JOURNAL OF BACTERIOLOGY, Nov. 1981, p. 712-715
0021-9193/81/110712-04$02.00/0

Vol. 148, No. 2

# NOTES

## Role of Lipopolysaccharide in the Receptor Function for Bacteriophage TuIb in *Escherichia coli*

FUJIO YU, HISAMI YAMADA, AND SHOJI MIZUSHIMA*

*Laboratory of Microbiology, Faculty of Agriculture, Nagoya University, Chikusa-Ku, Nagoya 464, Japan*

Received 12 May 1981/Accepted 20 July 1981

Bacteriophage TuIb required lipopolysaccharide in addition to the OmpC trimer as a receptor component. Both the fatty acid and polysaccharide regions of lipopolysaccharide were shown to participate in the receptor function. The roles of lipopolysaccharide and outer membrane proteins in the receptor function for T-even type bacteriophages are discussed.

Many outer membrane proteins of *Escherichia coli* are known to function as receptor components in bacteriophage infections. For example, the major outer membrane proteins OmpA, OmpC, and OmpF are receptor components for phages TuII* and K3, phages TuIb, MeI, and PA2, and phages T2, TuIa, and TP1, respectively (12). Although it has been believed that lipopolysaccharide (LPS) is the sole component of the receptor for phage T4 (13), recent studies revealed that in the K-12 strain both LPS and OmpC are required for the receptor function (8, 10). Furthermore, by using a technique for reconstitution of the cell surface of *E. coli*, the roles of cell surface components including the peptidoglycan layer in the T4 infection process were studied in detail (4). Phage TuIb, another phage that requires OmpC as a receptor component, has a structure which is very similar to that of T-even phages including T4 (3). Phage TuIb was also suggested to require LPS as a receptor component (3). In the present work, we used a reconstitution technique (14, 15) to study the roles of LPS and OmpC in TuIb infection in more detail. The roles of outer membrane proteins and LPS in the receptor function for phages having the T-even structure are also discussed in general in this paper.

Under the conditions for cell surface reconstitution, the OmpC trimer and LPS were assembled into a sheet or a vesicle with an ordered hexagonal lattice structure (14, 15). Figure 1 shows that in addition to OmpC, LPS is essential for expression of the receptor function. LPS alone showed no receptor activity. The minimum amount of LPS required for the receptor function was around one to three molecules per OmpC trimer. Consistent with a previous observation (15), electron microscopic observation of reconstituted samples having LPS/OmpC ratios higher and lower than this ratio revealed a sheet structure with the hexagonal lattice and amorphous aggregates, respectively (data not shown). The results suggest that at least a part of the role of LPS is to enable OmpC to form a sheet which is large enough to interact with individual distal ends of long tail fibers of a single phage particle.

To determine the moiety of LPS required for the receptor function, the reconstitution was carried out with LPS derivatives (Fig. 2). The heptoseless LPS, which is absolutely inactive as a substitute for the wild-type LPS in the receptor function for T4 (10), was partially active. The result is consistent with the fact that the heptoseless mutant used here was sensitive to phage TuIb. Although lipid A and fatty acid enable OmpC to form a flat sheet with a hexagonal lattice structure (15), they were absolutely inactive. These results indicate that in addition to enabling OmpC to assemble into a sheet with a hexagonal lattice structure, LPS participates more directly in the interaction with the phage through its polysaccharide moiety, most likely through both the 3-deoxy-D-manno-octulosonate region and the distal end region including heptose. However, it is still unclear whether LPS is directly involved in the interaction with TuIb or rather indirectly through the interaction with OmpC. It is indicated that the polysaccharide moiety of LPS interacts with OmpC to induce a conformational change of the protein (15).

Interaction between the reconstituted vesicles and TuIb was also examined under an electron microscope (Fig. 3). Phage TuIb has a structure that is very similar to that of T-even phages.

TABLE 1. *Receptor components for E. coli K-12 for phages having the T-even type structure*

| Phage | Receptor components | Reference |
|---|---|---|
| T2 | OmpF, LPS | 7 |
| T4 | OmpC, LPS | 8, 10 |
| T6 | Tsx | 9 |
| TuIa | OmpF, LPS | 3 |
| TuIb | OmpC, LPS | 3, this study |
| TuII* | OmpA, LPS | 3 |

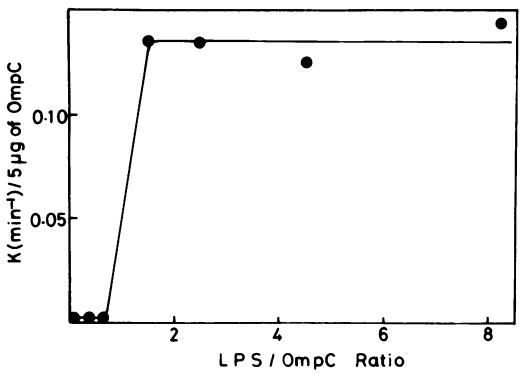


FIG. 1. *Requirement of LPS for receptor function for TuIb. The OmpC protein was purified from E. coli WA4, a TuIa-resistant mutant of E. coli W4626phe (16), as described previously (11). The protein thus purified exists as a trimer (17). LPS was prepared from E. coli W4626phe according to the method of Galanos et al. (5). The reconstitution of an ordered hexagonal lattice structure from OmpC and LPS was carried out with various LPS/OmpC ratios as described previously (14, 15). Receptor activity for TuIb of the reconstituted samples was determined as the ability to inactivate the phage. The method was essentially the same as that for T4 (10). Briefly, the reconstituted sample (5 μg of OmpC) was incubated with 1 × 10⁵ to 1.5 × 10⁵ plaque-forming units of TuIb at 37°C in 1 ml of an adsorption buffer supplemented with 50 μg of L-tryptophan. After different incubation periods, samples were diluted 50-fold with the adsorption buffer, 0.1 ml portions were plated with indicator bacteria (E. coli YA21), and the pseudo-first-order rate constant (K) for the phage inactivation was determined. The abscissa represents the molar ratio of LPS to OmpC trimer.*

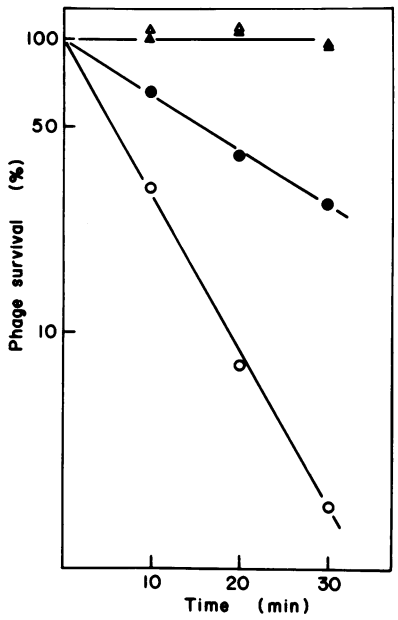


FIG. 2. *Receptor activity of samples reconstituted from OmpC and different LPS derivatives. The reconstitution was carried out from OmpC and LPS derivatives. Lipid A was prepared from LPS of E. coli YA21 by acetic acid hydrolysis as described previously (6), and the heptoseless LPS was prepared from E. coli YA21-6 (10) by the method of Galanos et al. (5). The molar ratio of LPS derivatives to OmpC trimer was 30:1. Three β-hydroxymyristic acid molecules (a product of Tokyo Kasei Kogyo) were regarded as being equivalent to one LPS molecule. Phage inactivation by the reconstituted samples was determined as described in the legend to Fig. 1. The LPS derivatives used were: △, β-hydroxymyristic acid; ▲, lipid A; ●, heptoseless LPS; and ○, wild-type LPS.*

Upon mixing with a vesicle reconstituted with OmpC and LPS, almost all of the phages were found on the vesicle surface with a contracted tail sheath. However, the phages were preferably adsorbed on larger vesicles, smaller vesicles being almost free from phages. The results are consistent with a previous finding for phage T4 that the phages require a flat surface which is large enough to interact with several long tail fibers of a single phage particle (4). Figure 3 also shows that phage heads were still filled with an electron-dense substance, indicating that DNA ejection did not take place.

Table 1 is a summary of the phages having the T-even structure and the receptor components of *E. coli* K-12 which are recognized by them. In all cases except T6, two components were found to constitute individual receptors. One is an outer membrane protein which is specific for individual phages, and the other is LPS which is common to them all. Although only the Tsx protein is known as a receptor component for T6, involvement of LPS in the receptor function can not be ruled out (9). In the intial stage of infection, the T-even type phages interact with the host cell surface first with long tail fibers and then with short tail fibers. Evidence has accumulated that in T-even phages long tail fibers seem to play an important role in the specific attachment of the phage to sensitive

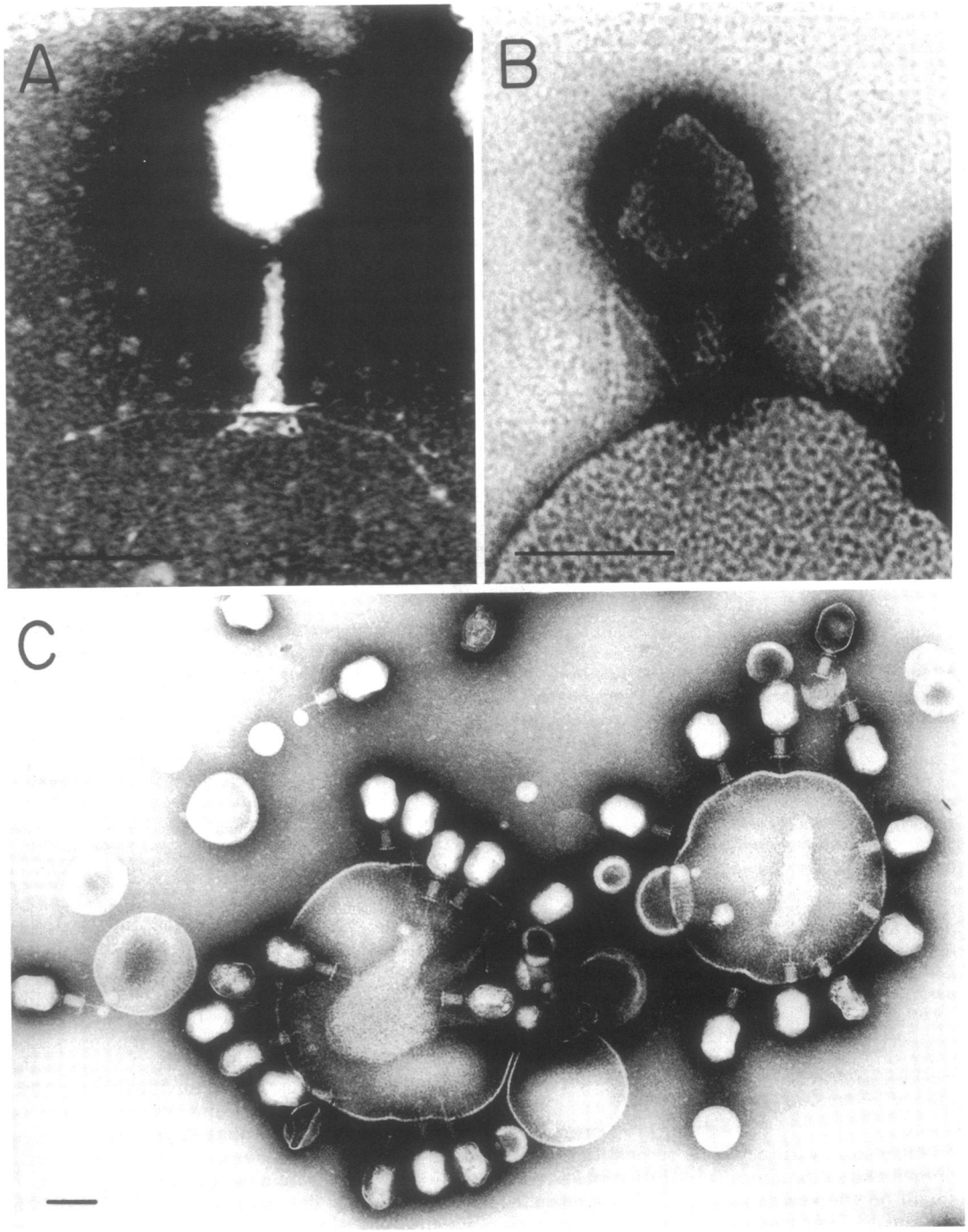


FIG. 3. *Electron microscopic observation of TuIb adsorbed on reconstituted OmpC-LPS vesicles. The molar ratio of LPS to OmpC trimer in the reconstitution mixture was 30:1. Reconstituted samples (5 μg of OmpC) were incubated with 1 × 10⁶ to 5 × 10⁶ phaque-forming units of TuIb at 37°C for 10 min in 50 μl of adsorption buffer (0.5 mM $MgSO_4$) supplemented with 2.5 μg of tryptophan, negatively stained with 1% sodium phosphotungstate (pH 6.2), and examined under a Hitachi HS9 electron microscope (B and C). (A) TuIb alone. Bars, 100 nm.*

bacteria (1, 2). Therefore, one can speculate that individual proteins listed in Table 1 may be involved in the specific interaction with the long tail fibers, whereas LPS, which is the component common to all of the receptors, may act as a receptor for the short tail fibers. However, this does not exclude the involvement of LPS in the interaction with the long tail fibers. In *E. coli* B the initial stage of T4 infection takes place on the LPS micelle, indicating that LPS can be a receptor for both short and long tail fibers (13). In addition to the possible role of LPS as a receptor component for short tail fibers, the results of experiments presented here with TuIb suggest that LPS also plays two possible roles in the interaction with the long tail fibers. One is to enable OmpC to form a flat sheet which is large enough to interact with several long tail fibers. The fatty acid region is an essential component for this role (15). The other role is to constitute a part of the receptor for the tail fibers. The polysaccharide region is assumed to be important for this either through a direct interaction with the fibers or through an interaction with OmpC. Thus, LPS may play a trial role in the receptor function for TuIb.

We thank U. Henning for the TuIb.

This work was supported by grants from the Ministry of Education, Science and Culture of Japan and the Toray Science Foundation.

## LITERATURE CITED

1. **Beckendorf, S. K., J. S. Kim, and I. Lielausis.** 1973. Structure of bacteriophage T4 genes 37 and 38. J. Mol. Biol. **73**:17-35.
2. **Crawford, J. T., and E. B. Goldberg.** 1980. The function of tail fibers in triggering base plate expansion of bacteriophage T4. J. Mol. Biol. **139**:679-690.
3. **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.
4. **Furukawa, H., H. Yamada, and S. Mizushima.** 1979. Interaction of bacteriophage T4 with reconstituted cell envelopes of *Escherichia coli* K-12. *J. Bacteriol.* **140**: 1071-1080.
5. **Galanos, C., O. Lüderitz, and O. Westphal.** 1967. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. **9**:245-249.
6. **Galanos, C., E. T. Rietschel, O. Lüderitz, and O. Westphal.** 1971. Interaction of lipopolysaccharides and lipid A with complement. Eur. J. Biochem. **19**:143-152.
7. **Hantke, K.** 1978. Major outer membrane proteins of *E. coli* K12 serve as receptors for the phages T2 (protein Ia) and 434 (protein Ib). Mol. Gen. Genet. **164**:131-135.
8. **Henning, U., and K. Jann.** 1979. Two-component nature of bacteriophage T4 receptor activity in *Escherichia coli* K12. J. Bacteriol. **137**:664-666.
9. **Manning, P. A., and P. Reeves.** 1978. Outer membrane proteins of *Escherichia coli* K-12: isolation of a common receptor for bacteriophage T6 and colicin K. Mol. Gen. Genet. **158**:279-286.
10. **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.
11. **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.
12. **Schwartz, M.** 1980. Interaction of phages with their receptor proteins, p. 59-94. *In* L. L. Randall and L. Philipson (ed.), Virus receptors part 1, bacterial viruses. Chapman and Hall, London.
13. **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.
14. **Yamada, H., and S. Mizushima.** 1978. Reconstitution of and ordered structure from major outer membrane constituents and the lipoprotein-bearing peptidoglycan sacculus of *Escherichia coli*. J. Bacteriol. **135**:1024-1031.
15. **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.
16. **Yamagata, H., and H. Uchida.** 1972. Chromosomal mutations affecting the stability of sex-factors in *Escherichia coli*. J. Mol. Biol. **63**:281-294.
17. **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.