Polymannose O-Antigens of *Escherichia coli*, the Binding Sites for the Reversible Adsorption of Bacteriophage T5$^+$ via the L-Shaped Tail Fibers

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A study of the adsorption kinetics of T5$^+$ and the tail fiber-less mutant hd-2 to lipopolysaccharides of various *Escherichia coli* strains demonstrated T5$^+$ binding to the O-antigen of the O8 and O9 types. Incorporation of radioactive mannose into the phosphomannose isomerase-deficient strain *E. coli* F860 O9 *pmi* allowed the derivation of the number of O-antigens per cell required to increase T5 adsorption. With more than 500 O-antigen molecules, acceleration of T5$^+$ adsorption was observed. The highest adsorption rate was obtained when nearly all lipopolysaccharide molecules were substituted with a polymannose O-antigen. Inhibition studies with purified components of an enzymatically degraded lipopolysaccharide of the O8 type showed that among the mannosides tested the smallest unit, the trimannoside, was the strongest inhibitor of T5$^+$ binding. We conclude that the reversible preadsorption to the O8 and O9 polymannose antigens increases the rate of infection via the cellular receptor protein encoded by the *fhuA* (formerly *tonA*) gene.

For infection of *Escherichia coli*, bacteriophage T5 requires the protein in the outer membrane encoded by the *fhuA* (formerly *tonA*) gene (1, 2, 11). Binding of the phage to the receptor protein is irreversible and leads to the release of DNA from the phage (24). In addition to this irreversible interaction, phage T5$^+$ shows reversible binding to the lipopolysaccharide (LPS) of its host *E. coli* F (8). This binding is mediated by the L-shaped tail fibers (20), and it accelerates the adsorption by a factor of 15 (8).

In this communication we report that the L-shaped tail fibers bind to the O-antigenic side chain of LPS, a polymannose, and we present evidence that the binding site of the tail fibers resides in a trimannoside.

MATERIALS AND METHODS

Bacterial strains, phage, media, and growth conditions. Bacterial strains used are listed in Table 1. The bacteria were grown in tryptone-yeast extract medium as described previously (8). For studies of mannose incorporation into LPS, M9 medium (1) supplemented with glycerol and histidine was used.

Phages T5$^+$ and hd-2 were kindly supplied by K. Saigo (20). Throughout the experiments, heat-stable mutants (9) of these phages were used. Phage Ω8 was a gift from K. Jann. Phages were routinely purified on CsCl gradients.

LPS analyses. LPS from *E. coli* F and phage C21-resistant mutants were isolated by the method of Galanos et al. (6). Gas chromatographic analyses were carried out as described by U. Feige (thesis, Universität Freiburg, Freiburg, Germany, 1977).

LPS from *E. coli* F492 was extracted and purified as described by Westphal and Jann (23).

Hydrolysis of O8 LPS by phage Ω8. The procedure of Reske et al. (19) was applied for the hydrolysis of O8 LPS by phage Ω8, with the exception that the time of incubation of the phage with LPS was extended to 36 h. The oligomannosides obtained from the supernatant after centrifugation of the phage and the residual LPS were purified on a Bio-Gel P-2 column (95 by 1.6 cm) with bidistilled water as the eluent. The mannosides were identified by thin-layer chromatography (22). The hexa- and trimannosides were obtained from the column in pure form, whereas the nonamannoside contained about 10% impurities of high-molecular-weight mannosides.

Sugar analyses of the mannoside preparations were performed after hydrolysis in 0.1 N HCl for 48 h at 105°C on a Biotronic sugar analyzer ZA 5100. Amino acids were determined after hydrolysis of 200 nmol (based on mannose) of each of the preparations with bidistilled 6 N HCl under nitrogen for 18 h at 105°C, using a Biotronic amino acid analyzer LC 6000 E.

Determination of the number of O-antigens per bacterial cell. To growing cells of *E. coli* F860 in M9-glycerol medium, [^{14}C]mannose was added. After incubation at 37°C, the optical density was measured and the incorporated radioactivity was determined after precipitation of the cells in 5% trichloroacetic acid at 0°C (17). Since in this strain the mannose is exclusively incorporated into the O-antigen, the number of mannose residues incorporated per cell can be calculated from the total incorporated radioactivity. This value divided by 60 (the average number of mannose residues per O-antigen [7, 15]) gives the number of LPS molecules bearing O-antigens per cell. This calculation relies on the observation that *E. coli*

TABLE 1. Strains of *E. coli* used

Strain(s)	Properties	Source/reference
F	O9[a]; wild type	11
F/5	*fhuA*	8
F/21-1, F/21-3, F/21-4	C21 resistant	This study
F492[b]	O8:K27⁻:H⁻ *his fhuA*	19
F719[b]	O9:K31⁻:H⁻ *his*	K. Jann
O9/5	As F719, but *fhuA*	This study
F860[b]	O9:K29⁻:H⁻ *his pmi*	10

[a] *E. coli* F cells are readily agglutinated with monospecific O9 antiserum at the same concentrations that *E. coli* O9 cells are agglutinated. No agglutination occurs with O8 antiserum. Preadsorption of the O9 antiserum with *E. coli* F results in loss of agglutination of O9 cells, whereas preadsorption with *E. coli* F/21-1 does not. Alkali-treated LPS isolated from *E. coli* F, in contrast to alkali-treated O9 LPS, does not show any reaction when tested by passive hemagglutination.

[b] Strains were obtained from B. Jann and K. Jann, Max Planck Institut für Immunbiologie, Freiburg.

O9 (K29⁻ as well as K30⁻) synthesizes complete O-antigenic side chains of nearly uniform length (7, 15).

Inhibition of phage adsorption by oligomannosides. The lyophilized mannosides were dissolved in M9 buffer just before the experiment was started, since storage at 4°C or freezing of the solutions resulted in a loss of biological activity. A total of 10^7 phage particles per ml were incubated with the mannosides. After 30 min at 0°C, 3×10^8 *E. coli* F cells per ml were added and the incubation was continued for another 10 min at 0°C. The incubation mixture was then diluted 500-fold into ice-cold phosphate-buffered saline and centrifuged. Remaining phage particles in the supernatant were plated on *E. coli* F.

When hd-2 was tested, the concentration of bacteria was raised to 1.5×10^9 per ml and the time of adsorption was prolonged up to 30 min.

RESULTS

E. coli F mutants with altered LPS were isolated by selecting for strains resistant to phage C21, which requires rough LPS for adsorption (13). The resistant strains were tested for adsorption of T5⁺. Strains F/21-1, F/21-3, and F/21-4 showed different adsorption of T5⁺ (Fig. 1). From these strains and from the parental strain, the sugar composition of the LPS was determined (Table 2). All LPS preparations contained heptose, galactose, glucose, and mannose. The first three sugars are common constituents of *E. coli* LPS cores. Mannose is found exclusively in O-antigenic side chains (14). (The exact structure of *E. coli* F LPS is still under investigation.) Hence, the amount of mannose in the LPS preparations reflects the amount of O-antigenic material on the surface of the bacteria.

The adsorption rate of T5⁺ increased with the number of O-antigens on the surface of the different F strains, whereas the adsorption rate of the fiberless phage hd-2 decreased (Fig. 1).

To confirm the finding that T5⁺ binds to the O-antigen of *E. coli* F, we tested *E. coli* strains with known O-antigens for T5 adsorption. *E. coli* F492 and O9/5 synthesize O-antigens with mannose as the sole sugar constituent (15, 18).

They showed binding of T5⁺ although they lacked the *fhuA*-coded receptor protein. No binding of hd-2 could be observed (Fig. 2). As already described for *E. coli* F/5 *fhuA* (8), binding to *E. coli* O9/5 *fhuA* and F492 was irreversible at 0°C and reversible at 32°C (data not shown).

A phosphomannose isomerase-deficient mutant of *E. coli*, F860 *pmi*, was used to vary the mannose content. This *pmi* strain is unable to synthesize O-antigen unless mannose is added to the growth medium. Upon addition of mannose, the strain immediately starts to synthesize O-antigen. Synthesis was accompanied by an increase in the adsorption of T5⁺ and a decrease in
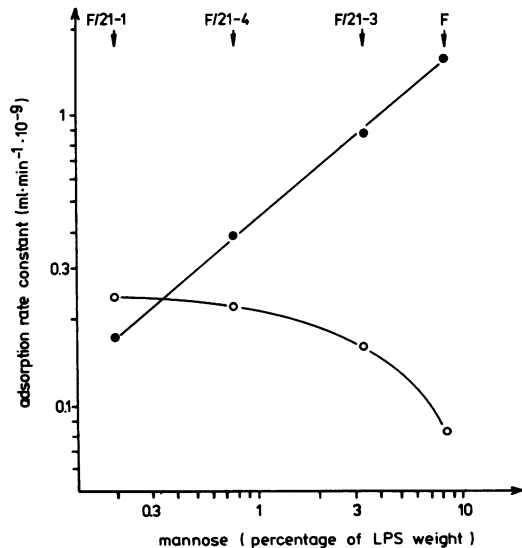

FIG. 1. Adsorption of T5⁺ (●) and hd-2 (○) to *E. coli* F and LPS mutants. The adsorption rate constants were determined as described previously (8). The values for the amounts of mannose were taken from Table 2 and are indicated by arrows for the corresponding bacterial strains.

TABLE 2. Sugar composition of LPS prepared from E. coli F and phage C21-resistant mutants

Strain	% of dry wt			
	Heptose	Glucose	Galactose	Mannose
F	8.9	9.9	5.3	9.0
F/21-1	10.1	6.3	6.7	0.2
F/21-3	10.2	7.2	3.2	3.6
F/21-4	10.8	6.9	3.3	0.8

the adsorption of hd-2 (Fig. 3a). Less than 1 μM mannose enhanced the adsorption of T5$^+$, whereas higher concentrations were needed to inhibit hd-2 adsorption (Fig. 3b).

By use of radiolabeled mannose, the number of O-antigens on the cell surface was determined and compared with the adsorption rate of T5$^+$. Adsorption started to increase at about 500 O-antigens per cell. Maximal adsorption occurred when nearly all (about 10^6) LPS molecules were substituted with an O-antigen (Fig. 4).

To define the binding site of T5$^+$ within the O-antigen, isolated LPS of E. coli F492 was hydrolyzed with phage Ω8. This phage is known to cleave O8 LPS into tri-, hexa-, nona-, and dodecamannosides (19). Purified preparations of these oligomannosides were used to inhibit the adsorption of T5$^+$ to E. coli F. Contaminants were negligible, since analyses of the mannoside preparations indicated no amino sugars, no ribose or deoxyribose, and only some amino acids in the range of 0.2 to 1.0 nmol, when 200 nmol of mannose was applied. The content of additional sugars was low; thus, the preparations used consisted of 90 to 97% mannose. The experiment was performed at 0°C. At this temperature T5$^+$ binds irreversibly to LPS, whereas at higher temperatures binding becomes reversible (8). To monitor nonspecific binding, we tested hd-2 under the same conditions. The term nonspecific is used, since the hd-2 phage mutant, lacking the L-shaped tail fibers, does not bind to E. coli fhuA mutants which contain polymannose O-antigens (Fig. 2) or to complete LPS (8). The trimannoside was the strongest inhibitor of T5$^+$ adsorption, even when equimolar amounts of the mannosides were applied (Table 3). In the case of nonamannoside, little specific inhibition of adsorption was observed. Phage hd-2 was inhibited to nearly the same extent as T5$^+$. In the series of mannosides tested, specific inhibition of T5$^+$ increased with decreasing chain length, whereas nonspecific inhibition increased with the length of the mannosides. Impurities of the preparations do not seem to be responsible for nonspecific inhibition. Mannose and α-methylmannose did not show any inhibition, even when applied at concentrations as high as 50 mg/ml (data not shown).

There is no evidence for hydrolytic activity of the tail fibers of T5. Incubation of E. coli F860 pmi cells, radiolabeled with [^{14}C]mannose, or of LPS isolated from these cells with T5 under conditions similar to those described for Ω8 (17, 19) did not result in a detectable liberation of radioactive, soluble compounds from the cells or the LPS (data not shown).

DISCUSSION

In this paper we showed that bacteriophage T5$^+$ binds to the O-antigens of E. coli F and to E. coli strains of the O8 and O9 LPS types. Binding was demonstrated by three different approaches. (i) The adsorption rate of T5$^+$ to LPS mutants of E. coli F decreased with decreasing amounts of complete (smooth) LPS in the mutant strains. (ii) Accelerated adsorption of T5$^+$ to a pmi mutant of E. coli O9 occurred only under conditions in which O-antigen was synthesized. (iii) Accelerated adsorption of T5$^+$ was inhibited by enzymatically cleaved subunits of O8 O-antigen. With the third approach it was possible to show that the binding site of the L-shaped tail fibers on the O-antigen resided in a sugar sequence of three mannoses, since the trimannoside was the strongest inhibitor of the accelerated adsorption. Further evidence came from the fact that T5$^+$ adsorbed to O8 as well as
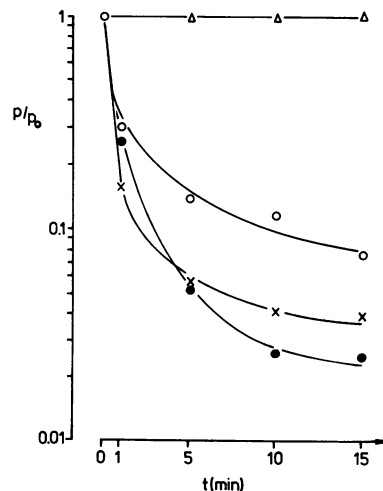

FIG. 2. Binding of T5$^+$ and hd-2 to different E. coli strains lacking the fhuA-coded receptor protein. A total of 7 × 10^7 cells were incubated with 2 × 10^5 phages at 0°C in 0.1 ml of M9 buffer. Binding was terminated by a 500-fold dilution into ice-cold phosphate-buffered saline. After centrifugation, the number of unbound phage was determined by titration on E. coli F. Binding of T5$^+$ to E. coli F/5 (○), F492 (O8) (×), and O9/5 (●) and of hd-2 (△) (binding was same to each of the three strains) was determined.
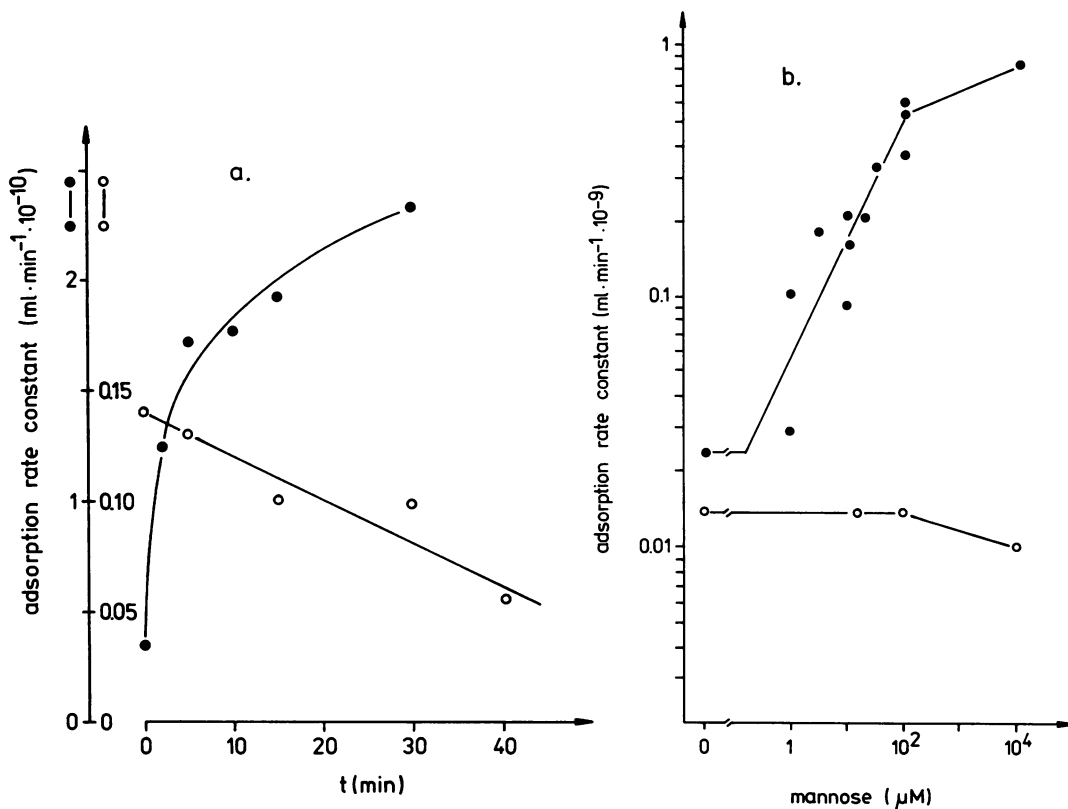
FIG. 3. Effect of O-antigen synthesis on the adsorption of T5$^+$ and hd-2 to *E. coli* F860 *pmi*. (a) To a growing culture of *E. coli* F860 *pmi* in M9–minimal-glycerol medium (optical density, 0.3), mannose was added at zero time. At the times indicated, samples were taken, immediately chilled to 0°C, and washed three times with ice-cold M9 buffer. The adsorption rate constants were determined as previously described (8). For T5$^+$ and hd-2 adsorption, the cells had been exposed to 20 μM (●) and 10mM (○) mannose, respectively. (b) In this experiment, growing cells were incubated with different mannose concentrations for 15 min. Treatment of the cells thereafter and adsorption of T5$^+$ (●) and hd-2 (○) were as described in the legend to (a).

to O9 O-antigen. The structures of both antigens are shown in Fig. 5. Although the structures are similar, the shape of the polysaccharide chain seems to be different because (i) there is no serological cross-reaction between the two antigens (10) and (ii) phage Ω8 does not cleave the O9 O-antigen (19). If the proper binding site for the L-shaped tail fibers of T5$^+$ is comprised of a sequence of more than three sugars, the tail fibers should bind to either O8 or O9 O-antigen but not to both.

An attempt to cleave the trimannoside by acid hydrolysis and to show binding activity of the dimannoside failed since even heating the trimannoside without detectable hydrolysis completely destroyed binding activity. Conformational changes may destroy the binding site.

For most LPS-specific phages, the receptor site consists of only a short sugar sequence (13). An essential constituent of the receptor for phage T4 in the LPS of *E. coli* B is a terminal glucose residue of the core (16). Binding is inhibited by certain mono- and disaccharides (5). Phages that recognize longer sequences are those which hydrolyze the LPS during adsorption.

According to Lindberg (13), those phages that bind to surface carbohydrates extending from the cell, such as exopolysaccharides or O-antigens, first must cleave these polysaccharides before they can bind to the cell surface. A two-step adsorption is also claimed for T4 and related phages such as C21, P1, and F0 (13). After reversible binding of the long tail fibers to a first receptor, anchoring of the baseplate to a second receptor on the cell surface is mediated by the short tail fibers. The first receptor for all of these phages has been identified in the core region of the LPS molecule. The presumed second receptor is unknown.

Among those phages interacting with LPS, phage T5 to our knowledge is the first phage for
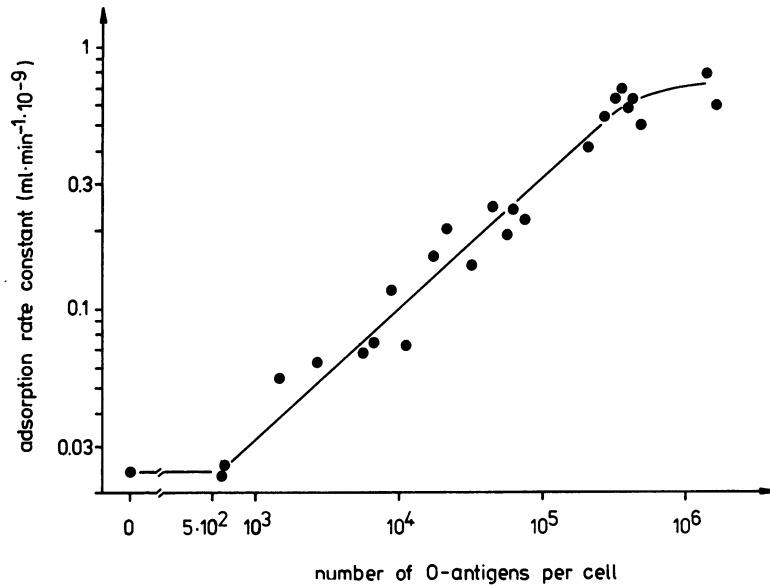
FIG. 4. Effect of the number of O-antigens per cell on the adsorption of T5+. Growing cells of *E. coli* F860 *pmi* were labeled with [14C]mannose. For each measuring point, the samples were divided. One sample was used for the determination of the number of O-antigens, and the other was used for the determination of adsorption rate constants at 0°C, as previously described (8).

which a two-step adsorption can be attributed to two different identified receptors. The first receptor is the O-antigen of *E. coli* F (this study), and the second receptor is the *fhuA*-coded protein in the outer membrane (1, 2, 11). T5 adsorption differs from that of the above-mentioned phages, since loss of the first receptor does not lead to T5 resistance. The decreased adsorption rate of the fiberless T5 mutant hd-2 to the

TABLE 3. Inhibition of phage T5+ and hd-2 adsorption by isolated mannosides

Mannoside (mM)	mg/ml	% Inhibition	
		T5+	hd-2
Tri-			
1.02	5.0	65	2
0.31	1.5	45	0
0.10	0.5	32	0
0.03	0.15	12	ND[a]
Hexa-			
0.50	5.0	29	6
0.15	1.5	18	2
0.05	0.5	13	0
0.015	0.15	6	ND
Nona-			
0.33	5.0	25	19
0.10	1.5	13	3
0.03	0.5	1	3
0.01	0.15	0	ND

[a] ND, Not determined.

polymannose-containing strains is probably caused by steric hindrance.

Loss of infectivity due to a lack of the first receptor appears to be quite well understood for bacteriophage T4. According to a model of Crowther et al. (4), binding of some of the long tail fibers to the first receptor induces a "cocked" state in the baseplate. As soon as the short tail fibers bind to the second receptor, the cocked state is discharged, resulting in the hexagon-star transition, tail contraction, and DNA injection. In the absence of the first receptor, binding of the short tail fibers does not lead to infection because the baseplate has not been cocked and is therefore not discharged. Proof for this model came from T4 mutants producing infective fiberless particles (3). These particles, lacking the long tail fibers, have mutations in some of the baseplate genes which allow these particles to infect not only sensitive bacteria but also bacteria resistant to wild-type T4 phage.

The complicated mechanism of T4 adsorption makes it likely that some of the steps in this sequence of events of cocking, discharging, and triggering are control steps preventing unsuccessful triggering of DNA release.

Compared with T4, the adsorption of T5 appears to be rather simple. Since neither loss of the tail fibers nor lack of the appropriate O-antigen of the host has any effect on the plating efficiency of T5, it seems unlikely that the two-step adsorption of T5+ involves control elements as suggested for T4. The most probable

O8-antigen $\left[\begin{array}{c} \underline{3} \ \text{Man} \ \xrightarrow[\alpha]{1,2} \ \text{Man} \ \xrightarrow[\alpha]{1,2} \ \text{Man} \ \xrightarrow{1}_{\alpha} \end{array}\right]_{n \approx 20}$

O9-antigen $\left[\begin{array}{c} \underline{3} \ \text{Man} \ \xrightarrow[\alpha]{1,3} \ \text{Man} \ \xrightarrow[\alpha]{1,2} \ \text{Man} \ \xrightarrow[\alpha]{1,2} \ \text{Man} \ \xrightarrow[\alpha]{1,2} \ \text{Man} \ \xrightarrow{1}_{\alpha} \end{array}\right]_{n=12-14}$
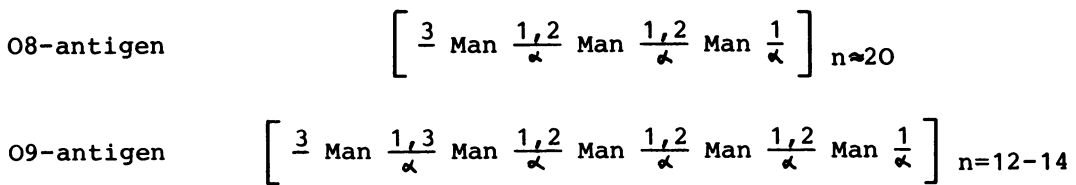
FIG. 5. Structures of the O-antigens of *E. coli* O8 and O9 LPS. The structure for O8 LPS was described by Reske and Jann (18), and that for O9 was described by Prehm et al. (15).

interpretation is that the interaction of the tail fibers with the *E. coli* F O-antigen accelerates the adsorption of T5$^+$ to the cell and keeps the phage at the surface of the host for a considerably longer time than T5 phage lacking the fibers. Binding to the *fhuA*-coded receptor protein becomes more likely the longer the phage remains near the surface (21). It is also important that the preadsorption to the polymannose is a reversible process so that the phage can move along the cell surface. Efficient surface attachment occurs when more than one of the three tail fibers binds to an O-antigen at the same time. This is possible when the mean distance between O-antigens corresponds to the distance between the tips of the fibers. If the latter distance is assumed to be 100 nm and the cell surface is assumed to be 5 μm^2, then about 700 evenly distributed O-antigens on the surface are needed for the mean distance between them to be 100 nm. This estimate is consistent with the results shown in Fig. 4.

LITERATURE CITED

1. **Braun, V., K. Schaller, and H. Wolff.** 1973. A common receptor protein for phage T5 and colicin M in the outer membrane of *Escherichia coli* B. Biochim. Biophys. Acta 323:87–97.
2. **Braun, V., and H. Wolff.** 1973. Characterization of the receptor protein for phage T5 and colicin M in the outer membrane of *E. coli* B. FEBS Lett. 31:77–80.
3. **Crowther, R. A.** 1980. Mutants of bacteriophage T4 that produce infective fiber-less particles. J. Mol. Biol. 137:159–174.
4. **Crowther, R. A., E. V. Lenk, Y. Kikuchi, and J. King.** 1977. Molecular reorganization in the hexagon to star transition of the baseplate of bacteriophage T4. J. Mol. Biol. 116:489–523.
5. **Dawes, J.** 1975. Characterization of the bacteriophage T4 receptor site. Nature (London) 256:127–128.
6. **Galanos, C., O. Lüderitz, and O. Westphal.** 1969. A new method for the extraction of R lipopolysaccharides. Eur. J. Biochem. 9:245–249.

7. **Goldemann, G., S. Kanegasaki, and K. Jann.** 1979. A common intermediate in the biosynthesis of the O8 and O9 antigens of *Escherichia coli*. FEMS Microbiol. Lett. 5:443–445.
8. **Heller, K., and V. Braun.** 1979. Accelerated adsorption of bacteriophage T5 to *Escherichia coli* F, resulting from reversible tail fiber-lipopolysaccharide binding. J. Bacteriol. 139:32–38.
9. **Hertel, R., L. Marchi, and K. Müller.** 1962. Density mutants of phage T5. Virology 18:576–581.
10. **Jann, K., B. Jann, V. Winter, and C. Wolf-Ullisch.** 1979. On the serological specificity of the *Escherichia coli* O8 and O9 antigens. J. Gen. Microbiol. 110:203–210.
11. **Kadner, R. J., K. Heller, J. W. Coulton, and V. Braun.** 1980. Genetic control of hydroxamate-mediated iron uptake in *Escherichia coli*. J. Bacteriol. 143:256–264.
12. **Lanni, Y. T.** 1958. Lysis inhibition with a mutant of bacteriophage T5. Virology 5:481–501.
13. **Lindberg, A. A.** 1977. Bacterial surface carbohydrates and bacteriophage adsorption, p. 289–356. *In* I. W. Sutherland (ed.), Surface carbohydrates of the prokaryotic cell. Academic Press, Inc., New York.
14. **Ørskow, I., F. Ørskov, B. Jann, and K. Jann.** 1977. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev. 41:667–710.
15. **Prehm, P., B. Jann, and K. Jann.** 1976. The O9 antigen of *Escherichia coli*: structure of the polysaccharide chain. Eur. J. Biochem. 67:53–56.
16. **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.
17. **Prehm, P., and K. Jann.** 1976. Enzymatic action of coliphage Ω8 and its possible role in infection. J. Virol. 19:940–949.
18. **Reske, K., and K. Jann.** 1972. The O8 antigen of *Escherichia coli*. Structure of the polysaccharide chain. Eur. J. Biochem. 31:320–328.
19. **Reske, K., B. Wallenfels, and K. Jann.** 1973. Enzymatic degradation of O-antigenic lipopolysaccharide by coliphage Omega 8. Eur. J. Biochem. 36:167–171.
20. **Saigo, K.** 1978. Isolation of high-density mutants and identification of non-essential structure proteins in bacteriophage T5: dispensability of L-shaped tail fibres and a secondary major head protein. Virology 85:422–433.
21. **Schwartz, M.** 1976. The adsorption of coliphage lambda to its host: effect of variations in the surface density of receptor and in phage-receptor affinity. J. Mol. Biol. 103:521–536.
22. **Wandersman, C., M. Schwartz, and T. Ferenci.** 1979. *Escherichia coli* mutants impaired in maltodextrin transport. J. Bacteriol. 140:1–13.
23. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharide extraction with phenol-water and further application of the procedure. Methods Carbohydr. Chem. 5:83–91.
24. **Zarnitz, M. L., and W. Weidel.** 1963. Uber die Rezeptorsubstanz für den Phagen T5. VI. Die Thermodynamik der Kontaktbildung zwischen Phage und Rezeptor sowie deren mögliche Bedeutung als morphogenetischer Modellmechanismus. Z. Naturforsch. Teil B 18:276–280.