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Proteins of Ribosome-Bearing and Free-Membrane Domains in *Bacillus subtilis*

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In lysates of *Bacillus subtilis* a free-membrane fraction without ribosomes can be separated from the denser membrane-ribosome complexes. As determined by one-dimensional sodium dodecyl sulfate gel electrophoresis, these two fractions differ markedly in protein composition; at least six major bands (molecular weights, 130,000, 92,000, 68,000, 64,000, 45,000, and 31,000) are essentially unique to the complexed-membrane fraction (CM proteins), and two are unique to the free-membrane fraction. After growth was slowed, the proportion of the free-membrane fraction increased, but the composition of this fraction was the same, whereas after puromycin treatment, which abruptly increased the proportion of the free-membrane fraction, this fraction contained CM proteins. Thus, it appears that the two fractions recovered from growing cells represent topographically and functionally distinct domains. In addition, the effect of growth rate suggests that formation of the complexed domain is regulated at least roughly in parallel with the formation of ribosomes. The separation of these membrane fractions should facilitate the study of protein secretion, membrane topography, and morphogenesis in bacteria.

Certain basic features of protein translocation into or across membranes were discovered in animal cells; these include the participation of membrane-associated ribosomes (reviewed in reference 8) and the synthesis of many proteins as precursors with a cleavable N-terminal signal sequence (1, 7). When these phenomena were also observed in bacteria, the simplicity of these organisms offered experimental advantages for some kinds of studies. For example, because bacteria secrete proteins directly to the exterior or into the periplasmic space rather than into the inaccessible endoplasmic reticulum, these proteins could be labeled extracellularly while they were still growing on membrane-associated polysomes (12–15), and this finding established unequivocally the process of cotranslational secretion, which was suggested many years earlier (8, 9). On the other hand, in the study of membrane morphogenesis the apparent uniformity of the bacterial cytoplasmic membrane seems to be a disadvantage. However, in this paper we show that different domains within the membrane are readily separated.

Our approach was based on an incidental finding encountered in the course of studies on extracellular labeling; as with animal cells, the centrifugation of lysates of *Escherichia coli* in a biphasic gradient separated not only free polysomes and the membrane-polysome complexes, but also a less dense free-membrane fraction without ribosomes (15). Although the latter might have consisted only of outer membrane, it also seemed possible that it might contain ribosome-free portions of the cytoplasmic membrane. If so, this material might lack proteins involved in protein secretion. Therefore, we carried out a similar fractionation of the gram-positive bacterium *Bacillus subtilis*, which has only a cytoplasmic membrane. As we show in this paper, not only does this organism yield both a small ribosome-free membrane fraction and a complexed-membrane fraction, but these fractions differ markedly in their patterns of proteins. Thus, it is clear that the disrupted bacterial cytoplasmic membrane forms classes of vesicles of varying composition, and the presence or absence of associated ribosomes provides a convenient tool for separating two such classes.

Although the proteins unique to the complexed-membrane fraction (CM proteins) are not necessarily all involved in protein secretion, subsequent work has provided strong evidence that one of these proteins is so involved, because it is tightly associated with secreting ribo-

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somes (S. Horiuchi, P. C. Tai, and B. D. Davis, Proc. Natl. Acad. Sci. U.S.A., in press). Thus, the fractionation procedure described below has opened up a direct approach to the search for the apparatus of protein secretion. In addition, this fractionation procedure may be useful for studying the topography of the bacterial cytoplasmic membrane and the factors that guide membrane morphogenesis.

MATERIALS AND METHODS

Bacterial strain and growth. *B. subtilis* ATCC strain 6051a was grown at 37°C with vigorous aeration in minimal medium A (2) supplemented with 0.4% glucose and 0.4% Casamino Acids, unless otherwise stated. Cells were harvested at the mid-log phase of growth, and chloramphenicol (0.1 mg/ml) and excess ice were added to prevent polysome runoff. Cells were labeled by growth in the presence of radioactive compounds (see below) for two generations.

Preparation of membrane fractions. The complexed- and free-membrane fractions were prepared by the method of Smith et al. (15), with some modifications. Briefly, cells harvested from 1 liter of a culture were suspended in cold buffer A (10 mM Tris-hydrochloride [pH 7.6], 50 mM KCl, 10 mM magnesium acetate, 1 mM phenylmethylsulfonyl fluoride, 1 mM *o*-phenanthroline) supplemented with 1 mM dithiothreitol and with 0.1 mg of chloramphenicol per ml to stabilize the polysomes. The cells were incubated with lysozyme (0.3 to 0.4 mg/ml) at 0°C to form protoplasts and then were lysed either by osmotic shock or by treatment with a French press (10,000 lb/in^2). Unlysed protoplasts and cell debris were removed by centrifugation at 3,000 × *g* for 10 min, and the membranes were then pelleted by centrifugation at 40,000 × *g* for 20 min or, in some cases, at 200,000 × *g* for 1 h to recover additional small membrane vesicles and dispersed in buffer A supplemented as described above. Portions (0.5 ml) of the supernatant were layered on discontinuous sucrose gradients (2 ml of 1.35 M sucrose and 2.3 ml of 1.8 M sucrose) in buffer A. After centrifugation for 18 h at 42,000 rpm in an SW50.1 rotor, two bands were visible; these were the free-membrane band just below the sample layer and the complexed-membrane band in the interface between the two sucrose layers. Each band was removed, and it was sometimes put through a second gradient to minimize cross-contamination. The collected bands were diluted in buffer A and centrifuged at 42,000 rpm for 1 h, and each pelleted membrane fraction (complexed or free) was stored at −75°C, unless specified otherwise.

To facilitate identification of the proteins unique to the complexed-membrane fraction, the ribosomes were released from that fraction by treatment with a low Mg^{2+} concentration, yielding a "derived" membrane fraction. For this purpose the complexed-membrane fraction was suspended in and dialyzed overnight against buffer B (same as buffer A, except that the magnesium acetate concentration was 0.01 mM). The resulting preparation was then centrifuged in a sucrose gradient as described above, except that buffer A was replaced by buffer B. The resulting upper band (the derived membrane fraction) was recovered and stored as described above.

Gel electrophoresis. Samples were solubilized at 100°C for 3 min in 50 to 100 μl of sample buffer (50 mM Tris-hydrochloride [pH 6.8], 4% sodium dodecyl sulfate [SDS], 4% glycerol, 2% mercaptoethanol, 0.001% bromphenol blue). After clarification for 5 min in an Eppendorf centrifuge, the supernatant was electrophoresed on an SDS–7 to 12% polyacrylamide slab gel (unless otherwise specified) by using the buffer system of Maizel (6). The bands were visualized either by staining with Coomassie brilliant blue R-250 or by fluorography performed with impregnation with Enhance (New England Nuclear Corp.) according to the instructions of the manufacturer.

Sonication of membranes. In a 200-ml culture of *B. subtilis* the lipid and RNA were labeled by adding 50 μCi of [^{14}C]oleic acid and 400 μCi of [^{3}H]uracil, or the protein was labeled by adding 200 μCi of [^{35}S]methionine. The complexed-membrane fraction was prepared as described above and was suspended in 3 ml of buffer A containing chloramphenicol. A portion (0.5 ml) of this preparation was sonicated on ice for three 10-s periods (at 1-min intervals) in a Braun sonicator set at the maximal intensity. A control sample was treated identically, except that sonication was omitted. After centrifugation on a biphasic sucrose gradient as described above, 5-drop portions (approximately 0.17 ml) were collected in 0.2 ml of buffer A and counted.

Reagents. [^{14}C]oleic acid (250 mCi/mmol), [^{3}H]uracil (50 Ci/mmol), [^{35}S]methionine (1,000 Ci/mmol), and a ^{3}H-labeled amino acid mixture were obtained from New England Nuclear Corp. All other chemicals were of reagent grade.

RESULTS

Separation of free- and complexed-membrane fractions. Although *E. coli* lysates yielded more free membrane than denser complexed membrane (15), *B. subtilis*, which lacks an outer membrane, yielded a relatively small band of presumably free membrane (just below the sample layer) and a larger band, previously studied (12), at the interface between 1.35 and 1.8 M sucrose. To determine more precisely the distribution of various components, *B. subtilis* was labeled with [^{3}H]uracil, [^{14}C]oleic acid, or ^{3}H-labeled amino acids, and after centrifugation in a discontinuous gradient, multiple samples were collected (Fig. 1).

Two peaks were sharply separated. Only the denser, complexed peak contained RNA ([^{3}H]uracil). The less dense peak contained roughly one-half as much lipid (Fig. 1A and B) and one-third as much protein (Fig. 1B) as the denser peak. Thus, it appears that in the complexed-membrane fraction the ribosomes provide perhaps one-third of the protein. In subsequent experiments the two peaks (designated free- and complexed-membrane fractions) were collected as visible bands, as described above.

Electron microscopy. It seemed possible that the ribosome-free membrane fraction was derived from mesosomes and that the complexed-

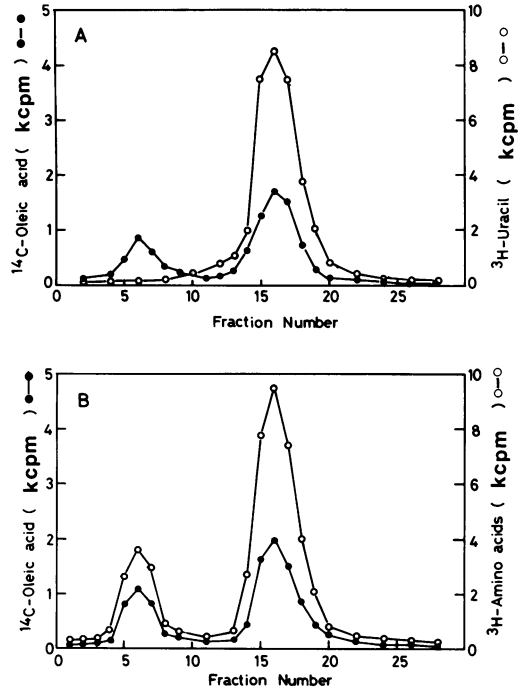

FIG. 1. Separation of complexed- and free-membrane fractions from *B. subtilis*. (A) Cells were grown for two generations in 200 ml of medium containing [^{3}H]uracil (200 μCi) and [^{14}C]oleic acid (25 μCi). The total membrane fraction was recovered, and a portion (0.5 ml; ^{3}H radioactivity, 5.3×10^4 cpm; ^{14}C radioactivity, 1.2×10^4 cpm) was centrifuged on a discontinuous sucrose density gradient (1.8 and 1.35 M sucrose) (see text). Then 5-drop samples were collected with an ISCO fractionator. The radioactivity of each sample was determined in a Triton X-100–xylene-based scintillant. (B) Cells were grown as described above, but with only 0.02% Casamino Acids, 15 ^{3}H-labeled amino acids (0.15 M each; total, 200 μCi), and [^{14}C]oleic acid (25 μCi). A portion of the membrane fraction (^{3}H radioactivity, 6.5×10^4 cpm; 1.5×10^4 cpm) was analyzed as described above.

membrane fraction came from the remainder of the membrane (lining the wall and uniformly studded with ribosomes). However, when cells were grown either with glucose or with glucose plus Casamino acids, harvested in the usual way, fixed with 2.5% glutaraldehyde and then 0.1 to 1.0% OsO_4, and sectioned, no mesosomes were observed.

Thus, it appears that in the cytoplasmic membrane the ribosome-bearing and ribosome-free domains form a mosaic. The sharp separation of these domains after lysis could be due either to preferential cleavage between the domains or to fragmentation mostly into vesicles much smaller than the domains. The latter possibility is supported by the results of electron microscopic observations. As Fig. 2 shows, the free vesicles ranged widely in size; some were as large as 0.5 μm in diameter, but most were much smaller. The presence of ribosomes prevented a clear answer to the question of whether the complexed-membrane fraction also contained very small vesicles.

Protein distribution in free-membrane, complexed-membrane, and derived membrane fractions. When the solubilized proteins of complexed- and free-membrane fractions were compared by one-dimensional SDS gel electrophoresis, followed by staining with Coomassie brilliant blue, the two fractions showed many bands in common, with moderate variations in the relative densities in different preparations; there were also striking differences at molecular weights above 30,000. Below this molecular weight any possible differences between the two membrane fractions were obscured by the ribosomal proteins present only in the complexed-membrane fraction.

For more detailed exploration, labeling of the proteins with radioactive amino acids proved to be more convenient and satisfactory than staining. Labeling with a mixture of 15 radioactive amino acids or with [^{35}S]methionine yielded essentially identical differences between the two fractions, and most experiments were carried out with the latter procedure. In a typical experiment (Fig. 3), 10 nonribosomal bands (out of about 25) (Fig. 3, open arrowheads) were present in the complexed-membrane fraction but

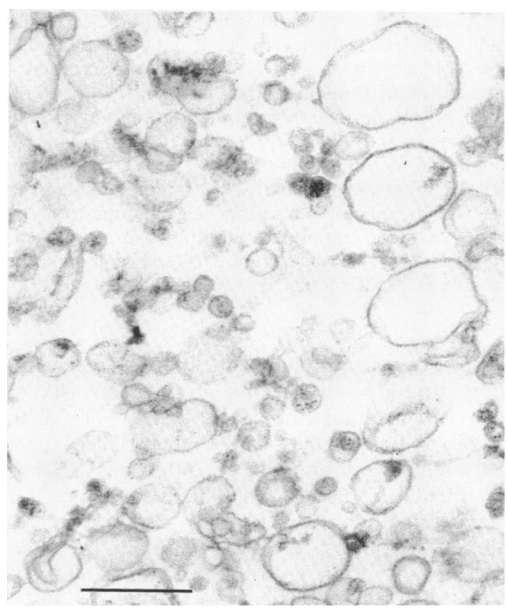

FIG. 2. Electron micrograph of a thin section of a free-membrane fraction. Bar = 0.5 μm.

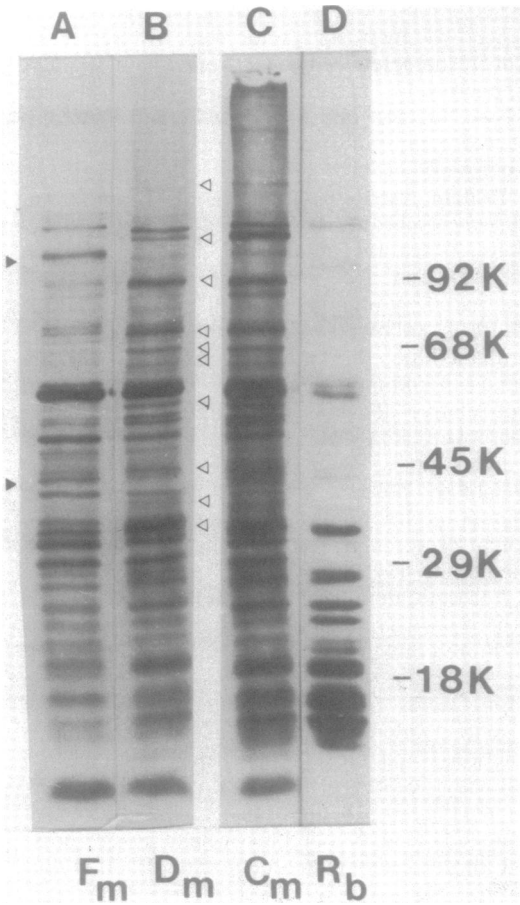

FIG. 3. Fluorogram of membrane fractions. The cells were grown with a mixture of ^{3}H-labeled amino acids, and the fractions were analyzed on an SDS–6 to 14% polyacrylamide gel; X-ray film was exposed 4 days. Lane A, Free-membrane fraction (F_m) (5×10^4 cpm); lane B, derived membrane fraction (D_m), isolated by using 0.01 mM Mg^{2+} (8×10^4 cpm); lane C, complexed-membrane fraction (C_m) (18×10^4 cpm); lane D, ribosomes (R_b) (6×10^4 cpm). The open arrowheads indicate bands that were unique to membrane-polysome complexes, and the solid arrowheads indicate bands that were unique to the free-membrane fraction. 92K, Molecular weight of 92,000.

absent, or virtually absent, in the free-membrane fraction. In various preparations six of these CM proteins (molecular weights, 130,000, 92,000, 68,000, 64,000, 45,000, and 31,000) most consistently exhibited large differences. Since the proteins having the highest molecular weights might have been SDS-resistant aggregates of the other proteins, we initially focussed only on the four with molecular weights of 68,000, 64,000, 45,000, and 31,000.

To search for possible additional differences at molecular weights below 31,000, we prepared a derived membrane fraction from the complexed-membrane fraction by releasing the ribosomes by dialysis against 10^{-5} M Mg^{2+} in buffer. For reasons that are not yet clear, the release of RNA and the resulting shift to a lower density were quite incomplete. Therefore, the derived membrane fraction was separated from the residual denser membrane fraction, as well as from the released ribosomes, by density gradient centrifugation. As expected, the derived membrane fraction contained the same six CM proteins as the membrane-polysome complexes. More significantly, the lower range of molecular weights that it exposed showed no additional differences from the free-membrane fraction (Fig. 3). A derived membrane fraction prepared by an alternative method (releasing ribosomes with puromycin) yielded similar results (Fig. 4, lane B). Most further work was done with the complexed-membrane fraction rather than with the derived membrane fraction.

Although most of the observed differences between the two fractions involved additional components in the complexed-membrane fraction, the free-membrane fraction also contained two virtually unique proteins (Fig. 3, solid arrowheads), which had molecular weights of 102,000 and 42,000 (Fig. 3). Clearly, the differences between the two domains that we separated are not limited to the secretory apparatus.

Effects of decreasing the ribosome concentration. If the free- and complexed-membrane fractions correspond to functionally different domains that exist in intact cell membranes, we would predict tht the proportions and compositions of these fractions might be affected by experimental manipulation that alters the attachment or the total concentration of ribosomes in cells. In fact, after abrupt release of part of the ribosomes by treatment of cells with puromycin, the free-membrane fraction not only expanded, but also contained detectable amounts of all six CM proteins (Fig. 4, lane C). Evidently, domains normally carrying ribosomes now formed vesicles without ribosomes. This result supports the conclusion that in growing cells the two fractions represent topographically separate domains and that when the functional difference in the binding of ribosomes is suppressed, the method used for fractionation no longer recognizes the topographical differences of the two fractions.

Quite a different result was obtained when the proportion of the complexed-membrane fraction was decreased by lowering the growth rate, which is known to lower the cellular concentration of ribosomes. When cells were grown in glucose-minimal medium (doubling time, 72 min) rather than in medium supplemented with Casamino Acids (doubling time, 35 min), the ratio of

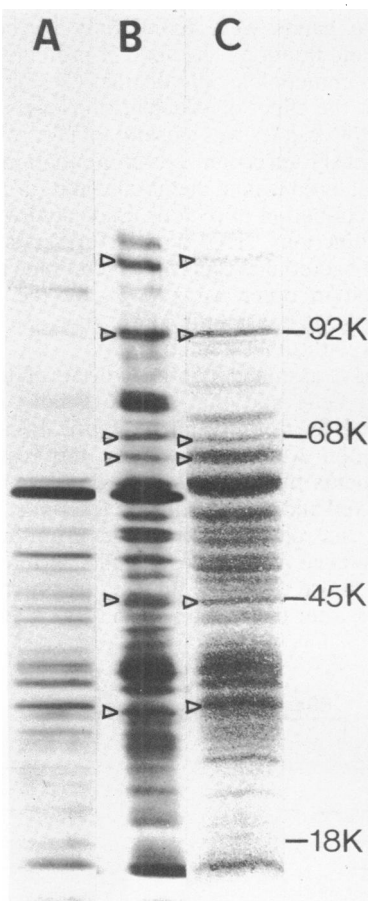

FIG. 4. Effect of puromycin treatment of cells and of the complexed-membrane fraction. The membrane fractions were obtained from unlabeled cells, and the gels were stained. Lane A, Free-membrane fraction isolated from growing cells; lane B, derived membrane fraction isolated after treatment of membrane-polysome complexes with puromycin (100 μg/ml), elongation factor EF-G, and GTP for 30 min; lane C, expanded free-membrane fraction isolated after treatment of cells with puromycin (40 μg/ml) for 30 min. The six CM proteins unique to the complexed-membrane fraction are indicated by arrowheads (lanes B and C). 92K, Molecular weight of 92,000.

free-membrane fraction to complexed-membrane fraction (measured as incorporated [^{14}C]oleic acid) increased from 1:7 to 1:3, but the complexed-membrane fraction continued to separate cleanly from the free-membrane fraction, and the latter continued to lack the unique CM proteins. This finding further supports the model of stable domains of complexed and free membranes. In addition, this finding suggests that the formation of the special proteins of the complexed-membrane fraction is coordinated, at least roughly, with the formation of ribosomes; i.e., the composition of a growing cell membrane responds adaptively to an altered need for sites that interact with ribosomes.

In the slower-growing cells not only was the proportion of complexed-membrane fraction smaller, but the ratio of RNA to lipid in that fraction was reduced about 50%. This difference may reflect a sparser population of ribosomes in the complexed domains, or these domains may be smaller and so the portions of adjacent free domains incorporated into the vesicles might seem larger.

Another kind of fractionation (fractionation by isopycnic centrifugation in a medium that releases ribosomes) reportedly separates the cytoplasmic membrane of *E. coli* into two kinds of vesicles, vesicles rich in ordered lipid domains and vesicles rich in disordered lipid domains; the proteins are concentrated in the latter (5). Moreover, the proportion of ordered domains increases markedly as the temperature is lowered. However, this kind of differentiation in membranes does not seem important for the fractionation that we describe here (which is based on much larger differences in density); when *B. subtilis* cells were lysed at 37°C instead of at the usual 0°C, the ratio of free-membrane fraction to complexed-membrane fraction was not significantly altered, and the patterns of proteins in these fractions were not changed.

Sonication of complexed-membrane fraction. It seemed possible that sonication might be another method for freeing the complexed-membrane fraction from its ribosomes or for improving the separation of free domains from complexed domains. In fact, sonication did not detectably release ribosomes from the complexed-membrane fraction. However, in some experiments (Fig. 5) sonication separated about one-half of the membrane (labeled with [^{14}C]oleic acid) from the membrane-polysome complexes; the released membrane now sedimented at the position of free membrane, whereas the remaining complexes shifted to a somewhat higher density. With some preparations, however, little membrane was separated from the complexes.

In all experiments sonication considerably narrowed the breadth of the band of the complexed-membrane fraction, as in Fig. 5, suggesting that this procedure made the vesicles more uniform in size or density. In a similar experiment the complexed-membrane fraction remaining after sonication and separated by centrifugation from a substantial amount of released free membrane retained its original content of the four CM proteins (Fig. 6) labeled with [^{35}S]methionine, as measured by an immunological procedure described in the accompanying paper (3). This finding provides further evidence that these proteins are located near sites of ribosome at-

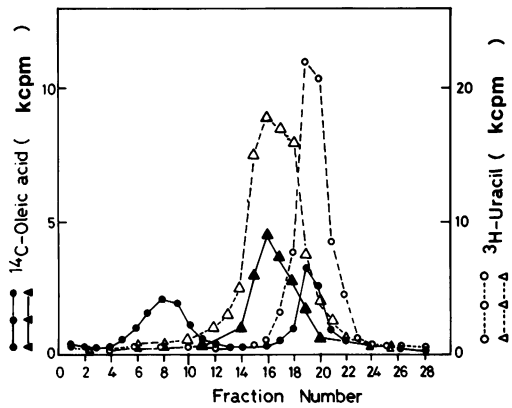

FIG. 5. Effect of sonication on the distribution of the complexed-membrane fraction. Cells were labeled with [^{14}C]oleic acid and [^{3}H]uracil, and the complexed-membrane fraction was sonicated as described in the text and then centrifuged and analyzed as described in the legend to Fig. 1. The centrifuged samples contained ^{3}H (1.3×10^{5} cpm) and ^{14}C (2.9×10^{4} cpm). Symbols: △ and ▲, control sample (no sonication); ○ and ●, sonicated sample.

tachment. In addition, it appears that sonication may be a useful procedure for obtaining more homogeneous preparations of membrane-polysome complexes with no loss of CM proteins.

Retention of membrane proteins by ribosomes after extraction by detergents. If the CM proteins or at least some of them are components of an apparatus of protein secretion, it seemed possible that they might remain associated with the ribosomes after gentle extraction of the membrane by detergents. We found that after a single extraction with 1% deoxycholate, which removed well over 90% of the lipid, the pelleted ribosomes retained variable amounts of some CM proteins. When the nonionic detergent Triton X-100 was used instead of deoxycholate, the pellet (which may have included membrane fragments) contained detectable amounts of all six CM proteins, although most of the other membrane proteins were removed (data not shown). These results indicate that the CM proteins are less easily solubilized from the complexed-membrane fraction than the other proteins.

DISCUSSION

In lysates of *B. subtilis* a complexed-membrane fraction carrying ribosomes can be separated sharply from a small, less dense free-membrane fraction (Fig. 1). These two fractions differ markedly in protein composition. As determined by one-dimensional gel electrophoresis, six major protein bands (CM proteins) were found to be almost unique to the complexed-membrane fraction (apart from its ribosomes), and two bands were found only in the free-membrane fraction. The six CM proteins identified are immunologically distinct (3) (Fig. 6).

Thus, the effect of attached ribosomes on the density of vesicles was a useful tool for revealing a previously unrecognized differentiation in the bacterial cytoplasmic membrane and for permitting fractionation of regions associated with different functions. This finding further suggests that the bacterial cytoplasmic membrane may be dissected in other ways (e.g., by the use of reagents, such as antibodies, that selectively interact with other components).

We have assumed that the two sets of domains exist in cells and that they are not artifacts produced by rearrangement during lysis. This assumption was supported by the results of experiments that altered the proportions of the complexed and free-membrane fractions. As expected, the release of ribosomes from domains of complexed membranes in cells by puromycin shifted those domains to the free-membrane fraction; after this treatment the free-membrane

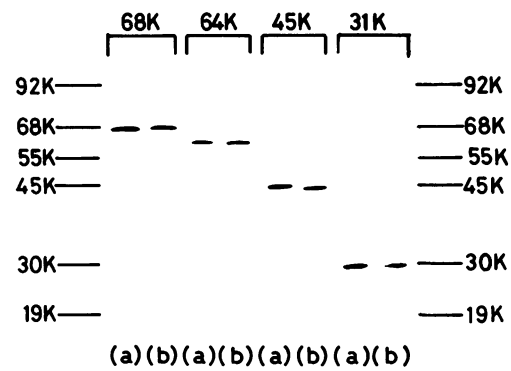

FIG. 6. Effect of sonication on the content of CM proteins in the complexed-membrane fraction. Cells were labeled with [^{35}S]methionine, and the complexed-membrane fraction was sonicated as described in the legend to Fig. 5. After centrifugation of the sonicated and control samples, the visible band at the interface between 1.8 and 1.35 M sucrose was collected; about 40% of the membrane of the sonicated sample had shifted to a lower density, as shown in Fig. 5. The complexed-membrane bands were collected, diluted in 5.0 ml of buffer A, and centrifuged at 42,000 rpm for 1 h, and the resulting pellets were solubilized. Samples corresponding to equal amounts of the initial membrane were immunoprecipitated with each antibody (indicated at the top), electrophoresed, and autoradiographed, as described in the text. (Details of antibody preparation and immunoprecipitation are described in the accompanying paper [3].) The film was exposed for 4 days for the precipitate obtained by using antibody to the 68-kilodalton (68K) protein and for 10 days for the precipitates obtained by using the three other antibodies. Lanes a, Control sample; lanes b, sonicated sample.

fraction increased in amount and contained CM proteins (Fig. 4, lane C). In contrast, after slow growth, which increased the proportion of the free-membrane fraction physiologically, the composition of this fraction did not change significantly. This result has the further important implication that the synthesis of CM proteins is regulated at least roughly in parallel with the synthesis of ribosomes.

At present, we have only limited evidence on the location of the two membrane domains. The free-membrane vesicles appear to have arisen, like the complexed membrane, from regions of cytoplasmic membrane adjacent to the wall. One possibility is that secreting ribosomes are not attached to the portion of the membrane adjacent to the septum of a dividing cell; indeed, it might be inefficient for a gram-positive bacterial cell that is exporting proteins to the exterior to secrete against the septum. However, membrane-associated ribosomes are involved not only in secretion but also in incorporating their products into the membrane, and the septal membrane might well share this function. Another possibility is that the sites of ribosome binding are clustered and that the regions containing these clusters are interspersed with regions of free membrane, forming a mosaic. This view is supported by the finding that slowing of growth decreases the fraction of membrane that is complexed rather than simply causing a decrease in the ratio of ribosomes to lipids in that fraction. In addition, we have begun to compare the free- and complexed-membrane fractions for their contents of proteins with known functions, and we have found that the bulk of the penicillin-binding proteins, which are involved in the synthesis and reshaping of peptidoglycan, are present in the free-membrane fraction (M. Caulfield, P. C. Tai, and B. D. Davis, manuscript in preparation).

The rather sharp difference in the protein compositions of the two fractions might suggest that the membranes break into vesicles preferentially at the borders between the differentiated regions. However, since very small vesicles predominate in the free-membrane fraction (Fig. 2), the separation observed might be mostly a separation of highly comminuted fragments rather than a separation of larger domains. Therefore, we can draw no conclusions about the sizes of the domains. It is of interest that sonication of the complexed-membrane fraction sometimes improved the separation by cleaving a small amount of free membrane, thus increasing the density of the remainder. Sonication also caused the complexed vesicles to sediment as a narrower band (Fig. 5), presumably by making either their size or their ratio of ribosomes to membrane more homogeneous.

With the recognition that differentiated regions of the bacterial membrane can be separated, the topography of the membrane becomes a challenging problem. Moreover, fractionation may help to make bacteria useful in the study of regional differentiation in membrane morphogenesis, a problem so far largely restricted to the complex, visible system of differentiated membranes in eucaryotic cells. Gram-negative cells have provided a limited bacterial model for such studies, since they form a grossly different, readily separable outer membrane and inner membrane, which are connected by multiple adhesion zones, and newly synthesized molecules of two different outer membrane proteins show quite different distributions (10, 11). The separation of adjacent regions of the cytoplasmic membrane of bacteria, as described here, may provide a more generalized approach.

In a formal sense, the two fractions described here correspond to the rough and smooth microsomes of eucaryotic cells, and one might have expected them to differ only in the presence of proteins involved in protein secretion. However, since both the free-membrane fraction and the complexed-membrane fraction of bacteria contain unique proteins, it is clear that the procedure which we used separates proteins involved in multiple functions. In addition, in contrast to the large number of CM proteins of bacteria, the rough microsomes of animal cells contain only two major bands (molecular weights, 65,000 and 63,000) that are absent from the smooth microsomes (4). As we show elsewhere (Horiuchi et al., in press), the 64-kilodalton bacterial CM protein is almost certainly involved in protein secretion, since it is located at sites in the membrane that are covered by ribosomes; however, the possible role(s) of other CM proteins in this function remains to be determined.

ACKNOWLEDGMENTS

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