosyl on total membrane in the presence of Mg^{2+}. However, on the basis of morphological studies, the detergent sensitivities of the L and cytoplasmic membrane of whole cells suspended in buffer containing Mg^{2+} differ from those described here. Treatment of such cells with ionic detergents, including Sarkosyl and SDS, has been reported to result in the dissolution of the cytoplasmic membrane (15, 16).

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