0021-9193/78/0133-0329$02.00/0
JOURNAL OF BACTERIOLOGY, Jan. 1978, p. 329-335

Vol. 133, No. 1
Printed in U.S.A.

Interaction Between Two Major Outer Membrane Proteins of *Escherichia coli*: the Matrix Protein and the Lipoprotein

MARC DEMARTINI AND MASAYORI INOUYE*

State University of New York at Stony Brook, Stony Brook, New York 11794

Received for publication 8 July 1977

The affinity of the matrix protein, one of the major outer membrane proteins of *Escherichia coli*, for the peptidoglycan was examined by extracting the cell envelope complex at 55°C with 2% sodium dodecyl sulfate containing different amounts of NaCl. It was found that the matrix protein was extracted from the peptidoglycan of a mutant strain (*lpo*) that lacks another major membrane protein, the lipoprotein, at a lower NaCl concentration than was the matrix protein of the wild-type cell (*lpo*$^+$). When the envelope fraction of the wild-type strain was treated with trypsin, which is known to cleave the bound-form lipoprotein from the peptidoglycan, the affinity of the matrix protein for the peptidoglycan decreased to the same level as that of the affinity of the matrix protein for the peptidoglycan of the mutant strain. It was further shown that the free-form lipoprotein was also retained in the matrix protein-peptidoglycan complex, although the extent of retention of the free form of the lipoprotein was less than that of the matrix protein. These results indicate that both the free and the bound forms of the lipoprotein are closely associated with the matrix protein and that the bound form of the lipoprotein plays an important role in the association between the matrix protein and the peptidoglycan.

The cell envelope of *Escherichia coli* consists of two distinct membranes, the outer membrane and the inner, or cytoplasmic, membrane (8, 26). The cell wall, or peptidoglycan layer, is located between the two membranes (26). The cytoplasmic membrane is known to have many important functions, such as active transport, energy metabolism, and synthesis of macromolecules. On the other hand, the function of the outer membrane is still rather obscure. However, the outer membrane contains a protein composition much simpler than that of the cytoplasmic membrane. Because of this simpler composition of membrane proteins, the outer membrane provides a good system for studying biosynthesis and assembly of biomembranes (18). The outer membrane consists of three major proteins, the lipoprotein (2, 18), the matrix protein (29), and the *tolG* protein (6). Among them, the lipoprotein is the most abundant in *E. coli* and exists in two different forms in the outer membrane, a free form and a bound form, which is covalently linked to the peptidoglycan (15, 20). The entire chemical structure of the lipoprotein has been determined (3, 11), and the mechanism of biosynthesis of the lipoprotein has been well documented (9, 10, 14, 15, 18, 20, 22). On the basis of the primary structure of the lipoprotein, three-dimensional molecular assembly models have been proposed (2, 17). The matrix protein was originally described by Rosenbusch (29). It has a very strong affinity for the peptidoglycan, thus remaining firmly bound to the peptidoglycan when the whole envelope of *E. coli* is solubilized in 2% sodium dodecyl sulfate (SDS) at 55°C. Currently, the matrix protein is claimed to be a protein that forms passive diffusion pores in the outer membrane (27). Another major outer protein, the *ompA* (*tolG*) protein, has been shown by Chai and Foulds (6) to be required for susceptibility to a certain class of colicins. The matrix and *tolG* proteins are sometimes described as proteins I and II* (13).

It is intriguing to know how these major proteins are assembled in the outer membrane and whether there are any interactions between these proteins. In the present paper, we have studied interactions between the lipoprotein and the matrix protein and have found that the bound-form lipoprotein plays an important role in the affinity of the matrix protein for the peptidoglycan. Furthermore, we have found that the free-form lipoprotein is also retained by the peptidoglycan along with the matrix protein, suggesting that there are close associations among the free and bound forms of the lipoprotein and the matrix protein.

MATERIALS AND METHODS

Bacterial strains. The following two *E. coli* K-12 strains were obtained from Y. Hirota, National Institute of Genetics, Mishima, Japan: JE5505 F$^-$ *lpo pps*

his proA argE thi gal lac xyl mtl tsx; JE5506 F⁻ *pps his proA argE thi gal lac xyl mtl tsx* (16).

Culture medium. Cells were grown in L-broth at 37°C (25) and harvested at about 5×10^8 cells per ml.

Preparation of cell envelope fractions. The envelope fractions were prepared as described previously (19). In some experiments, the envelope fraction from about 10^{10} cells was treated for 1 h at 37°C with 100 μg of trypsin per ml in 5 ml of 10 mM sodium phosphate buffer, pH 7.2. The trypsin treatment was terminated by adding 100 μg of soybean trypsin inhibitor (Sigma) per ml (22).

SDS extraction. The final envelope fractions from about 10^{10} cells were suspended in 5 ml of 2% SDS in 10 mM tris(hydroxymethyl)aminomethane buffer, pH 7.4, containing 10% glycerol and various amounts of NaCl (29). The suspensions were incubated at 55°C for 30 min and then centrifuged at 20°C and 100,000 × *g* for 60 min. The pellets were solubilized in a boiling-water bath for 13 min in 200 μl of 2% SDS in 10 mM tris(hydroxymethyl)aminomethane buffer, pH 7.2, containing 20% glycerol.

SDS-gel electrophoresis. SDS-polyacrylamide slab gel electrophoresis was carried out with 17.5% acrylamide according to the method of Anderson et al. (1). To obtain better separation of smaller-molecular-weight proteins, NaCl was added at a final concentration of 0.07 M in the running gel. Gels were scanned with a Joyce-Lobel 3CS microdensitometer. Areas of the peaks were determined by tracing with a Numonics Corp. electronic graphics calculator.

RESULTS

Retention of matrix protein by peptidoglycan. Rosenbusch showed that the matrix protein was retained by the peptidoglycan when the cell envelope fraction was extracted with 2% SDS as 60°C (29). Figure 1A-2 shows an SDS-gel pattern of the envelope proteins of the wild-type (*lpo*⁺) cells retained by the peptidoglycan after extraction with the same SDS solution as described by Rosenbusch (29), except the extraction was carried out for 30 min at 55 instead of 60°C. It can be seen that a substantial amount of the matrix protein (band a) was retained by the peptidoglycan when compared with total envelope proteins (Fig. 1A-1). Longer extraction periods did not decrease the amount of retained

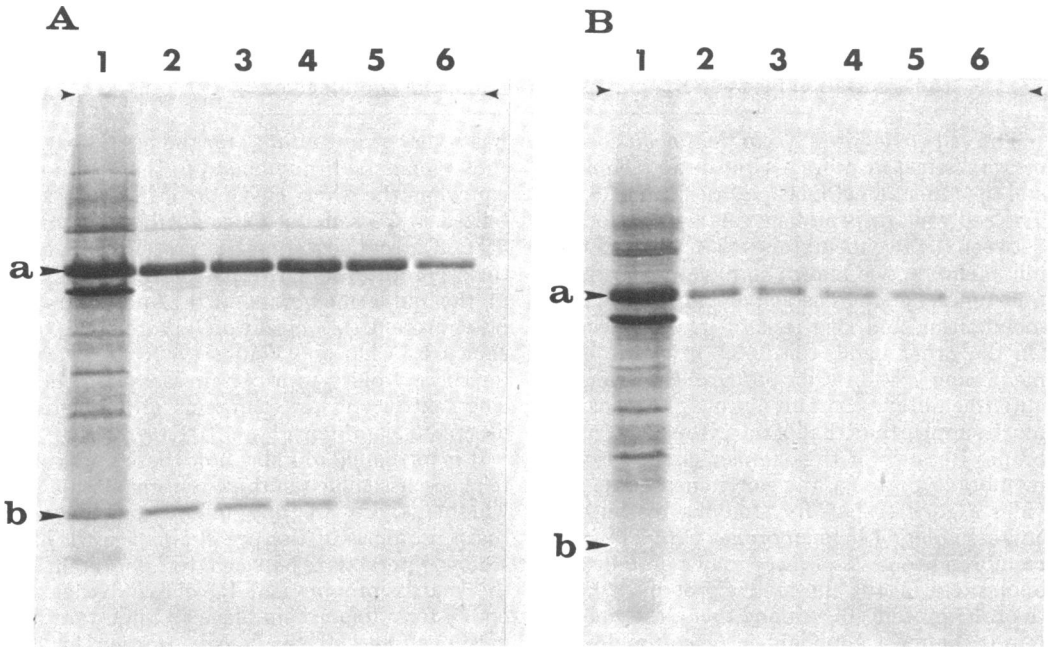

FIG. 1. *SDS-polyacrylamide gel electrophoresis of the matrix protein and the lipoprotein retained by the peptidoglycan from lpo⁺ and lpo cells after extraction with 2% SDS in the presence of various concentrations of NaCl. Cell envelopes were prepared as described in the text. They were then extracted for 30 min at 55°C with 2% SDS in 10 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.2, containing 10% glycerol and various concentrations of NaCl (see text). (A) Samples from lpo⁺ cells: whole cell envelope proteins without extraction (1); proteins retained by the peptidoglycan after the extraction in the absence of NaCl (2), or in the presence of 0.01 (3), 0.025 (4), 0.050 (5), or 0.100 (6) M NaCl. (B) Samples from lpo cells: whole cell envelope proteins without extraction (1); proteins retained by the peptidoglycan in the absence of NaCl (2), or in the presence of 0.010 (3), 0.025 (4), 0.050 (4), or 0.100 (6) M NaCl. Arrows a and b indicate the positions of the matrix protein and the lipoprotein, respectively. Small arrows on the top of the gel indicate the origin of gel electrophoresis.*

matrix protein (data not shown). It should be noticed that almost all other major envelope proteins except the lipoprotein (band b) were removed by the extraction. When NaCl was added to the extraction solution, it appears that the amounts of the matrix protein as well as the lipoprotein retained by the peptidoglycan gradually decreased as a function of NaCl concentration (Fig. 1A-3 to -6).

Similar experiments were performed with an *lpo* mutant that lacks both the free and the bound forms of the lipoproteins (16). Fig. 1B-1 shows an SDS-gel pattern of the total envelope proteins of the *lpo* cells, in which no lipoprotein band is apparent. Figure 1B-2 to -6 shows SDS-gel patterns of the envelope proteins retained by the peptidoglycan after SDS extraction in various concentrations of NaCl. It can be seen that a smaller amount of matrix protein is retained by peptidoglycan from *lpo* cells than is retained by peptidoglycan from *lpo*$^+$ cells.

To quantitate the amounts of the matrix protein retained by the peptidoglycan, the gel patterns of Fig. 1 were scanned by a microdensitometer, and the areas of the peaks were measured. Figure 2 shows the result expressed by percent retention, in which the amounts of the matrix protein in the whole cell envelope fractions (Fig. 1A-1 and B-1) were taken as 100%. In the case of the *lpo*$^+$ cells, about 70% of the matrix protein was retained in the absence of NaCl, whereas 20% was retained in the case of the *lpo* cells. When up to 0.025 M NaCl was added to the SDS, the percent retention values of the matrix protein of the *lpo*$^+$ cells remained almost constant at 60%. On the other hand, the percent retention values dropped to about 10% in the case of the *lpo* cells. This indicates that the affinity of the matrix protein for the peptidoglycan in the *lpo*$^+$ cells is markedly greater than that for the peptidoglycan in the *lpo* cells under these conditions. As the concentrations of NaCl were further increased, the percent retention values of the matrix protein of the *lpo*$^+$ cells gradually decreased. This indicates that the retention of the matrix protein is due to ionic interaction. It should be noted that the total amounts of the matrix protein in the whole cell envelope fractions were the same in both the *lpo*$^+$ and the *lpo* cells (Fig. 1A-1 and B-1, respectively).

Retention of the free-form lipoprotein. The complex retained not only the matrix protein but also the free form of the lipoprotein (Fig. 1A). Figure 3 shows the percent retention of the free-form lipoprotein obtained by quantitative measurement of Fig. 1A as described for Fig. 2. It can be seen that the free-form lipoprotein was dissociated from the matrix protein-peptidoglycan complex as the concentrations of NaCl were increased, as in the case of the matrix protein. However, it should be pointed out that the percent retention values for the lipoprotein were always lower than those for the matrix protein at any NaCl concentration. The ratios of the percent retention values of the free form lipoprotein to those of the matrix protein (Fig. 3) were calculated to be 0.91, 0.71, 0.46, and 0.29

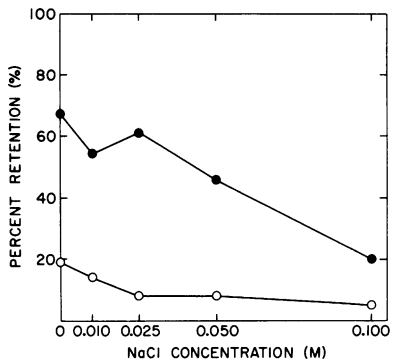

FIG. 2. *Retention of the matrix protein bound to the peptidoglycan of lpo*$^+$ *and lpo cells after the SDS extraction at different NaCl concentrations. Data were obtained from the gel shown in Fig. 1. The amounts of the matrix protein were measured by scanning the gel stained by Coomassie brilliant blue, as described in the text. Closed and open circles represent the percent retention of the matrix protein by the lpo*$^+$ *peptidoglycan and the lpo peptidoglycan, respectively. The amount of the matrix protein in the whole cell envelope (Fig. A-1 and B-1) is expressed as 100%.*

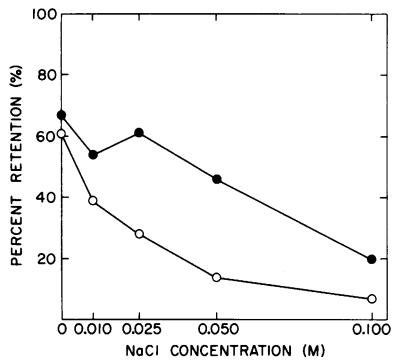

FIG. 3. *Comparison of the retention of the matrix protein and the free form of the lipoprotein by the peptidoglycan of the lpo*$^+$ *cells after SDS extraction at different NaCl concentrations. Data were obtained from the gel shown in Fig. 1A. Closed and open circles represent the percent retention of the matrix protein and the free form, respectively, by the peptidoglycan of lpo*$^+$ *cells. The amounts of the matrix protein and the lipoprotein in the whole cell envelope (Fig.1 A-1) are expressed as 100%.*

at 0, 0.010, 0.025, and 0.050 M NaCl, respectively. These results indicate that the free form of the lipoprotein is also retained by ionic interactions in the matrix protein-peptidoglycan complex and that the affinity of the lipoprotein for the complex becomes progressively weaker, in comparison with that of the matrix protein for the peptidoglycan, as the concentrations of NaCl increase.

Role of the bound-form lipoprotein. The above results suggest that the lipoprotein plays an important role in the retention of the matrix protein by the peptidoglycan. However, it is unlikely that the free-form lipoprotein is essential for the retention, since the extent of the retention was always greater for the matrix protein than for the free-form lipoprotein (Fig. 3). Therefore, it appears that the bound form of the lipoprotein plays a major role in the retention of the matrix protein by the peptidoglycan. To prove this, we treated the cell envelope fractions with trypsin. It is known that trypsin cleaves the bound-form lipoprotein from the peptidoglycan, although both the lipoprotein and the matrix protein are resistant to trypsin digestion (21). Figure 4A-4 and B-4 shows the gel patterns of trypsinized whole envelope fractions of the *lpo*$^+$ and the *lpo* cells, respectively. It was found that 100% of the matrix protein (band a) was recovered in both cases after the trypsin treatment, when compared densitometrically with the gel patterns of the undigested envelope fractions (Fig. 1A-2 and B-1). On the other hand, in the case of the *lpo*$^+$ cells, the amount of the lipoprotein increased by about 50% in the trypsin treatment (band b, Fig. 4A-4), because the bound form of the lipoprotein was cleaved from the peptidoglycan, to become free form. The amount of the bound form of the lipoprotein is known to be one-half of that of the free form (20). Furthermore, it should be noticed that another major band (band c, Fig.

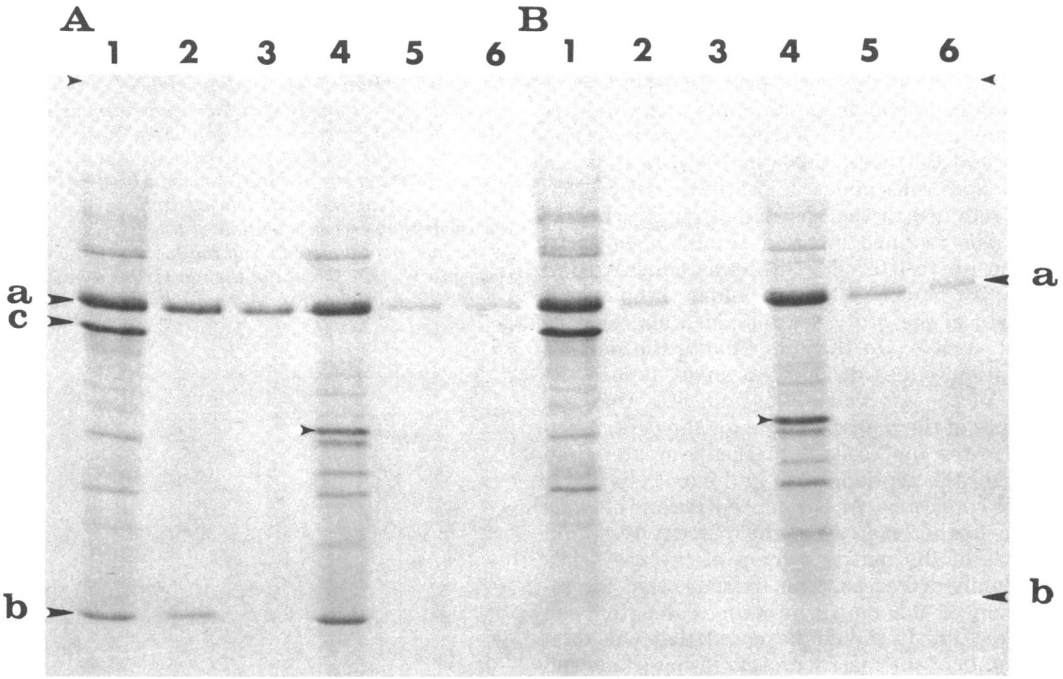

FIG. 4. *Effect of trypsin digestion on the retention of the matrix protein by the peptidoglycan. Preparation of cell envelope fractions and trypsin treatment of the envelope fractions were carried out as described in the text. They were then extracted with 2% SDS in the absence and presence of 0.05 M NaCl as described in the text. (A) samples from lpo*$^+$ *cells; (B) samples from lpo cells. (1) Whole cell envelope proteins without trypsin treatment; (2) retained proteins after extraction in the absence of NaCl; (3) retained proteins after extraction in the presence of 0.05 M NaCl; (4) whole cell envelope proteins after trypsin treatment; (5) same as (2), except the envelope fraction was treated with trypsin before the extraction; (6) same as (3), except the envelope fraction was treated with trypsin before the extraction. Arrows a, b, and c indicate the positions of the matrix protein, the lipoprotein, and the tolG protein, respectively. Small arrows on the top of the gel indicate the origin of gel electrophoresis. Other small arrows in (A-4) and (B-4) indicate new bands formed by the trypsin treatment.*

4A-1 and B-1) in the undigested envelope fractions disappeared after trypsin treatment (Fig. 1A-4 and B-4). This band has been identified as *tolG* protein (6). This protein has been shown to be sensitive to trypsin digestion, producing a smaller-molecular-weight band from the *ompA* (tolG) protein, shown by small arrows in Fig. 4A-4 and B-4 (18, 22).

Both the undigested and the digested envelope fractions were then extracted with 2% SDS with and without 0.05 M NaCl. It can be seen in Fig. 4A-5 and -6 that the affinity of the matrix protein of the *lpo*$^+$ cells for the peptidoglycan was reduced after trypsin treatment to the level of that of the *lpo* cells (Fig. 4B-2 and -3). In the absence of NaCl, the percent retention value of the matrix protein of the *lpo*$^+$ cells (obtained as described for Fig. 2) dropped drastically, from 48% (Fig. 4A-2) to 8% (Fig. 4A-5), as a result of trypsin treatment. The latter value is very close to those for the *lpo* cells, 4% for the undigested (Fig. 4B-2) and 12% for the digested (Fig. 4B-5) envelope fractions. In the presence of 0.05 M NaCl, the percent retention value was changed from 23% (Fig. 4A-3) to 6% (Fig. 4A-6) by trypsin treatment, again close to those for the *lpo* cells, 4% for the undigested (Fig. 4B-3) and 5% for the digested (Fig. 4B-6) envelope fractions. There was also a dramatic loss of retention of the free form of the lipoprotein after trypsin treatment (band b, Fig. 4A-5 and -6). From these results, we conclude that the bound form of the lipoprotein plays an essential role in the retention of the matrix protein and the free form of the lipoprotein by the peptidoglycan.

DISCUSSION

The matrix protein of the *E. coli* outer membrane is known to have a strong affinity for the peptidoglycan, so it can be retained on the peptidoglycan even after the envelope fraction has been treated at 55°C in 2% SDS (29). However, since the matrix protein can be dissociated from the peptidoglycan under the same conditions by adding NaCl (12), the two components are assumed to be held together mainly by ionic interaction. In the present paper, it has been shown that the free and bound forms of the lipoprotein are also closely associated with the matrix protein by ionic interactions and that the bound-form lipoprotein plays an important role in retaining the matrix protein on the peptidoglycan, according to the following facts. (i) The affinity of the matrix protein for the peptidoglycan layer was substantially reduced in a mutant strain (*lpo*) lacking the lipoprotein. (ii) The affinity of the matrix protein for the peptidoglycan in the wild-type strain (*lpo*$^+$) could be reduced to the level of the *lpo* mutant by pretreating the *lpo*$^+$ envelope fraction with trypsin; the trypsin treatment is known to cleave the bound-form lipoprotein from the peptidoglycan (21). (iii) The free-form lipoprotein was also retained in the matrix protein-peptidoglycan complex. (iv) The affinity of both the matrix protein and the free-form lipoprotein for the peptidoglycan decreased as NaCl concentrations in SDS solution for extraction increased.

Therefore, one can conclude that one of the major factors binding the matrix protein on the peptidoglycan is ionic interaction between the matrix protein and the bound-form lipoprotein. It is not certain at present to what extent this interaction contributes to maintaining the complex. However, the interaction between the matrix protein and the bound-form lipoprotein seems to be more important than that between the matrix protein and the peptidoglycan, at least under the condition of 2% SDS containing 0.025 M NaCl (Fig. 2), where about 60% of the matrix protein was retained on the peptidoglycan in the case of the *lpo*$^+$ strain, in contrast to about 10% in the case of the *lpo* strain. It should be noted that the phospholipids and lipopolysaccharides do not play a major role in the retention of the matrix protein by the peptidoglycan, since more than 95% of the phospholipids and lipopolysaccharides were extracted by 2% SDS, even in the absence of NaCl, for both the *lpo*$^+$ and the *lpo* cells (data not shown).

An interesting observation in this study is the retention of the free form of the lipoprotein within the matrix protein–bound-form lipoprotein–peptidoglycan complex. The free-form lipoprotein may be directly associated with the bound-form lipoprotein through ionic interactions, as previously proposed (17). Alternatively, the free-form lipoprotein may have ionic interactions with the matrix protein, with or without direct interactions with the bound-form lipoprotein. In this regard, it has recently been reported that lipoprotein molecules can be cross-linked in the intact membrane (28). Furthermore, we have shown that the purified lipoprotein can form paracrystals with an ordered ultrastructure (7), and the lipoprotein aggregates can be formed through ionic interactions (N. Lee, S. Tu, and M. Inouye, manuscript in preparation). These results imply the existence of direct interactions between the molecules of lipoprotein themselves.

It should be noted that all experiments in the present study were carried out in the absence of magnesium ions. When magnesium ions were added to 2% SDS used for extraction, it was found that the matrix protein became insoluble whether the envelope fractions were prepared

from *lpo*$^+$ or *lpo* strains (data not shown). More strikingly, the matrix protein was insoluble in the presence of magnesium ions even when the peptidoglycan had been digested with T4 phage lysozyme before extraction (data not shown). In a reconstitution experiment using the matrix protein and peptidoglycan of *E. coli*, it has been reported that, in the presence of 5 mM magnesium ions, the presence of the bound-form lipoprotein was not necessary for the reconstitution of the matrix protein-peptidoglycan complex (12). Furthermore, high concentrations of magnesium ions were sufficient to cause the precipitation of the matrix protein from the SDS solution, without the addition of peptidoglycan (12). Thus, it appears that magnesium ions are not only a factor in binding the matrix protein to the peptidoglycan, but they also appear to participate in the interaction between the matrix protein molecules themselves. Our preliminary experiments and the reconstitution experiments of other investigators (32) indicate that the lipopolysaccharide on the outside of the outer membrane also participates with the magnesium ions in these matrix protein interactions.

Besides the interactions between the matrix protein and the lipoprotein shown in the present paper, there are many other observations that pertain to the molecular assembly of the proteins of the outer membrane of *E. coli*. Among these are the observations of the relative positions of the matrix protein and the lipoprotein in the outer membrane. Both proteins have been shown to be partially exposed on the outside of the outer membrane (4, 23) as well as on the inner surface, associated with the peptidoglycan (2, 29, 31). In fact, the matrix protein was observed as an ordered, almost crystalline, array on the peptidoglycan (31), and it seems to be necessary for the penetration of β-lactam antibiotics into the cell (27). The possibility that either the matrix protein or the lipoprotein or both might be responsible for the passive diffusion of small molecules through the outer membrane is not refuted by these structural data, by our new data from the present work (17, 27), or by the existence of mutants lacking the lipoprotein or the matrix protein (16, 30). High contents of β-sheet structure in the matrix protein (29) and α-helical structure in the lipoprotein (5, 24) should also be taken into account when considering the functions and interaction of these two proteins.

ACKNOWLEDGMENTS

The work was supported by U.S. Public Health Service (USPHS) grant GM 19043 from the National Institute of General Medical Services (NIGMS) and by grant BC-67D from the American Cancer Society. M.D. was a recipient of a traineeship from the USPHS Health Service, GM 07212, from the NIGMS.

LITERATURE CITED

1. **Anderson, C. W., P. R. Baum, and R. F. Gestland.** 1973. Processing of adenovirses 2-induced proteins. J. Virol. **12**:241-252.
2. **Braun, V.** 1975. Covalent lipoprotein from the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **415**:335-337.
3. **Braun, V., and V. Bosch.** 1972. Repetitive sequences in the murein-lipoprotein of the cell wall of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **69**:970-974.
4. **Braun, V., V. Bosch, E. R. Klumpp, and I. Neff.** 1976. Antigenic determinants of murein lipoprotein and its exposure at the surface of enterobacteriaceae. 1976. Eur. J. Biochem. **62**:555-566.
5. **Braun, V., H. Rotering, J. P. Otims, and H. Hagenmaier.** 1976. Conformational studies on murein-lipoprotein from the outer membrane of *Escherichia coli*. Eur. J. Biochem. **70**:601-610.
6. **Chai, T., and J. Foulds.** 1974. Demonstration of a missing outer membrane protein in tolG mutants of *Escherichia coli*. J. Mol. Biol. **85**:465-474.
7. **DeMartini, M., S. Inouye, and M. Inouye.** 1976. Ultrastructure of paracrystals of a lipoprotein from the outer membrane of *Escherichia coli*. J. Bacteriol. **127**:564-571.
8. **DePetris, S.** 1976. Ultrastructure of the cell wall of *Escherichia coli* and chemical nature of its constituent layers. J. Ultrastruct. Res. **19**:45-83.
9. **Halegoua, S., A. Hirashima, J. Sekizawa, and M. Inouye.** 1976. Protein synthesis in toluene treated *Escherichia coli*. Exclusive synthesis of membrane proteins. Eur. J. Biochem. **69**:163-167.
10. **Halegoua, S., J. Sekizawa, and M. Inouye.** 1977. A new form of structural lipoprotein of outer membrane of *Escherichia coli*. J. Biol. Chem. **252**:2324-2330.
11. **Hantke, K., and V. Braun.** 1973. Covalent binding of lipid to protein. Diglyceride and amide-linked fatty acid at the N-terminal end of the murein-lipoprotein of the *Escherichia coli* outer membrane. Eur. J. Biochem. **34**:284-296.
12. **Hasegawa, Y., H. Yamada, and S. Mizushima.** 1976. Interactions of membrane proteins O-8 and O-9 with peptidoglycan sacculus of *Escherichia coli* K-12. J. Biochem. (Tokyo) **80**:1401-1409.
13. **Henning, V., K. Rehn, and B. Hoeln.** 1973. Cell envelope and shape of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **70**:2033-2077.
14. **Hirashima, A., S. Wang, and M. Inouye.** 1974. Cell-free synthesis of a specific lipoprotein of the *Escherichia coli* outer membrane directed by purified messenger RNA. Proc. Natl. Acad. Sci. U.S.A. **71**:4149-4153.
15. **Hirashima, A., H. C. Wu, P. C. Venkateswaran, and M. Inouye.** 1973. Two forms of a structural lipoprotein in the envelope of *Escherichia coli*. Further characterization of the free form. J. Biol. Chem. **248**:5654-5659.
16. **Hirota, Y., H. Suzuki, Y. Nishimura, and S. Yesuda.** 1977. On the process of cellular division in *E. coli*. A mutant of *E. coli* lacking a murein-lipoprotein. Proc. Natl. Acad. Sci. U.S.A. **74**:1417-1420.
17. **Inouye, M.** 1974. A three-dimensional molecular assembly model of a lipoprotein from the *Escherichia coli* outer membrane. Proc. Natl. Acad. Sci. U.S.A. **71**:2396-2400.
18. **Inouye, M.** 1975. Biosynthesis and assembly of the outer membrane proteins of *Escherichia coli*, p. 351-391. *In* A. Tzagoloff, (ed.), Membrane biogenesis. Plenum Publishing Corp., New York.
19. **Inouye, M., and J. P. Guthrie.** 1969. A mutation which changes a membrane protein of *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **64**:957-961.
20. **Inouye, M., J. Shaw, and C. Shen.** 1972. The assembly of a structural lipoprotein in the envelope of *Escherichia coli*. J. Biol. Chem. **247**:8154-8159.
21. **Inouye, M., and M.-L. Yee.** 1972. Specific removal of proteins from the envelope of *Escherichia coli* by pro-

tease treatments. J. Bacteriol. **112**:585–592.

22. **Inouye, S., S. Wang, J. Sekizawa, S. Halegoua, and M. Inouye.** 1977. Amino acid sequence for the peptide extension on the prolipoprotein of the *Escherichia coli* outer membrane. Proc. Natl. Acad. Sci. U.S.A. **74**:1004–1008.
23. **Kamio, Y., and H. Nikaido.** 1977. Outer membrane of *Salmonella typhimurium*. X. Identification of proteins exposed on cell surface. Biochim. Biophys. Acta **464**:589–601.
24. **Lee, N., E. Cheng, and M. Inouye.** 1977. Optical properties of an outer membrane lipoprotein from *Escherichia coli*. Biochim. Biophys. Acta **465**:650–656.
25. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**:190–260.
26. **Murray, R. G. E., P. Steed, and H. H. Elson.** 1965. The location of the mucopeptide in sections of the cell wall of *Escherichia coli* and other gram-negative bacteria. Can. J. Microbiol. **11**:547–560.
27. **Nikaido, H., S. A. Song, L. Shaltiel, and M. Nurminen.** 1977. Outer membrane of *Salmonella*. XIV. Reduced transmembrane diffusion rates in porin-deficient mutants. Biochem. Biophys. Res. Commun. **76**:324–330.
28. **Reithmeier, R. A. F., and P. D. Bragg.** 1977. Cross-linking of the proteins in the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **466**:245–256.
29. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*. Regular arrangement in the peptidoglycan and unusual dodecyl sulfate binding. J. Biol. Chem. **249**:9019–9029.
30. **Schmityes, C. J., and U. Henning.** 1976. The major proteins of the *Escherichia coli* outer membrane. Eur. J. Biochem. **63**:47–52.
31. **Steven, A. C., B. Heggeler, R. Muller, J. Kistler, and J. P. Rosenbusch.** 1977. Ultrastructure of a periodic protein layer in the outer membrane of *Escherichia coli*. Biologe **72**:292–301.
32. **Yu, F., and S. Mizushima.** 1977. Stimulation by lipopolysaccharide of the binding of outer membrane proteins O-8 and O-9 to the peptidoglycan layer of *Escherichia coli*. Biochem. Biophys. Res. Commun. **74**:1397–1402.