Interaction of Bacteriophage T4 with Reconstituted Cell Envelopes of *Escherichia coli* K-12

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The interaction with bacteriophage T4 of the cell surface of *Escherichia coli* K-12 reconstituted from outer membrane protein O-8, lipopolysaccharide, and the lipoprotein-bearing peptidoglycan sacculus was studied. The reconstituted cell surface was active as a receptor for the phage, resulting in the contraction of the tail sheath, a morphological change in the base plate which was accompanied by the extension of short tail pins down to the cell surface and the penetration of the needle through the cell surface. However, the ejection of phage deoxyribonucleic acid did not take place. Both O-8 and lipopolysaccharide were essential for the interaction. In the reconstitution, the wild-type lipopolysaccharide could not be replaced by either heptoseless lipopolysaccharide or lipid A. The lipoprotein-bearing peptidoglycan sacculus was also found to be an active component for the phage adsorption. The sacculus most likely functioned as a basal framework on which O-8 and lipopolysaccharide assembled to form a flat sheet which is large enough to interact with individual distal ends of long tail fibers of a single phage particle.

The outer membrane, the outermost layer of gram-negative bacteria, consists of proteins, lipopolysaccharide (LPS) and phospholipids (15). In *Escherichia coli*, the outer membrane has a close association with the underlying peptidoglycan layer through the lipoprotein which is covalently bound to the peptidoglycan layer (13, 38, 39; for the lipoprotein see reference 8) and probably also through some of the outer membrane proteins (17, 18, 33, 41).

Recently, we succeeded in reconstituting an ordered hexagonal lattice structure with a lattice constant of about 7 nm on the entire surface of the lipoprotein-bearing peptidoglycan sacculus from outer membrane proteins O-8 or O-9 or both and LPS (39). Proteins O-8 and O-9 are identical with Ib and Ia, c and b; and 1b and 1a, respectively (20). We further showed that when the peptidoglycan layer lacking the bound form of the lipoprotein was used, the lattice structure was formed independently of the peptidoglycan layer (39). These results strongly indicate that O-8 or O-9 or both and LPS provide the framework of the outer membrane and that the bound form of the lipoprotein plays a role in the holding of the framework on the peptidoglycan layer, thus forming the basal structure of the *E. coli* cell surface. The lattice structure resembled that observed in the cell envelopes which had been treated with sodium dodecyl sulfate (SDS) (33), indicating that the molecular arrangement in

the reconstituted cell surface is similar to that in the native cell envelope. However, it was uncertain whether the reconstituted cell surface was functionally active or not.

The bacterial cell surface functions in the process of bacteriophage infection (23). For example, an early stage of phage T4 infection involves the interaction of the cell surface with long tail fibers, tail pins, and the needle (1, 9, 12, 32, 34). Recently, we showed that in *E. coli* K-12 both LPS and O-8 are required to obtain the receptor activity for phage T4 (28). The same conclusion was obtained by Henning and Jann (19). The present study was undertaken to determine whether the cell surface reconstituted from LPS and O-8 with or without the lipoprotein-bearing peptidoglycan sacculus functions in the process of the phage infection or not. The cell surface reconstituted from LPS and O-8 allowed the phage to adsorb onto the cell surface with long tail fibers and tail pins, contract the tail sheath, and insert the tail needle into the cell surface. However, it did not allow the phage to eject DNA. The cell surface was more active as the receptor than that with only LPS and O-8.

MATERIALS AND METHODS

Preparation of envelope components. Outer membrane proteins O-8 and O-9 were purified from *E. coli* YA21 (K-12, F⁻ *met leu* λ ⁻) as described previ-

ously (29) and kept in 1% SDS. These proteins exist as trimers in these preparations (31, 40). The LPS content of the purified preparations was about 10 $\mu\text{g}/\text{mg}$ of protein. LPS was prepared from YA21 by the method of Galanos et al. (16). Heptoseless LPS was prepared from T4-resistant mutant YA21-6 (28) by the same method. Lipid A was prepared from LPS by acetic acid hydrolysis as described (27). The lipoprotein-bearing peptidoglycan sacculus was prepared from *E. coli* YA21 as described previously (39). The free form of the lipoprotein was prepared from the same strain by the method of Inouye et al. (22).

Preparation of bacteriophage T4. Phage T4 was prepared as described previously (28).

Reconstitution of cell surface. The reconstitution of the cell surface from 100 μg of O-8, 100 μg of LPS, and the lipoprotein-bearing peptidoglycan sacculus (25 μg of glucosamine) was carried out with an SDS solution by dialysis against a solution containing 10 mM Tris-hydrochloride (pH 7.8), 5 mM MgCl_2 , and 0.02% sodium azide at 25°C. The method was basically the same as that described previously (39).

Assay of receptor activity for phage T4. The method was the same as that described previously (28). Briefly, the sample to be assayed was incubated for 10 min with 3×10^5 plaque-forming units of phage T4 at 37°C in 1 ml of an adsorption buffer supplemented with 50 μg of L-tryptophan. After a 10-min incubation, 0.1 ml of the sample was withdrawn to determine the plaque-forming ability. *E. coli* YA21 was used as indicator. Receptor activity is expressed as the ability to inactivate the phage.

Electron microscopy. The interaction between the reconstituted cell surface and phage T4 was examined under a Hitachi HS-9 electron microscope. Negative staining was carried out as described previously (38). Samples for thin sectioning were prefixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 20 min at 0°C, followed by a 4-h fixation with 1% OsO_4 in the same buffer at 0°C. The samples were dehydrated through an ascending series of ethanol and embedded in Epon. Sections, which were cut on a Porter-Blum MT-1 ultramicrotome, were first stained with 2% uranyl acetate solution overnight and subsequently stained with lead citrate solution for 5 min.

Analytical methods. The amount of protein was determined by the method of Lowry et al. (24). The amount of LPS was estimated from the 2-keto-3-deoxyoctonate content, which was determined by the method described by Osborn et al. (30).

RESULTS

Requirement of O-8 and LPS for receptor activity. We showed in the previous work (28) that the receptor for phage T4 was reconstituted from outer membrane protein O-8 and LPS dissolved in deoxycholate solution. Two other methods of reconstitution have been examined. One of them involved the solubilization of these components with Triton X-100 followed by precipitation by the addition of magnesium ions (10), and the other involves solubilization in an SDS solution followed by dialysis against a mag-

nesium chloride solution as described above. Of these three methods, the reconstitution from the SDS solution, which results in the assembly of O-8 and LPS into a hexagonal lattice, was found to be the best. Therefore, details of the interaction between cell surface components and phage T4 were examined by the use of this reconstitution method.

The amounts, as a ratio, of O-8 and LPS required for the expression of the receptor activity for phage T4 were investigated (Fig. 1). No receptor activity was observed with LPS alone. The addition of O-8 resulted in the appearance and increase of the receptor activity, which levelled off when the O-8/LPS ratio in weight reached about 0.5. The molar ratio of O-8 to LPS at this point was calculated to be 0.05, assuming the molecular weights of O-8 and LPS to be 38,000 (29, 40) and 4,000 (26), respectively. Although chemical and physical properties of O-9 are very similar to those of O-8 in many respects (17, 21, 29, 39, 41), only a very little receptor activity was reconstituted with O-9 (Fig. 1). Since the O-9 preparation used was contaminated with 10% of protein O-8, the reconstituted receptor activity could be accounted for by the contaminating O-8. Therefore, we concluded that O-9 was completely inactive as a component of the receptor. This conclusion is consistent with the result of Henning and Jann (19).

The role of LPS in the receptor activity was

FIG. 1. Requirement of O-8 for receptor activity for phage T4. Reconstitution of the receptor activity was carried out with 100 μg of wild-type LPS and different amounts of O-8 (●). Reconstituted cell surface structures containing 10 μg of LPS were used for the assay of receptor activity. The abscissa represents the weight ratio O-8/LPS in the reconstituted cell surface structure. The receptor activity was represented by the phage inactivation during a 10-min incubation. In the other experiment (○), O-8 was replaced by O-9.

also examined (Fig. 2). No receptor activity appeared with O-8 alone, although this protein preparation was contaminated with 1% LPS. The addition of LPS to the reconstitution mixture resulted in an increase in the receptor activ-

FIG. 2. Requirement of LPS for receptor activity for phage T4. Reconstitution of the receptor activity was carried out with 100 μ g of O-8 and different amounts of LPS or its derivatives. Reconstituted cell surface structures containing 5 μ g of O-8 were used for the assay of receptor activity. ■, Wild-type LPS; □, heptoseless LPS; ▽, lipid A. The abscissa represents the weight ratio of LPS/O-8 in the reconstituted cell surface structures. The amounts of heptoseless LPS and lipid A were expressed as weight of the equivalent amount of wild-type LPS.

ity until the ratio in weight of LPS to O-8 reached about 1.5. The molecular ratio of O-8 to LPS at this point was calculated to be 0.07. This value is roughly the same as that estimated from the results shown in Fig. 1. Neither heptoseless LPS nor lipid A was found to be active as a component of the receptor.

Role of peptidoglycan sacculus in receptor function. When the reconstitution was carried out in the presence of the lipoprotein-bearing peptidoglycan sacculus, a hexagonal lattice consisting of O-8 and LPS was assembled on the entire surface of the peptidoglycan sacculus (39; Fig. 3). As shown in Table 1, the lattice structure reconstituted on the peptidoglycan sacculus showed appreciably higher receptor activity than that consisting solely of O-8 and LPS. Here are some possible explanations for the stimulative effect of the lipoprotein-bearing peptidoglycan. Either the bound form of the lipoprotein or the peptidoglycan may be involved in the receptor function. Alternatively, the peptidoglycan sacculus may play a role by allowing O-8 and LPS to assemble widely onto its surface so that the lattice structure can interact with individual distal ends of long tail fibers of a single phage particle. To determine the possible role of the lipoprotein, the reconstitution was carried out in the presence of the free form of the lipoprotein. However, the lipoprotein did not stimulate the

FIG. 3. Reconstitution of an ordered lattice structure from O-8 and LPS on the lipoprotein-bearing peptidoglycan sacculus. The reconstituted structure was negatively stained and examined with an electron microscope. The length of the bar in this and in subsequent micrographs represents 100 nm.

TABLE 1. *Reconstitution of receptor for phage T4*

Addition			Phage survival ^a (%)	
O-8	LPS	Lipoprotein-bearing peptidoglycan	Expt 1	Expt 2
+	—	—	99	90
—	+	—	100	94
+	+	—	29	52
+	+	+	8	23
—	—	+	97	87

^a Two typical experiments are shown. After the reconstitution, the amount of LPS or other components recovered, in case of experiments without LPS, were determined, and the portion equivalent to one-tenth of the original amount was used for the assay of the receptor activity.

receptor activity (data not shown). On the other hand, the second possibility was supported by the following evidence. As will be shown later, the reconstitution from O-8 and LPS occasionally produced extraordinarily large vesicles in addition to the normal-sized vesicles whose diameter was 100 to 200 nm (see Fig. 7). Such large vesicles adsorbed appreciably larger numbers of phages than smaller ones. Therefore, it is strongly suggested that reconstituted vesicles of the usual size could not provide a flat surface large enough to interact with individual distal ends of long tail fibers of single phage particle which are 160 nm distant from each other (34).

Electron microscopic observation. The interaction between the reconstituted cell surface and phage T4 was examined with an electron microscope. Figure 3 shows the cell surface reconstituted from O-8, LPS, and the lipoprotein-bearing peptidoglycan. Although the profile was essentially the same as that shown in the previous work (39), the lattice structure covering the entire surface of the peptidoglycan sacculus can be seen more clearly. Figures 4 and 5 show the mode of adsorption of phage T4 to the reconstituted cell surface. Due to the presence of the medium used for the phage adsorption, the lattice structure on the peptidoglycan sacculus was not clearly seen. Almost all of the phages on the reconstituted cell surface were found to have a contracted tail sheath, whereas those independent of the cell surface had a long sheath with a clearly visible base plate which is a typical profile of an uninfected phage. The base plate of phages adsorbed on the reconstituted cell surface underwent a morphological change which was accompanied by the extension of short tail pins from it to the cell surface (Fig. 4B and 5). However, as negatively stained specimens clearly show, phage heads were still filled with an electron-dense substance, indicating

that DNA ejection did not take place on the synthetic cell surface. However, in thin-sectioned specimens, some phage particles adsorbed on the reconstituted cell surface had an empty head indicating that the release of phage DNA took place to some extent during the preparation of thin-sectioned specimens. On the other hand, unadsorbed phages did not release their DNA during the preparation of thin-sectioned specimens (data not shown). The results suggest that although the reconstituted cell surface does not cause the ejection of DNA from phages adsorbed on it, it causes a certain modification on phages so that they lose their DNA during the preparation of thin-sectioned specimens.

The mode of interaction of hexagonally latticed vesicle reconstituted from O-8 and LPS with phage T4 was almost the same as that of the reconstituted sacculus with peptidoglycan (Fig. 6 and 7). That is, the contraction of the tail sheath and the extension of tail pins to the surface of vesicles were observed. However, many phages having a contracted sheath (about 50% of the total phages) were observed independent of vesicles (Fig. 6). A fraction of such free contracted phages in the original phage preparation was less than 2%. Therefore, the results most probably indicate that the interaction between the vesicle and the phage was rather unstable so that many of the adsorbed phages were released from the vesicle during the preparation procedures for electron microscopy. The unstability of binding was most probably due to the limited extent of a flat surface on the vesicle. An extraordinarily large vesicle could adsorb and retain a large number of phage particles (Fig. 7A), whereas only a small portion of the small vesicles could retain the phage (Fig. 6 and 7). Since the reconstitution was carried out with purified O-8 and LPS, such large vesicles could not be peptidoglycan-containing. The requirement of certain extent of a flat surface for the phage adsorption was further supported by the fact that nonvesicular flat fragments, even though they were small, were able to interact with a phage particle (Fig. 7B and C). Although the phage preparation used contained a small number of cell debris which resembled the non-vesicular flat fragments, such cell debris, if any, could account for less than 1% of the small fragment in the reconstituted preparations. Furthermore, when the reconstitution was carried out in the presence of the peptidoglycan sacculus, such small fragments were hardly observed.

To elucidate whether needles of adsorbed phages penetrated the surface or not, we measured the distance between the base plate and the reconstituted cell surface. The distance be-

FIG. 4. Interaction with phage T4 of cell surface reconstituted from O-8, LPS, and the lipoprotein-bearing peptidoglycan sacculus as seen in negatively stained specimens. Reconstituted cell surface was incubated at 37°C for 30 min with 3×10^8 phage T4 in 20 μ l of the adsorption buffer. The multiplicity of infection was about 100 phage particles per reconstituted sacculus. The incubated mixture was directly negatively stained and examined with an electron microscope.

tween the base plate and the tip of the needle of an unattached contracted phage particle was taken as a control. A phage particle with contracted sheath can also be prepared artificially

in the presence of 6 M urea (36). This phage particle was also taken as a control. As shown in Fig. 8, needles of adsorbed phages were appreciably shorter than those of unattached con-

FIG. 5. Interaction with phage T4 of cell surface reconstituted from O-8, LPS, and the lipoprotein-bearing peptidoglycan sacculus as seen in thin-sectioned specimens. The cell surface-phage T4 complexes obtained in Fig. 4 were recovered by centrifugation, fixed, and thin-sectioned. Two small pictures are parts of such specimens where the mode of attachment of phage T4 to the cell surface can be seen more clearly.

FIG. 6. Interaction with phage T4 of cell surface reconstituted from O-8 and LPS as seen in negatively stained specimens. Conditions were the same as these given in Fig. 4. The incubated mixture was directly negatively stained and examined with an electron microscope. Three typical profiles are given.

FIG. 7. Interaction with phage T4 of cell surface reconstituted from O-8 and LPS as seen in thin-sectioned specimens. The cell surface-phage T4 complexes in Fig. 6 were recovered by centrifugation, fixed, and thin-sectioned. Arrows indicate "tips" of the needle of phage penetrated through the reconstituted cell surface structure. Two small pictures (B and C) show that phage T4 could be retained on a small flat surface.

tracted phages irrespective of the presence of the peptidoglycan layer. It should be noted that the external length of needles of unattached contracted phages showed a wide distribution with two peaks, and the second peak almost coincided with that of the urea-treated phages. The results suggest that many phages may have lost the tip of the needle during detachment from the reconstituted cell surface. Provided that the external length of needles of urea-

treated phage particles represents those after the sheath contraction in normal conditions, the needle of adsorbed phages must have penetrated about 23 nm into the reconstituted cell surface, since the average external lengths of needles of adsorbed phages and urea-treated phages were about 28 nm (27 nm with peptidoglycan and 29 nm without peptidoglycan) and about 51 nm, respectively. On the other hand, the thickness of the reconstituted cell surface structure shown in

FIG. 8. Histograms of the lengths of needles of unattached contracted phage particles, urea-treated phage particles, and adsorbed phage particles. Lengths of the needles of unattached contracted phages and urea-treated phages were measured with negatively stained specimens from the base plate to the distal tips of the needle. Lengths of the needles of adsorbed phages were measured with thin-sectioned specimens from the base plates to the cell surface on which they had adsorbed. In both cases, lengths of contracted sheath (45 nm from the top of the sheath to the bottom of the base plate) were used as the standard length. This length was determined by using uniform latex particles (Dow Chemicals) as an internal magnification standard. ■, Unattached contracted phages; ■, urea-treated phages; □, phages adsorbed on the cell surface reconstituted with peptidoglycan; ▨, phages adsorbed on the cell surface reconstituted without peptidoglycan.

Fig. 7 was about 10 nm. Therefore, the tip of needles must have penetrated to the other side of the reconstituted cell surface structure. Indeed, as indicated by arrows in Fig. 7, a structure, most probably the tip of the needle, was sometimes observed at the other side of the reconstituted cell surface structure on which phages were adsorbed. From these results we concluded that the phage needle penetrated through the reconstituted cell surface.

DISCUSSION

Both LPS and outer membrane protein of *E. coli* K-12 are required for phage T4 receptor function (19, 28), whereas in the case of *E. coli*

B only LPS is required (37). The present work showed that the early process of phage T4 infection takes place on the synthetic *E. coli* K-12 cell surface reconstituted from O-8, LPS, and the lipoprotein-bearing peptidoglycan sacculus. In addition to O-8 and LPS which were essential for the process, the lipoprotein-bearing peptidoglycan sacculus stimulated the process. The sacculus most likely functioned as a basal framework on which O-8 and LPS assembled to form a flat sheet which is large enough to interact with individual distal ends of long tail fibers of a single phage particle. Without the peptidoglycan sacculus or with a sacculus carrying no lipoprotein, O-8 and LPS assembled into vesicles with a diameter of 100 to 200 nm (39, and this paper), which cannot supply a flat surface large enough to interact with a phage particle. Crawford and Goldberg showed that several long tail fibers must be bound simultaneously for a phage particle to adsorb irreversibly (9). The importance of a flat surface for phage T2 infection has been suggested by DePamphilis (14).

Either the lipoprotein or peptidoglycan moiety of the sacculus may also play a role in the infection process. The free form of the lipoprotein did not stimulate the reconstitution of the receptor activity. However, this does not exclude the possible participation of this protein, since the purification process involved heat treatment of the lipoprotein in SDS solution (22). The possibility of direct participation of the peptidoglycan layer in the infection process remains uncertain.

It is well known that *E. coli* mutants having heptoseless LPS are resistant to phage T4 infection. From the analyses of mutants of *E. coli* B resistant to phage T4, Dawes (11) and Prehm et al. (32) showed that the glucose-heptose region of LPS is responsible for the interaction with the phage. In the K-12 strain, the essential requirement of the heptose-containing distal end of the oligosaccharide region for the phage adsorption was also indicated (28). These results were confirmed by the present reconstitution experiment which showed that neither the heptoseless LPS nor lipid A was active as a component of the phage receptor (Fig. 2), despite the fact that both of them were able to interact with O-8 to form a hexagonal lattice structure (H. Yamada and S. Mizushima, submitted for publication).

The infection process of phage T4 may be divided into four stages: (i) binding of individual distal ends of long tail fibers to the receptor on the cell wall, (ii) pinning down of tail pins to the cell surface, (iii) elongation of tail pins and insertion of the needle into the cell surface as the tail sheath contracts, and (iv) ejection of phage

DNA to the opposite side of the cell surface (34). The present study revealed that the reconstituted cell surface functions in the first three stages. This indicates that receptors for both long tail fibers and tail pins consist of either or both LPS and O-8. However, the roles of individual components in the two receptor functions remain unknown.

Phage particles adsorbed on the reconstituted cell surface penetrated about 23 nm into the surface with their needle (Fig. 8). Since the reconstituted cell surface contained only O-8 and LPS with or without peptidoglycan, it is most probable that the penetration was simply caused by mechanical force of the contraction of the tail sheath which was held on the cell surface by both tail pins and long tail fibers. The importance of tail pins in holding of the phage on the cell surface during the penetration was indicated by the fact that phage mutants having a defect in the tail pin proteins were released from the cell surface upon contraction of the tail sheath (35).

It is apparent that in gram-negative bacteria such as *E. coli* or *Salmonella typhimurium*, outer and cytoplasmic membranes adhere to one another at 200 to 400 localized areas (3, 5). The adhesion area has been studied in relation to the mechanism of phage infection (4, 5, 6). At low multiplicity of infection phages T1 to T7 were reported to be selectively adsorbed to the adhesion area, whereas at high multiplicity of infection phages were adsorbed to the entire surface of bacteria. The present study shows that the artificial cell surface reconstituted from O-8, LPS, and the peptidoglycan sacculus has an ability to induce the phage T4 infection to the stage of the needle insertion. Since O-8 and LPS are major components of the outer membrane and believed to cover the entire surface of the intact bacterial cell (33), the result suggests that every part of the cell surface is eligible to be the receptor for phage T4. The high affinity adsorption of phages to the adhesion zones may be due to a special conformation of the receptor in this region which is determined by the adhered cytoplasmic membrane. Alternatively, phage T4 may not be selectively adsorbed to the adhesion zone, since experimental evidence to support the selective adsorption of T4 is rather weak (4).

The reconstituted cell surface was unable to induce the ejection of phage DNA, indicating that certain additional factors are required for the ejection. Goldberg et al. showed that phosphatidyl glycerol induced the DNA ejection from contracted phages which were prepared by 6 M urea treatment or by infecting a bacterial cell with gene 12⁻ phage T4 (2, 7). The adhesion zone may provide phages with the phospholipid

that is enriched in the cytoplasmic membrane (25). We hope in the future to reconstitute a cell surface that can perform the whole process of phage infection and to reveal the roles of individual cell surface components in the process of the phage infection.

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LITERATURE CITED

1. Arscott, P. G., and E. B. Goldberg. 1976. Cooperative action of the T4 tail fibers and baseplate in triggering conformational change and in determining host range. *Virology* **69**:15-22.
2. Baumann, L., W. C. Benz, A. Wright, and E. B. Goldberg. 1970. Inactivation of urea-treated phage T4 by phosphatidyl glycerol. *Virology* **41**:356-364.
3. Bayer, M. E. 1968. Areas of adhesion between wall and membrane of *Escherichia coli*. *J. Gen. Microbiol.* **53**:395-404.
4. Bayer, M. E. 1968. Adsorption of bacteriophages to adhesions between wall and membrane of *Escherichia coli*. *J. Virol.* **2**:346-356.
5. Bayer, M. E. 1975. Role of adhesion zones in bacterial cell-surface function and biogenesis, p. 393-427. In A. Tzagoloff (ed.), *Membrane biogenesis: mitochondria, chloroplasts, and bacteria*. Plenum Publishing Corp., New York.
6. Bayer, M. E., and T. W. Starkey. 1972. The adsorption of bacteriophage ϕ x174 and its interaction with *Escherichia coli*, a kinetic and morphological study. *Virology* **49**:236-256.
7. Benz, W. C., and E. B. Goldberg. 1973. Interactions between modified phage T4 particles and spheroplasts. *Virology* **53**:225-235.
8. Braun, V. 1975. Covalent lipoprotein from the outer membrane of *Escherichia coli*. *Biochim. Biophys. Acta* **415**:335-377.
9. Crawford, J. T., and E. B. Goldberg. 1977. The effect of baseplate mutations on the requirement for tail-fiber binding for irreversible adsorption of bacteriophage T4. *J. Mol. Biol.* **111**:305-313.
10. Datta, D. B., B. Arden, and U. Henning. 1977. Major proteins of the *Escherichia coli* outer cell envelope membrane as bacteriophage receptors. *J. Bacteriol.* **131**:821-829.
11. Dawes, J. 1975. Characterization of the bacteriophage T4 receptor site. *Nature (London)* **256**:127-128.
12. Dawes, J. 1977. The function of carboxyl groups in phage T4 adsorption. *FEMS Microbiol. Lett.* **1**:187-192.
13. 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.
14. DePamphilis, M. L. 1971. Isolation of bacteriophages T2 and T4 attached to the outer membrane of *Escherichia coli*. *J. Virol.* **7**:683-686.
15. DiRienzo, J. M., K. Nakamura, and M. Inouye. 1978. The outer membrane proteins of gram-negative bacteria: biosynthesis, assembly, and functions. *Annu. Rev. Biochem.* **47**:481-532.
16. Galanos, C., O. Lüderitz, and O. Westphal. 1969. A new method for the extraction of R lipopolysaccharides. *Eur. J. Biochem.* **9**:245-249.
17. 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.
18. Hazumi, N., H. Yamada, and S. Mizushima. 1978. Two new peptidoglycan-associated proteins in the outer membrane of *Escherichia coli* K-12. FEMS Microbiol. Lett. **4**:275-277.
 19. Henning, U., and K. Jann. 1979. Two-component nature of bacteriophage T4 receptor activity in *Escherichia coli* K-12. J. Bacteriol. **137**:664-666.
 20. Ichihara, S., and S. Mizushima. 1977. Strain specificity of outer membrane proteins in *Escherichia coli*. J. Biochem. (Tokyo) **81**:1525-1530.
 21. Ichihara, S., and S. Mizushima. 1978. Characterization of major outer membrane proteins O-8 and O-9 of *Escherichia coli* K-12. Evidence that structural genes for the two proteins are different. J. Biochem. (Tokyo) **83**:1095-1100.
 22. Inouye, S., K. Takeishi, N. Lee, M. DeMartini, A. Hirashima, and M. Inouye. 1976. Lipoprotein from the outer membrane of *Escherichia coli*: purification, paracrystallization, and some properties of its free form. J. Bacteriol. **127**:555-563.
 23. Lindberg, A. A. 1973. Bacteriophage receptors. Annu. Rev. Microbiol. **27**:205-241.
 24. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
 25. Miura, T., and S. Mizushima. 1968. Separation by density gradient centrifugation of two types of membranes from spheroplast membrane of *Escherichia coli* K-12. Biochim. Biophys. Acta **150**:159-161.
 26. Mühlradt, P. F., V. Wray, and V. Lehman. 1977. A ³¹P-nuclear-magnetic-resonance study of the phosphate groups in lipopolysaccharide and lipid A from *Salmonella*. Eur. J. Biochem. **81**:193-203.
 27. Müller-Seitz, E., B. Jann, and K. Jann. 1968. Degradation studies of the lipopolysaccharide from *E. coli* O71:k?:H12. Separation and investigation of O-specific and core polysaccharides. FEBS Lett. **1**:311-314.
 28. 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.
 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* K-12. 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 membranes. J. Biol. Chem. **247**:3962-3972.
 31. 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.
 32. Prehm, P., B. Jann, K. Jann, G. Schmidt, and S. Stirm. 1976. On a bacteriophage T3 and T4 receptor region within the cell wall lipopolysaccharide of *Escherichia coli* B. J. Mol. Biol. **101**:277-281.
 33. 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.
 34. Simon, L. D., and T. F. Anderson. 1967. The infection of *Escherichia coli* by T2 and T4 bacteriophages as seen in the electron microscope. 1. Attachment and penetration. Virology **32**:279-297.
 35. Simon, L. D., J. G. Swan, and J. E. Elatgaard. 1970. Functional defects in T4 bacteriophages lacking the gene 11 and gene 12 products. Virology **41**:77-90.
 36. Wais, A. C., and E. B. Goldberg. 1969. Growth and transformation of phage T4 in *Escherichia coli* B/4, *Salmonella*, *Aerobacter*, *Proteus*, and *Serratia*. Virology **39**:153-161.
 37. Wilson, J. H., R. B. Luftig, and W. B. Wood. 1970. Interaction of bacteriophage T4 tail fiber components with a lipopolysaccharide fraction from *Escherichia coli*. J. Mol. Biol. **51**:423-434.
 38. 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* K-12. J. Biochem. (Tokyo) **81**:1889-1899.
 39. 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.
 40. 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.
 41. 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* K-12. Biochem. Biophys. Res. Commun. **74**:1397-1402.