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Arrangement of Bacteriophage Lambda Receptor Protein (LamB) in the Cell Surface of *Escherichia coli*: a Reconstitution Study

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The LamB protein purified in a solution of sodium dodecyl sulfate was assembled into an ordered hexagonal lattice structure with a lattice constant of about 7.8 nm in the presence of lipopolysaccharide. The LamB alone formed aggregates with some lattice structure. However, the regularity of the lattice was only maintained within a very small area. An ordered hexagonal lattice was also formed when the wild-type lipopolysaccharide was replaced by heptoseless lipopolysaccharide, lipid A, and even fatty acid. However, the lattice constants were appreciably smaller than that with the wild-type lipopolysaccharide. The results suggest that the heptose-containing polysaccharide region, as well as the fatty acid region, are involved in the interaction with the LamB protein. The LamB-lipopolysaccharide lattice was preferably formed on the peptidoglycan layer when the lipoprotein was covalently bound to this layer. These results indicate that the molecular arrangement of the LamB protein in the outer membrane is similar to that of matrix proteins, OmpC and OmpF, which exist as trimers. The ordered hexagonal lattice was active in the receptor function for lambda, resulting in phage adsorption and deoxyribonucleic acid ejection. Thus, this reconstitution system should provide a useful means of studying the mechanism of lambda infection.

The outer membrane and the peptidoglycan layer constitute the surface structure of *Escherichia coli* and other gram-negative bacteria. In *E. coli* the lipoprotein covalently bound to peptidoglycan and major outer membrane components were shown to play roles in the interaction between these two organelles (4, 6, 12, 18, 38, 42, 43). In a previous paper we showed that a membrane with an ordered hexagonal lattice can be reconstituted on the entire surface of the lipoprotein-bearing peptidoglycan from outer membrane proteins OmpC or OmpF or both and lipopolysaccharides (43). Since this reconstitution system includes most of the major constituents of the *E. coli* cell surface, this provides a unique system for studying interactions between these constituents. Proteins OmpC and OmpF are identical with O-8/O-9, Ib/Ia, c/b, and 1b/1a, respectively (31). Taking advantage of the fact that OmpC or OmpF or both proteins and lipopolysaccharide are assembled into an ordered hexagonal lattice structure even in the absence of the peptidoglycan layer (43), the mode of interaction between the OmpC protein and lipopolysaccharide was studied in detail (44).

In maltose-induced cells the receptor for phage lambda (LamB protein) is also a major outer membrane protein (5). This protein is also known to facilitate the transport of maltose, maltooligosaccharides, and other sugars (5, 39-41). Evidence has accumulated that LamB, OmpC, and OmpF proteins share a number of unique physical and biochemical properties. These include resistance to denaturation by sodium dodecyl sulfate (SDS), characteristic association to the peptidoglycan layer, and formation of a channel for small hydrophilic molecules (3, 20, 26). In addition, the LamB protein is supposed to exist as oligomers, either dimers or trimers, in the outer membrane (3, 17, 33),while OmpC and OmpF proteins have been proved to exist as trimers (27, 32, 45).

In the present work, we show that the LamB protein behaves almost the same as the OmpC and OmpF proteins in the reconstitution system; that is, the LamB protein interacts with lipopolysaccharide to form a hexagonal lattice sheet or vesicles and, in the presence of the lipoprotein-bearing peptidoglycan layer, this assembly takes place on this layer. This reconstitution system will provide a means of studying the mechanism of lambda adsorption and other early steps in the phage-host interaction.

MATERIALS AND METHODS

Preparation of envelope components. The LamB protein was prepared by the method of Nakae (26) from *E. coli* T19 (K-12, *tsx supE kmt*), a strain producing very reduced amounts of OmpC and OmpF proteins. Lipopolysaccharide was prepared by the method of Galanos et al. (10) from *E. coli* YA21 (K-12, F⁻ *met leu*) grown in glucose-Casamino Acids medium containing 3% NaCl as described previously (12). The heptoseless lipopolysaccaride was prepared from T4-resistant mutant YA21-6 (25) by the same method. Both lipopolysaccharide preparations were kept in 1% SDS. Lipid A was prepared from lipopolysaccharide by acetic acid hydrolysis as described (11) and kept in 100 mM Tris-hydrochloride (pH 8.0)-1% SDS-0.5 M NaCl. The preparation was essentially free from 3-deoxy-D-manno-octulosonate. Phospholipid was prepared from YA21 as described previously (28). A mixture of myristic acid and 3-hydroxymyristic acid (1:1) was used as a substitute for fatty acid from lipopolysaccharide. Phospholipid and fatty acid were kept in a small volume of chloroform-methanol (2:1) under N_2 gas at −20°C.

The lipoprotein-bearing peptidoglycan sacculus was prepared from *E. coli* JE5512 (Hfr Cavalli, *lpp*⁺ *man-1 pps*) as described previously (43). The peptidoglycan sacculus free from the bound form of the lipoprotein was prepared by the same procedure from *E. coli* JE5513, a lipoprotein-negative (*lpp*) mutant derived from JE5512 (14).

Reconstitution experiments. Reconstitution of the cell surface structure was carried out as described previously (43) with the LamB protein instead of the OmpC protein. Briefly, the LamB protein (80 μg) was incubated with 0.5 M NaCl in 100 μl of 100 mM Tris-hydrochloride (pH 8.0)-1% SDS-0.1% 2-mercaptoethanol at 37°C for 30 min. It was then mixed with lipopolysaccharide dissolved in 100 μl of 1% SDS, and when indicated, 20 μg of the peptidoglycan sacculus was suspended in 200 μl of 1% SDS and dialyzed against 5 mM $MgCl_2$-10 mM Tris-hydrochloride (pH 8.0)-0.025% 2-mercaptoethanol at 25°C for 48 h. The resultant pellet was recovered by centrifugation and examined under a Hitachi HS-9 electron microscope. Uniform latex particles (Dow Chemicals) and a carbon grating (Radd Research Ind.) were used as magnification standards. The method of negative staining with 1% sodium phosphotungstate (pH 6.2) was the same as that described previously (42). Electron micrographs were further analyzed with an optical diffractometer that was basically the same as that described by Erickson et al. (8).

Assay of receptor activity for phage lambda. Phage lambda was heat-induced from *E. coli* CSH45 [K-12, *lac thi trpR* (λ *c*I857 *S*7)] and purified on a CsCl gradient (23). The receptor activity of reconstituted samples for phage lambda was assayed in the presence and absence of chloroform as described by Randall-Hazelbauer and Schwartz (36). Briefly, reconstituted samples or native cell envelopes (about 20 μg of the LamB protein) were incubated with 5×10^5 PFU of lambda at 37°C in 1 ml of 10 mM Tris-hydrochloride (pH 7.3)-10 mM $MgSO_4$ in the presence and absence of 5 drops of chloroform. Receptor activity is expressed as the ability to inactivate the phage. *E. coli* CSH25a (K-12, F⁻ *supF thi*) was used as the indicator strain for plaque counting. For electron microscopic observation, reconstituted samples (about 20 μg of the LamB protein) were treated with 3×10^6 PFU of lambda in 100 μl of 10 mM Tris-hydrochloride (pH 7.3)-10 mM $MgSO_4$ in the presence of 10 μl of chloroform at 37°C for 5 min and negatively stained with 1% sodium phosphotungstate (pH 6.2). A Hitachi HS-9 electron microscope was used.

Analytical methods. Protein content was determined by the method of Lowry et al. (19). The amount of lipopolysaccharide was estimated from the 3-deoxy-D-manno-octulosonate content, which was determined by the method described by Osborn et al. (30). The amount of lipid A was determined by weighing. The value was almost the same as that estimated from the 3-hydroxymyristic acid content, which was determined by gas chromatography (37). The amount of phospholipid was calculated by assuming 25 μg of phospholipid per μg of lipid phosphorus, which was assayed by the method of Bartlett (2). Glucosamine in peptidoglycan preparations was assayed by the Elson-Morgan reaction (13), and the amount of peptidoglycan was calculated by assuming 5 μg of peptidoglycan per μg of glucosamine.

RESULTS

Interaction between the LamB protein and lipopolysaccharide. The LamB protein is resistant to SDS and has been purified to homogeneity in SDS solution (26). The purified protein retains the oligomeric structure and is active in forming permeability channels that allow the diffusion of maltose and other sugars (26). The LamB protein purified in this work was almost homogeneous, as judged by polyacrylamide gel electrophoresis, and the contamination by lipopolysaccharide was less than 2 μg per mg of protein. Assuming molecular weights of the LamB monomer and lipopolysaccharide to be 48,000 (7, 26) and 4,300 (34), respectively, contamination of the preparation by lipopolysaccharide was estimated to be less than 0.03 lipopolysaccharide molecules per LamB monomer.

Under the reconstitution conditions the LamB protein alone formed aggregates that consisted of small fragments (Fig. 1A). Although their shapes were quite heterogeneous, they showed some lattice structure. The regularity of the lattice, however, was only maintained within a very small area. The optical diffractogram showed very faint hexagonally disposed reflections, indicating that the lattice structure, if any, was hexagonal. Assuming that these reflections were derived from the basic lattice repeat and therefore represented the first order of a hexagonal reciprocal lattice, we estimated the lattice constant to be about 6.7 nm (Table 1).

The addition of lipopolysaccharide together with the LamB protein resulted in the formation

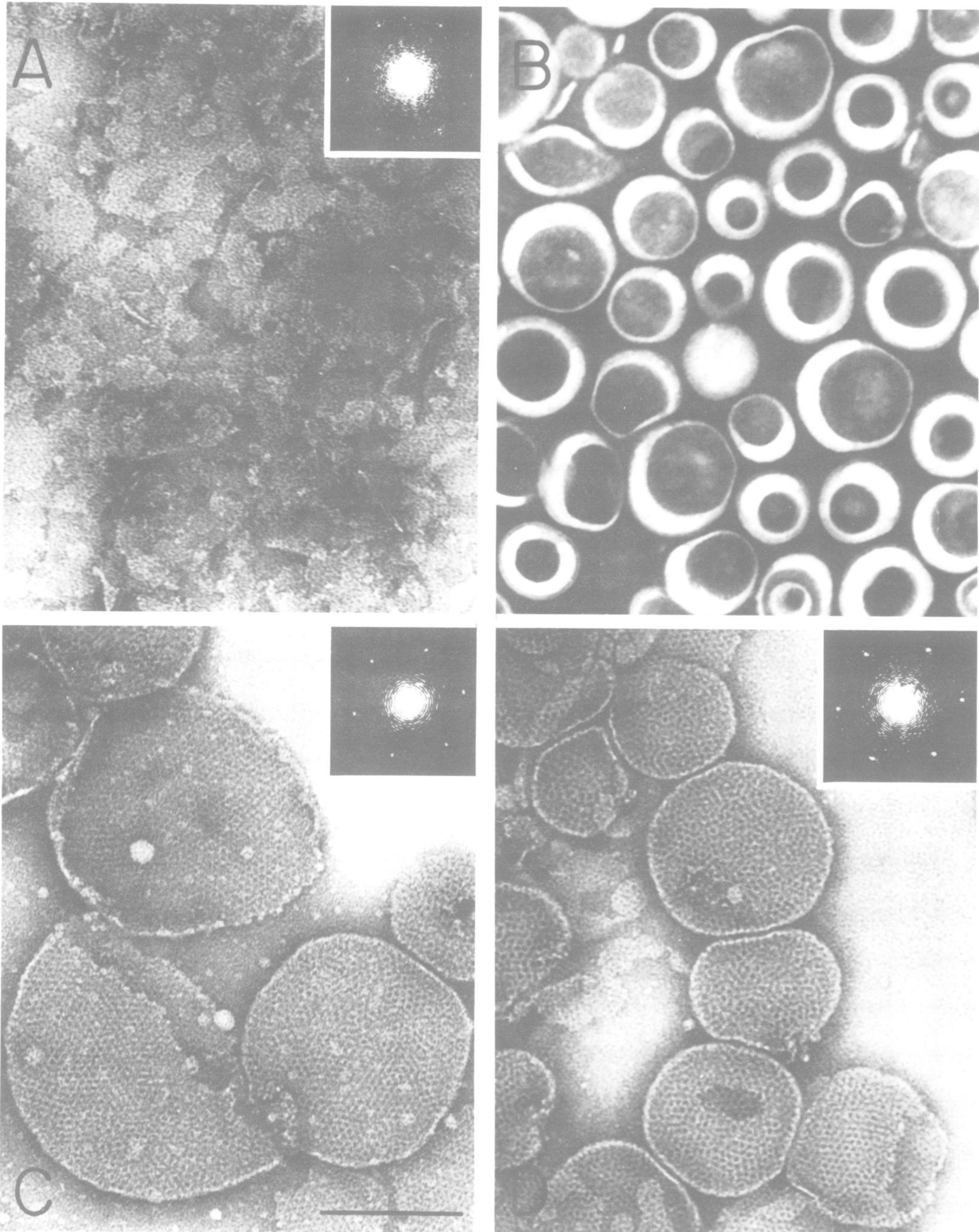

FIG. 1. *Assembly of an ordered lattice structure from the LamB protein and lipopolysaccharide. The LamB protein and the wild-type lipopolysaccharide were incubated under the reconstitution conditions. The resultant structures were negatively stained and examined with an electron microscope. The typical optical diffractograms taken from the electron micrographs are also presented. Envelope components added to the reaction mixtures were as follows: (A) 80 μg of the LamB protein alone; (B) 20 μg of lipopolysaccharide alone; (C) 80 μg of the LamB protein and 7 μg of lipopolysaccharide (molar ratio was about 1:1); (D) 80 μg of the LamB protein and 20 μg of lipopolysaccharide (molar ratio was about 1:3). Bars represent 200 nm.*

of vesicles with a much clearer and highly ordered hexagonal lattice (Fig. 1C and D). The appearance was very much like that observed with the OmpC protein and lipopolysaccharide

TABLE 1. *Lattice constants of reconstituted hexagonal lattice structures*[a]

Envelope components in reconstitution mixture	Molar ratio (LamB/LPS)[b]	No. of measurements (*n*)	Lattice constant (nm)
LamB	—	24	6.7 ± 0.2
LamB + wild-type LPS	1:1	15	7.6 ± 0.2
	1:3	26	7.8 ± 0.3
LamB + heptoseless LPS	1:3	32	7.4 ± 0.2
LamB + lipid A	1:3	18	7.1 ± 0.1
LamB + fatty acid	1:3	14	7.3 ± 0.1
LamB + phospholipid	1:3	27	7.3 ± 0.2
LamB + wild-type LPS + lipoprotein-bearing peptidoglycan[c]	1:3	23	7.9 ± 0.2

[a] Electron micrographs of negatively stained reconstituted samples were subjected to optical diffractometry, and lattice constants were determined. Lattice constants are given as means and standard errors of means of *n* measurements.

[b] LPS, Lipopolysaccharide. One lipopolysaccharide molecule was regarded as being equivalent to six fatty acid or three phospholipid molecules. The average molecular weight of *E. coli* phospholipid was regarded as 750. —, None.

[c] Reconstitution conditions were the same as those given in the legend to Fig. 3.

(43, 44). The addition of lipopolysaccharide also resulted in a considerable increase in the lattice constant (Table 1). It was about 7.6 nm when the molar ratio of the LamB monomer to lipopolysaccharide was 1:1. With a larger amount of lipopolysaccharide smaller vesicles with a slightly larger lattice constant (7.8 nm) were obtained. Lattice constants with the OmpC protein also varied depending on the amount of lipopolysaccharide (44). However, they were significantly smaller (6.5 to 7.5 nm) than those with the LamB protein. In agreement with previous results (43, 44), lipopolysaccharide alone did not yield any ordered lattice structure (Fig. 1B).

Lattice formation with compounds derived from lipopolysaccharide. To study the roles of various moieties of the lipopolysaccharide molecule in LamB assembly, the reconstitution experiment was carried out with heptoseless lipopolysaccharide, lipid A, or fatty acid (Fig. 2 and Table 1). The heptoseless lipopolysaccharide also produced vesicles with a highly ordered hexagonal lattice structure, as in the case of the wild-type lipopolysaccharide. However, the lattice constant was slightly smaller than that with the wild-type lipopolysaccharide (Table 1). The ordered lattice structure was also produced with lipid A and even with fatty acid (Fig. 2B and C). The optical diffractograms clearly showed the hexagonal arrangement. However, in these cases, they were assembled into a flat sheet. The lattice constants with these compounds were also smaller than that with the wild-type lipopolysaccharide (Table 1). Furthermore, phospholipid was found to act as a substitute for lipid A or fatty acid (Fig. 2D and Table 1), indicating that 3-hydroxymyristic acid, which is peculiar to lipopolysaccharide, is not necessarily required for the hexagonal arrangement of the LamB protein. In agreement with results in a previous paper (44), the lipopolysaccharide derivatives and phospholipid alone did not produce any lattice structure (data not shown). Together with our previous findings concerning the interaction between the OmpC protein and lipopolysaccharide, these results indicate that the fatty acid region of the lipopolysaccharide primarily participates in the hexagonal arrangement of the LamB protein and that the heptose-containing polysaccharide moiety may be involved in the interaction with the LamB protein to give the protein a particular conformation that is reflected in the lattice constant.

Assembly of the lattice on the peptidoglycan layer. In the presence of the lipoprotein-bearing peptidoglycan, the assembly of the OmpC protein and lipopolysaccharide into the hexagonal lattice takes place on the peptidoglycan surface (43). This was also the case with the LamB protein. When the reconstitution experiment was carried out in the presence of the lipoprotein-bearing peptidoglycan sacculus, assembly of the LamB protein and lipopolysaccharide into the hexagonal lattice took place on the entire surface of the peptidoglycan sacculus (Fig. 3A). The lattice constant was almost the same as that without peptidoglycan (Table 1). The lattice formation predominantly took place on the peptidoglycan sacculus, indicating that the surface of the peptidoglycan is the preferred site for the assembly of the hexagonal lattice. The lipoprotein covalently bound to the peptidoglycan was responsible for this assembly, since when the lipoprotein-free peptidoglycan sacculus was used, no lattice structure was formed on it; instead, vesicles with a lattice structure were formed independently of the sacculus (Fig. 3B).

Adsorption of phage lambda on the reconstituted cell surface. The LamB protein is essential as a receptor component for phage lambda infection (36). The isolated receptor requires chloroform to function (22, 36). Even the native cell envelope required chloroform for the receptor function (Fig. 4). Reconstituted samples were tested for their receptor activity (Fig. 4). None of them showed receptor activity in the absence of chloroform (data not shown). In the

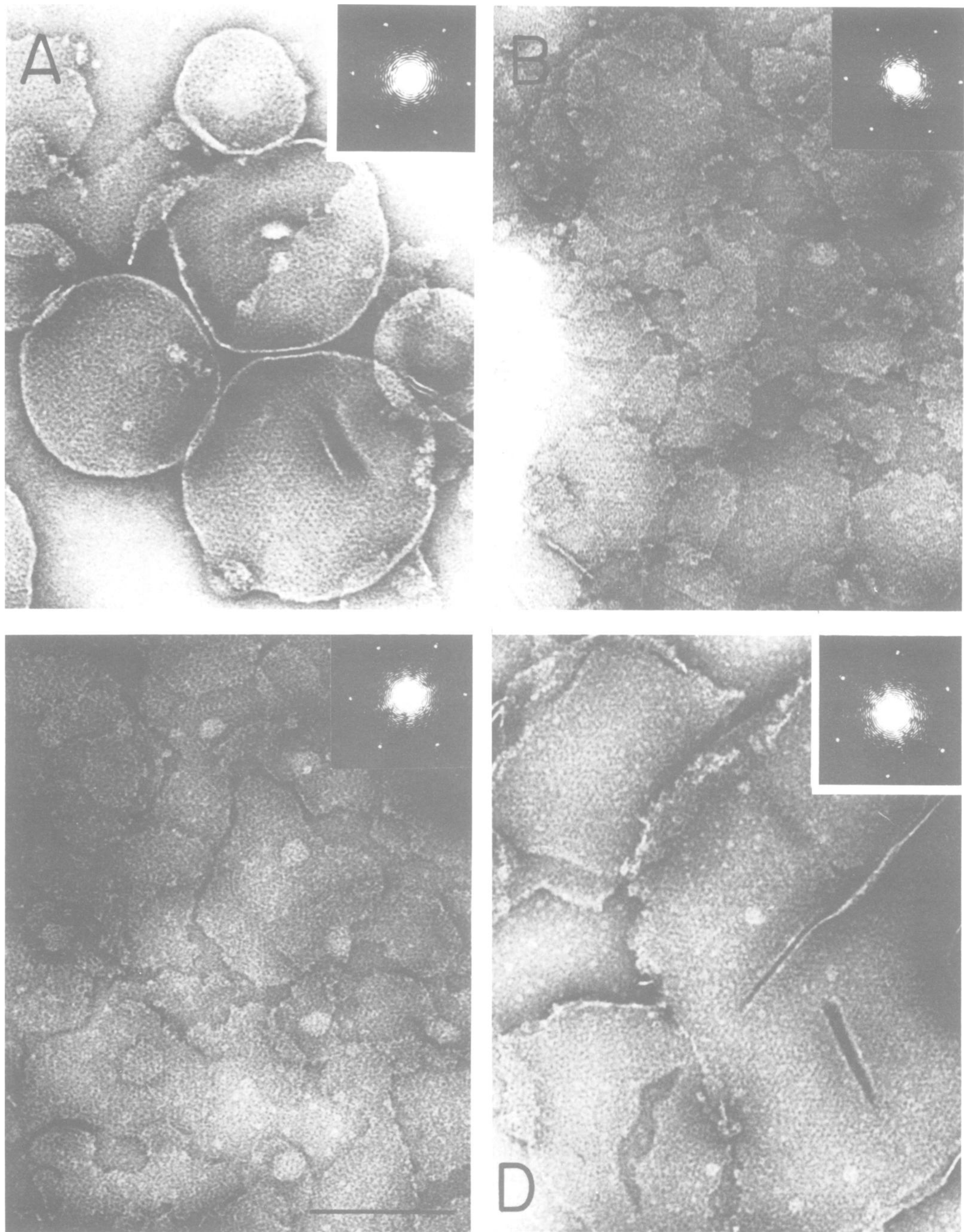

FIG. 2. *Assembly of ordered lattice structure from the LamB protein and lipopolysaccharide derivatives. Experimental procedures were the same as those described in the legend to Fig. 1. In addition to the LamB protein, the reaction mixture contained heptoseless lipopolysaccharide (A), lipid A (B), fatty acid (C), and phospholipid (D). Molar ratio of the LamB monomer to lipopolysaccharide derivatives was 1:3. Six fatty acid or three phospholipid molecules were regarded as being equivalent to one lipopolysaccharide molecule. Bars represent 200 nm.*

presence of chloroform, the purified LamB protein was as active as the native cell envelope on the basis of the LamB content. The vesicle reconstituted from the LamB protein and lipopolysaccharide was also active as the receptor. However, the activity was always slightly lower

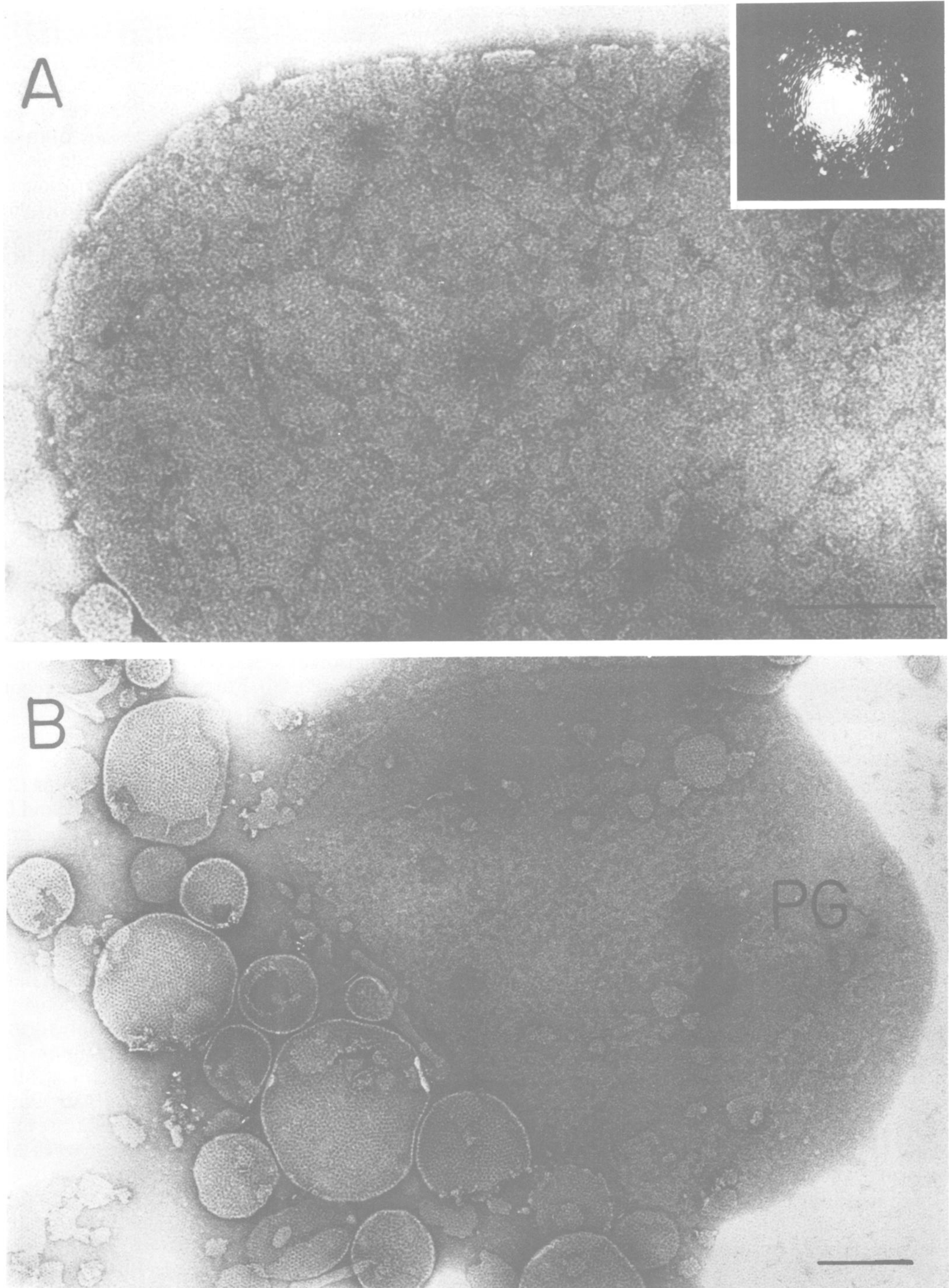

FIG. 3. *Assembly of an ordered lattice structure from the LamB protein and lipopolysaccharide on the lipoprotein-bearing peptidoglycan sacculus. Experimental procedures were the same as those described in the legend to Fig. 1. Envelope components added to the reaction mixtures were as follows: (A) 80 μg of the LamB protein, 20 μg of the wild-type lipopolysaccharide and the lipoprotein-bearing peptidoglycan sacculus (20 μg as peptidoglycan); (B) 80 μg of the LamB protein, 20 μg of the wild-type lipopolysaccharide and the lipoprotein-free peptidoglycan sacculus (20 μg as peptidoglycan). Bars represent 200 nm. PG, lipoprotein-free peptidoglycan sacculus.*

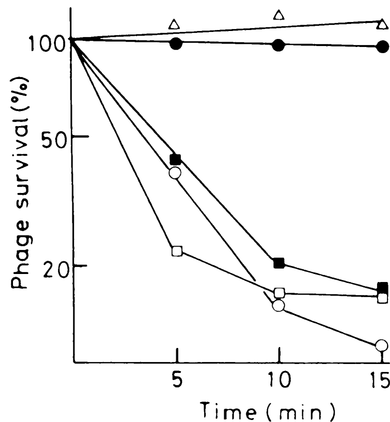

FIG. 4. *The receptor activity for phage lambda of native and reconstituted envelopes. Reconstitutions were carried out in the absence of the peptidoglycan sacculus. Samples assayed for the receptor activity were as follows: △, none; ○ and ●, native cell envelopes prepared as described previously (29) (the amount of LamB in the cell envelope was estimated from the polyacrylamide gel profile of envelope proteins); □, sample reconstituted with the LamB protein alone; ■, sample reconstituted with LamB protein and wild-type lipopolysaccharide (molar ratio 1:3). Native cell envelopes or reconstituted samples were incubated for indicated times in the presence (△, ○, □, ■) and absence (●) of chloroform. Receptor activity was represented by the phage inactivation.*

than that with the LamB protein alone. Under conditions employed in Fig. 4, about 10% of the phage always survived even after prolonged incubation, although the reason is unclear. Interaction between phage lambda and the reconstituted cell surface can also be observed under an electron microscope in the presence of chloroform (Fig. 5). No interaction was observed in the absence of chloroform (data not shown). When reconstitution of the LamB-lipopolysaccharide lattice was carried out on the lipoprotein-carrying peptidoglycan sacculus, the entire surface of the sacculus was covered by lambda. Almost all of the phages on the reconstituted cell surface were found to have an empty head and an empty sheath, indicating that DNA ejection had already taken place. The results were consistent with previous observations for crude receptor preparations in that the LamB protein induces DNA ejection in the presence of chloroform (22). The phage adsorption and the DNA ejection were also observed for vesicles reconstituted from the LamB protein and lipopolysaccharide. However, the adsorption occurred predominantly on some vesicles, whereas other vesicles were almost devoid of phages. This may be due to possible asymmetric assembly of the LamB protein in the vesicles; the LamB protein may face outside in some vesicles and inside in others. The fact that the receptor activity of the LamB-lipopolysaccharide vesicle is weaker than that of the LamB protein alone may also be due to the possible asymmetric assembly. For a control experiment, we reconstituted a vesicle from the OmpC protein and lipopolysaccharide as described (43). Neither the phage adsorption nor the DNA ejection was observed on it. Although the reason is unclear, it should also be noted in Fig. 5B that the phage tended to bind to a limited area of a vesicle as a cluster. Such clustering was not observed in the absence of the vesicle, supporting the view that the clustering took place on the vesicle. Since the LamB protein alone did not form an ordered structure, it was difficult to study the interaction with the phage under an electron microscope.

DISCUSSION

Evidence has accumulated that the LamB protein shares a number of unique physical and biochemical properties with OmpC and OmpF proteins, major outer membrane proteins. These include resistance to denaturation by SDS, characteristic association to the peptidoglycan layer, and formation of a channel for small hydrophilic molecules (3, 20, 26). Furthermore, very recently Moreno and Wandersman showed that OmpC and LamB proteins can serve as substitute receptors for host-range mutants of phage Tula, which uses the OmpF protein as a receptor (24). In the present reconstitution work, we found the following additional evidence of the similarity between LamB and OmpC proteins: (i) these two proteins form a highly ordered hexagonal lattice structure in the presence of lipopolysaccharide, (ii) the fatty acid moiety of the lipopolysaccharide is essential for the lattice formation, (iii) the carbohydrate moiety most likely plays a role in determining the lattice constant, and (iv) the lattice structure is preferably formed on the peptidoglycan layer when the lipoprotein is covalently bound to this layer. Almost all of these properties are also shared by the OmpF protein (43; H. Yamada and S. Mizushima, manuscript in preparation). The present work also showed some differences between LamB and OmpC proteins. The lattice constant provided by the LamB protein was larger than that provided by the OmpC protein under the same conditions. This may be due to the fact that the molecular weight of the LamB protein is appreciably larger than that of the OmpC protein (7, 26, 29). Another possible difference between the two proteins is the fact that the LamB protein is seemingly able to take on a hexagonal structure by itself, whereas we failed to observe the

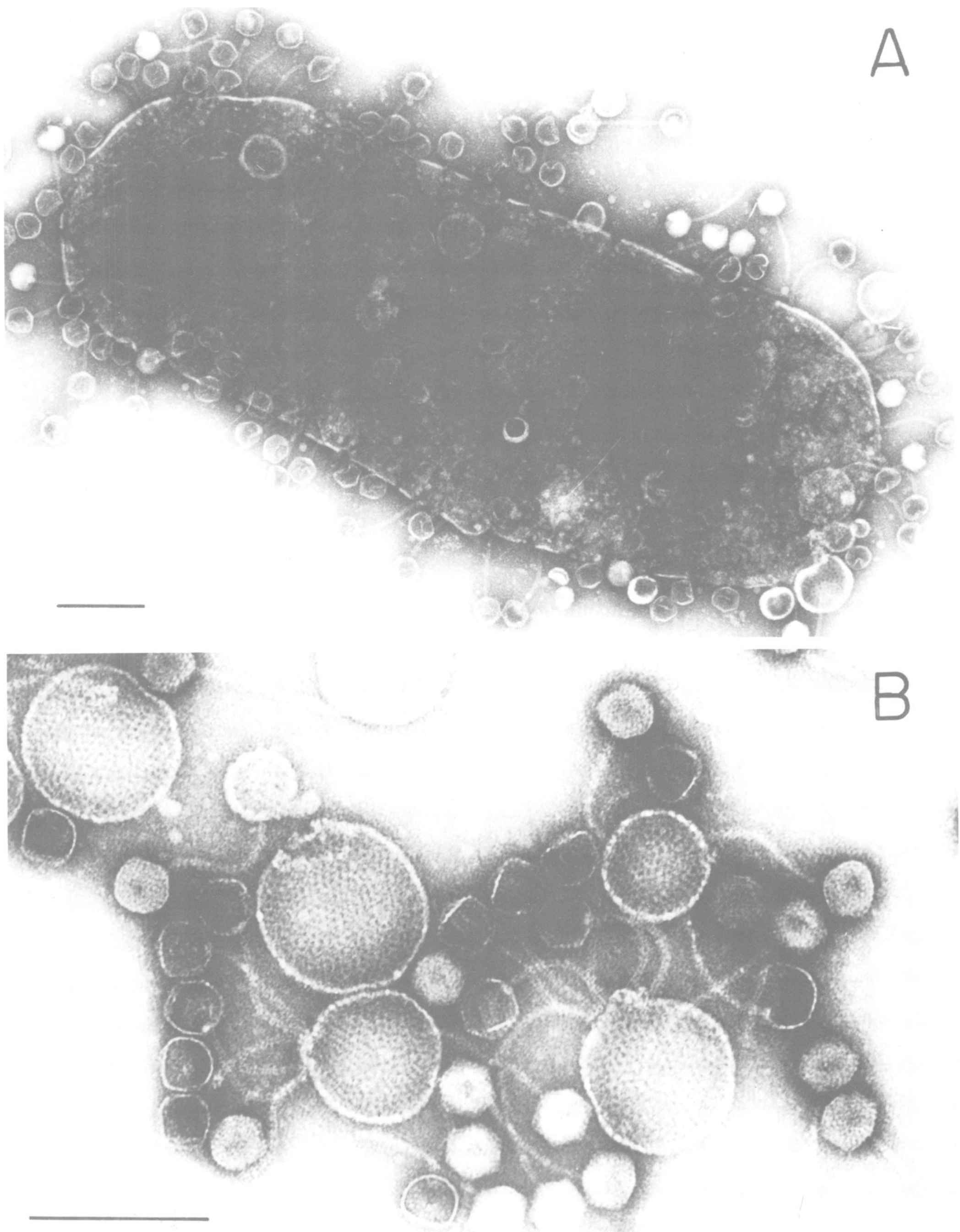

FIG. 5. *Adsorption of lambda phage on the reconstituted cell surface as seen in negatively stained specimens. Reconstitution experiments were carried out with the LamB protein and the wild-type lipopolysaccharide in the presence (A) or absence (B) of the lipoprotein-bearing peptidoglycan sacculus as described in the legend to Fig. 3. The reconstituted samples were treated with phage lambda, negatively stained, and examined with an electron microscope as described in the text. Bars represent 200 nm.*

hexagonal lattice structure with the OmpC protein alone (43, 44). However, the hexagonal arrangement of the LamB protein was limited to a very small area in the absence of lipopolysac-

charide, and lipopolysaccharide certainly played a role in LamB assembly. At present it is difficult to conclude whether the two proteins are different in the mode of assembly.

Proteins OmpC and OmpF exist as trimers and can be purified in SDS solution in this form (27, 45). The hexagonal assembly of these proteins with lipopolysaccharide is most probably due to their trimer condition. The LamB protein has been suggested to exist as either a dimer (3, 17) or a trimer (33). The striking similarities between the LamB protein and OmpC and OmpF proteins, especially in the ability to form a hexagonal lattice, strongly favor the idea that the LamB protein also exists as a trimer. Proteins OmpC and OmpF highly resemble each other and most probably are able to form heterotrimers (trimers consisting of both monomers) as well as homotrimers (15). We do not know whether the LamB monomer forms heterotrimers with OmpC and OmpF or both monomers.

The present work provided evidence on the molecular level at which the LamB protein interacts with lipopolysaccharide. Both the fatty acid region and the carbohydrate region of the lipopolysaccharide were shown to participate in the interaction with the LamB protein. The importance of the carbohydrate region in the assembly of the LamB protein in the outer membrane has been suggested by Randall (35), who showed loss of the LamB protein from the outer membrane of a lipopolysaccharide-deficient strain of *E. coli*. A similar phenomenon has been reported for the OmpF protein, the heptoseless mutation resulting in an appreciable decrease of the OmpF protein (1, 16, 21).

A reconstitution system generally provides a useful means of studying the structure-function relationship in organelles. By the use of *E. coli* cell surface reconstituted from purified molecular components, we have obtained information on the roles of lipopolysaccharide, the OmpC protein, the bound form of the lipoprotein, the peptidoglycan layer, and phospholipid in the cytoplasmic membrane in the process of phage T4 infection (9; H. Furukawa and S. Mizushima, manuscript in preparation). Differing from T4, lambda has a noncontractile flexible tail. Therefore, early steps of the lambda infection process must be largely different from those of T4, which has a contractile sheath. In the present work we succeeded in making a reconstituted cell surface containing the LamB protein. The cell surface has a highly ordered structure and is active in phage lambda infection. This must provide a useful means of studying the mechanism of lambda adsorption and other early steps in the lambda-host interaction.

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LITERATURE CITED

1. **Ames, G. F., E. N. Spudich, and H. Nikaido.** 1974. Protein composition of the outer membrane of *Salmonella typhimurium*: effect of lipopolysaccharide mutations. J. Bacteriol. **117**:406–416.
2. **Bartlett, G. R.** 1959. Phosphorus assay in column chromatography. J. Biol. Chem. **234**:466–468.
3. **Boehler-Kohler, B. A., W. Boos, R. Dieterle, and R. Benz.** 1979. Receptor for bacteriophage lambda of *Escherichia coli* forms larger pores in black lipid membranes than the matrix protein (porin). J. Bacteriol. **138**:33–39.
4. **Bosch, V., and V. Braun.** 1973. Distribution of murine-lipoprotein between the cytoplasmic and outer membranes of *Escherichia coli*. FEBS Lett. **34**:307–310.
5. **Braun, V., and H. J. Krieger-Brauer.** 1977. Interrelationship of the phage λ receptor protein and maltose transport in mutants of *Escherichia coli* K12. Biochim. Biophys. Acta **469**:89–98.
6. **DeMartini, M., and M. Inouye.** 1978. Interaction between two major outer membrane proteins of *Escherichia coli*: the matrix protein and the lipoprotein. J. Bacteriol. **133**:329–335.
7. **Endermann, R., I. Hindennach, and U. Henning.** 1978. Major proteins of the *Escherichia coli* outer cell envelope membrane: preliminary characterization of the phage λ receptor protein. FEBS Lett. **88**:71–74.
8. **Erickson, H. P., W. A. Voter, and K. Leonard.** 1978. Image reconstitution in electron microscopy: enhancement of periodical structure by optical filtering. Methods Enzymol. **49**:39–66.
9. **Furukawa, H., and S. Mizushima.** 1979. Interaction with bacteriophage T4 of reconstituted cell envelopes of *Escherichia coli* K-12. J. Bacteriol. **140**:1071–1080.
10. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. **9**:245–249.
11. **Galanos, C., E. Th. Rietschel, O. Lüderitz, and O. Westphal.** 1971. Interaction of lipopolysaccharides and lipid A with complement. Eur. J. Biochem. **19**:143–152.
12. **Hasegawa, Y., H. Yamada, and S. Mizushima.** 1976. Interactions of outer membrane proteins O-8 and O-9 with peptidoglycan sacculus of *Escherichia coli* K-12. J. Biochem. (Tokyo) **80**:1401–1409.
13. **Herbert, D., P. J. Phipps, and R. E. Strange.** 1971. Determinatioin of hexosamines by the Elson-Morgan reaction. Methods Microbiol. **5B**:284–285.
14. **Hirota, Y., H. Suzuki, Y. Nishimura, and S. Yasuda.** 1977. On the process of cellular division in *Escherichia coli*: a mutant of *E. coli* lacking a murine-lipoprotein. Proc. Natl. Acad. Sci. U.S.A. **74**:1417–1420.
15. **Ichihara, S., and S. Mizushima.** 1979. Arrangement of proteins O-8 and O-9 in outer membrane of *Escherichia coli* K-12: existence of homotrimers and heterotrimers. Eur. J. Biochem. **100**:321–328.
16. **Koplow, J., and H. Goldfine.** 1974. Alterations in the outer membrane of the cell envelopes of heptose-deficient mutants of *Escherichia coli*. J. Bacteriol. **117**: 527–543.
17. **Kühl, P. W.** 1978. The receptor of bacteriophage λ: evidence for its dimeric nature. FEBS Lett. **96**:385–388.
18. **Lee, N., and M. Inouye.** 1974. Outer membrane proteins of *Escherichia coli*: biosynthesis and assembly. FEBS Lett. **39**:167–170.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1959. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.

20. **Luckey, M., and H. Nikaido.** 1980. Specificity of diffusion channels produced by λ phage receptor protein of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:167–171.
21. **Lugtenberg, B., R. Peter, H. Bernheimer, and W. Berendsen.** 1976. Influence of cultural conditions and mutations on the composition of the outer membrane proteins of *Escherichia coli*. Mol. Gen. Genet. **147**:251–262.
22. **Mackay, D. J., and V. C. Bode.** 1976. Binding to isolated phage receptors and λ DNA release in vitro. Virology **72**:167–181.
23. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Moreno, F., and C. Wandersman.** 1980. OmpC and LamB proteins can serve as substitute receptors for host range mutants of coliphage TuIa. J. Bacteriol. **144**: 1182–1185.
25. **Mutoh, N., H. Furukawa, and S. Mizushima.** 1978. Role of lipopolysaccharide and outer membrane protein of *Escherichia coli* K-12 in the receptor activity for bacteriophage T4. J. Bacteriol. **136**:693–699.
26. **Nakae, T.** 1979. A porin activity of purified receptor protein from *Escherichia coli* in reconstituted vesicle membranes. Biochem. Biophys. Res. Commun. **88**:774–781.
27. **Nakae, T., J. Ishii, and M. Tokunaga.** 1979. Subunit structure of functional porin oligomers that form permeability channels in the outer membrane of *Escherichia coli*. J. Biol. Chem. **254**:1457–1461.
28. **Nakamura, K., and S. Mizushima.** 1975. In vitro reassembly of membrane vesicle from *Escherichia coli* outer membrane components: role of individual components and Mg ions in reassembly. Biochim. Biophys. Acta **413**:371–393.
29. **Nakamura, K., and S. Mizushima.** 1976. Effects of heating in dodecyl sulfate solution on the conformation and electrophoretic mobility of isolated major outer membrane proteins from *Escherichia coli*. J. Biochem. (Tokyo) **80**:1411–1422.
30. **Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson.** 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*: isolation and characterization of cytoplasmic and outer membrane. J. Biol. Chem. **247**:3962–3972.
31. **Osborn, M. J., and H. C. P. Wu.** 1980. Proteins of the outer membrane of gram-negative bacteria. Annu. Rev. Microbiol. **34**:369–422.
32. **Palva, E. T., and L. L. Randall.** 1978. Arrangement of protein I in *Escherichia coli* outer membrane: cross-linking study. J. Bacteriol. **133**:279–286.
33. **Palva, E. T., and P. Westermann.** 1979. Arrangement of the maltose-induced major outer membrane proteins, the bacteriophage λ receptor in *Escherichia coli* and the 44K protein in *Salmonella typhimurium*. FEBS Lett. **99**:77–80.
34. **Prehm, P., S. Stirm, B. Jann, and K. Jann.** 1976. Cell-wall lipopolysaccharides of ampicillin-resistant mutants of *Escherichia coli* K-12. Eur. J. Biochem. **66**:369–377.
35. **Randall, L. L.** 1975. Quantitation of the loss of the bacteriophage lambda receptor protein from the outer membrane of lipopolysaccharide-deficient strains of *Escherichia coli*. J. Bacteriol. **123**:41–46.
36. **Randall-Hazelbauer, L., and M. Schwartz.** 1973. Isolation of the bacteriophage lambda receptor from *Escherichia coli*. J. Bacteriol. **116**:1436–1446.
37. **Rietschel, E. Th., H. Gottert, O. Lüderitz, and O. Westphal.** 1972. Nature and linkages of the fatty acids present in the lipid-A component of *Salmonella* lipopolysaccharides. Eur. J. Biochem. **28**:166–173.
38. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*: regular arrangement on the peptidoglycan and unusual dodecyl sulfate binding. J. Biol. Chem. **249**:8019–8029.
39. **Szmelcman, S., and M. Hofnung.** 1975. Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. J. Bacteriol. **124**:112–118.
40. **Szmelcman, S., M. Schwartz, T. J. Silhavy, and W. Boos.** 1976. Maltose transport in *Escherichia coli* K12. A comparison of transport kinetics in wild-type and λ-resistant mutants with the dissociation constants of the maltose-binding protein as measured by fluorescence quenching. Eur. J. Biochem. **65**:13–19.
41. **von Meyenburg, K., and H. Nikaido.** 1977. Outer membrane of Gram negative bacteria. XVII. Specificity of transport process catalyzed by the λ-receptor protein in *Escherichia coli*. Biochem. Biophys. Res. Commun. **78**: 1100–1107.
42. **Yamada, H., and S. Mizushima.** 1977. Lipoprotein-bearing peptidoglycan sacculus as a preferred site for the in vitro assembly of membrane from dissociated components of outer membrane of *Escherichia coli* K12. J. Biochem. (Tokyo) **81**:1889–1899.
43. **Yamada, H., and S. Mizushima.** 1978. Reconstitution of an ordered structure from major outer membrane constituents and the lipoprotein-bearing peptidoglycan sacculus of *Escherichia coli*. J. Bacteriol. **135**:1024–1031.
44. **Yamada, H., and S. Mizushima.** 1980. Interaction between major outer membrane protein (O-8) and lipopolysaccharide in *Escherichia coli* K-12. Eur. J. Biochem. **103**:209–218.
45. **Yu, F., S. Ichihara, and S. Mizushima.** 1979. A major outer membrane protein, O-8, of *Escherichia coli* K-12 exists as a trimer in sodium dodecyl sulfate solution. FEBS Lett. **100**:71–74.