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O Antigen-Dependent Mutant of Bacteriophage T5

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A T5 mutant is described which showed normal infection of *Escherichia coli* F but virtually no infection of cells lacking the *E. coli* F O antigen. This was due to very poor adsorption to the O antigen-deficient cells. Inactivation kinetics with anti-T5 serum and adsorption and desorption kinetics to receptor-deficient *E. coli* F cells suggested that the mutation did not affect the L-shaped tail fibers which mediate binding to the O antigen. Proof was obtained from genetic data; the structural gene for the L-shaped tail fibers mapped at a different position on the T5 chromosome than did the mutated gene. Since binding of the T5 mutant to the FhuA receptor protein was strongly inhibited by ferrichrome and since the mutation could not be crossed into phage BF23, we conclude that the mutation affects the receptor binding protein of the T5 tail.

The receptor protein for bacteriophage T5 in the outer membrane of *Escherichia coli* is encoded by the *fhuA* (formerly *tonA*) gene (4, 5, 12). Binding to the FhuA protein is irreversible and leads to the injection of phage DNA. *E. coli* cells which lack the FhuA protein are resistant to phage T5.

On some *E. coli* strains, T5 shows a reversible binding to the O antigen of the lipopolysaccharide (LPS) before the interaction with FhuA (8, 9). These *E. coli* strains which are of the O8 and O9 serotype (9) contain polymannos O antigens (17). Binding of T5 to a short sequence of mannosyl residues within the O antigens is mediated by the L-shaped tail fibers (LTF) (9). Binding is irreversible at 0°C, whereas it becomes reversible at temperatures above 8°C (8, 9). At 32°C the interaction between LTF and O antigen accelerates adsorption of T5 by a factor of 15 (8, 9). The loss of LTF by mutation or lack of the appropriate O antigen on the cell surface only affects the adsorption rate, whereas the final number of phage bound and the number and size of the phage plaques remain almost unchanged.

In this communication we described a T5 mutant which is dependent on the polymannose O antigen of *E. coli* F, and we present evidence that the mutation is located in the gene encoding the receptor-binding protein of the T5 tail. The mutation will be called *oad* (*O a*ntigen *d*ependence).

MATERIALS AND METHODS

Bacterial strains, phages, media, and growth conditions. Bacteria and phages are listed in Table 1. Bacteria were grown in tryptone-yeast extract medium as previously described (8). The medium was supplemented with 1 mM $CaCl_2$ for phage propagation. Phages were routinely purified on CsCl gradients (22).

Autoradiography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Radiolabeled, infected cells were prepared as described previously (22). The radioactive amino acids were added 20 min after infection, and the cells were collected 30 min after infection. For separation of proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the system described by Lugtenberg et al. (14) was applied.

Two-factor crosses. Two-factor crosses were carried out in liquid culture at a cell concentration of 2×10^8/ml and an input of each phage at a multiplicity of infection (MOI) of 5 (10). In those experiments in which phenotypic mixing occurred (i.e., crosses between T5 and BF23, crosses with T5*oad*, and crosses between LTF^+ and LTF^- phages) samples of the lysates were incubated with *E. coli* F (10^9 cells/ml) at an MOI of 0.1. After 15 min at 37°C, the incubation mixtures were diluted 100-fold and plated together with the indicator bacteria. *E. coli* $Fsu\beta^+$ was commonly used for determination of the total number of phages.

Recombinant phages were characterized by picking single plaques and testing for phenotypic properties. *Oad* phages were detected by their poor plating efficiency on *E. coli* F/21-1. LTF^- phages were characterized by their different adsorption rates to *E. coli* F and F/21-1 (9). Amber defects were tested by complementation (10).

Phage inactivation by antiserum. Phages (10^7/ml) were mixed with an equal volume of rabbit anti-T5 serum. After incubation at 37°C for 1 h, the incubation mix was diluted 100-fold. A 100-μl amount was plated, with *E. coli* F as the indicator.

Antibody blotting. Antibody blotting was performed as follows (21). Phage proteins were separated by SDS-PAGE and transferred electrophoretically onto a nitrocellulose membrane. Anti-T5 serum (diluted 100-fold) was added. After incubation for 3 h at room temperature, surplus antibodies were removed by several washings with phosphate-buffered saline (PBS) plus 0.1% Triton X-100. Antibodies bound to phage proteins on the membrane were labeled with peroxidase coupled to *Staphylococcus aureus* protein A. As a substrate for peroxidase, 3-amino-9-ethylcarbazole was used (C. Komischke, personal communication), which forms an insoluble, colored complex on cleavage by peroxidase.

Phage adsorption and inhibition of adsorption. Phage adsorption was carried out as previously described (8). To test for inhibition by ferrichrome, 10 μl of phages (2×10^7/ml) was mixed with 10 μl of ferrichrome solution. Adsorption was started by the addition of 80 μl of *E. coli* F cells, which had been washed twice and then suspended in PBS at a cell density of 6×10^8/ml. After 10 min at 37°C, 10 μl of the incubation mix was diluted 500-fold and kept for a further 15 min at 37°C to allow desorption of phages not bound to the receptor (8). Adsorption kinetics were plotted as the number of unadsorbed phages (p_t) per input phage numbers (p_o) versus the time of adsorption.

Isolation and purification of phage tails. The procedure

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TABLE 1. Bacteria and phages used

Strains	Relevant properties	Source and reference
E. coli		
F	Wild type	13
Fsuβ$^+$	As F, but permissive host for amber mutants	10
F/21-1, -3, -4	Phage C21 resistant	9
F/5	*fhuA*	8
Phages		
T5*st*[a]	Wild type	Laboratory collection
T5*oad*	O antigen dependent	This study
T5*hd*-1, -2	LTF$^-$	18
Bf23$^+$	Wild type	Laboratory collection
Amber phages[b]		
T5*amN*4	*amN*4, O antigen dependent	22, this study
T5*amN4*-2	*amN*4	This study
T5*stamN*5[a]	*amN*5	22
T5*D*16*am*34a	*amD*16	10, 23
T5*D*17*am*34d	*amD*17	10, 23
T5*D*18*am*5	*amD*18	10, 23
T5*hd*-1*am*D18	*amD*18, LTF$^-$	This study
T5*oadamN*5	*amN*5, O antigen dependent	This study
BF23*amN*4[c]	*amN*4	This study
BF23*amN*5[c]	*amN*5	This study

[a] *st* denotes heat-stable phages (11) which contain an uncharacterized deletion in the deletable region of the genome (15).

[b] Amber mutation *N*5 affects a head protein (22); all other amber defects affect tail proteins (22, 23).

[c] Hybrid phages of T5 and BF23. The respective amber mutations of T5 were crossed into BF23$^+$.

described by Zweig and Cummings (22) was followed, with the exception that we used a sucrose step gradient (5% steps from 15 to 55%) in PBS instead of the glycerol-D_2O gradient.

Two-dimensional gel electrophoresis. The method of O'Farrell was applied (16) as modified by Ames and Nikaido (1).

RESULTS

Detection and characterization of the *oad* mutation. When preparing radiolabeled, infected strain F/21-1 cells we observed that phage T5*amN*4, in contrast to other T5 amber mutants, failed to induce synthesis of phage-specific proteins (Fig. 1). Monitoring of the optical densities of *E. coli* F and F/21-1 cultures infected with T5*amN*4 and T5*st* showed that T5*amN*4 was unable to cause lysis of strain F/21-1 cells (Fig. 2). Adsorption kinetics revealed that this was due to very poor adsorption of T5*amN*4 to strain F/21-1 compared with other amber phages (Fig. 3).

The *N*4 amber mutation affects the synthesis of the major tail protein (22, 23). If suppression of the *N*4 defect in Fsuβ$^+$ was responsible for the poor adsorption to strain F/21-1, the substitution of one amino acid in the major tail protein would have a direct influence on adsorption. To answer this question, we crossed the *N*4 mutation into phage BF23, which is a very close relative of T5 (15), but uses a different receptor. If the *N*4 mutation was responsible for the poor adsorption to strain F/21-1, this phenotype should be also present in BF23*amN*4, and on crossing back into T5*st* the resulting T5*amN*4 should again show this phenotype. If a different mutation than *N*4 was responsible for this phenotype, then there should be a good chance to lose this mutation on crossing *N*4 into BF23 owing to the different receptor of BF23. Two T5*amN*4 recombinants were isolated. They showed the same adsorption rate to strain F/21-1 as did T5*st* (data not shown), indicating that the *N*4 defect was not responsible for the poor adsorption of T4*amN*4 to strain F/21-1. Indeed, from a cross of T5*amN*4 × T5*amD*18 ca. 40% of the wild-type recombinants showed the phenotype of poor adsorption to strain F/21-1. These recombinants were called T5*oad*.

The normal adsorption of T5*oad* to *E. coli* F and the poor adsorption to strain F/21-1 suggested that the *E. coli* F O antigen played an essential role in the adsorption of T5*oad* to *E. coli* F. This was verified with a set of LPS mutants of *E. coli* F, differing in the amount of O antigen synthesized (9). The adsorption rate of T5*st* to these mutants was proportional to the amount of O antigen (9; Table 2). The same was true for T5*oad*. However, although a 90% reduction of the amount of O antigen reduced the adsorption rate of T5*st* by a factor of four, the adsorption rate of T5*oad* was reduced by a factor of 1,000 (Table 2). Simultaneous with the reduction of the adsorption rates, a reduction of the plating efficiencies was observed both for T5*st* and for T5*oad* (Table 2). The results indicated that the reduction of plating efficiency was a consequence of the reduced adsorption rate.

Phages isolated from plaques of T5*oad* on *E. coli* F/21-1 plated on *E. coli* F and F/21-1 with the same efficiency as T5*st*, indicating that they were wild-type revertants.

The *oad* mutation does not affect the LTF. The data obtained thus far show that the interaction with the *E. coli* F

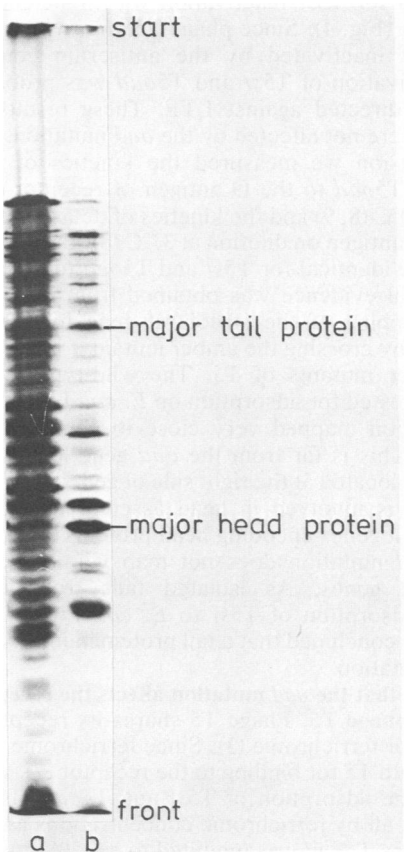

FIG. 1. Autoradiography of *E. coli* F/21-1 cells infected with T5*amN*4 and T5*stamN*5. Cells, infected in the log phase with phage at an MOI of 5, were labeled for 10 min with [^{14}C]amino acids 20 min after infection. a, T5*amN*4; b, T5*stamN*5.

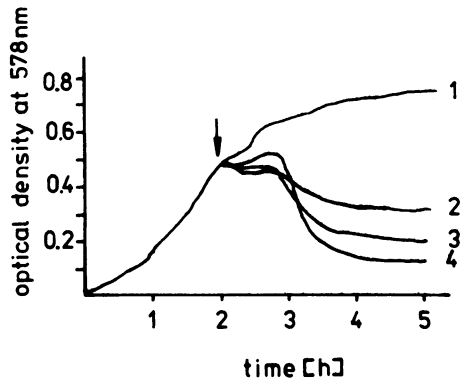

FIG. 2. Lysis of *E. coli* F and F/21-1 by T5*st* and T5*amN*4. T5 phages were added to growing cultures of strain F or F/21-1 at an MOI of 5 (indicated by the arrow). Growth and lysis were monitored by a biophotometer. Curve 1, strain F/21-1 infected with T5*amN*4; curve 2, strain F/21-1 infected with T5*st*; curve 3, strain F infected with T5*st*; and curve 4, strain F infected with T5*amN*4.

O antigen is essential for T5*oad* infection. As a consequence, the possession of the LTF should be a necessary prerequisite of T5*oad* infection. We did not try to verify this by isolating T5*oad* without LTF, since such phages will hardly form plaques. However, when T5*st* and T5*oad* were inactivated by an anti-T5 serum, about half of the antiserum concentration needed for T5*st* inactivation was sufficient for T5*oad* inactivation (Fig. 4). Since phage T5*hd*-2, which lacks LTF, was hardly inactivated by the antiserum concentrations used, inactivation of T5*st* and T5*oad* was probably due to antibodies directed against LTF. These results suggested that LTF were not affected by the *oad* mutation. To support this conclusion we measured the kinetics of irreversible binding of T5*oad* to the O antigen of receptor-deficient *E. coli* F/5 at 0°C (8, 9) and the kinetics of detachment of T5*oad* from the O antigen on dilution at 37°C (8). Both kinetics were found to be identical for T5*st* and T5*oad* (data not shown).

Additional evidence was obtained from mapping experiments. Mapping of the structural gene for the LTF was performed by crossing the amber mutant T5*hd*-1*amD*18 with other amber mutants of T5. The wild-type recombinants were then tested for adsorption on *E. coli* F and F/21-1. The *hd*-1 mutation mapped very close to genes *D*16 and *D*17 (Table 3). This is far from the *oad* gene locus, which was found to be located at the right side of gene *N*5 (Fig. 5). Gene *N*5, which is involved in head assembly, is located near other known genes encoding head proteins (15). Interestingly, the *oad* mutation does not map within the cluster of known tail genes. As isolated tails from T5*oadamN*5 inhibited adsorption of T5*st* to *E. coli* F/21-1 inefficiently (Fig. 6), we concluded that a tail protein indeed is affected by the *oad* mutation.

Evidence that the *oad* mutation affects the receptor binding protein of phage T5. Phage T5 shares its receptor with the iron chelator ferrichrome (3). Since ferrichrome is known to compete with T5 for binding to the receptor (7), we tested its effect on the adsorption of T5*st* and T5*oad*. T5*st* was not inhibited at all by ferrichrome concentrations as high as 100 μM, whereas T5*oad* was inhibited to ca. 50% by 1 μM ferrichrome (Fig. 7). The test was performed under conditions at which transport of ferrichrome occurred (7). The result may indicate that there was a considerable lag between reversible binding of T5*oad* to the O antigen and irreversible binding to the receptor, a lag which is not observed with T5*st*. To test for this possibility, T5*st* and T5*oad* were allowed to adsorb to *E. coli* F, and adsorption was then stopped by either dilution at 0°C or dilution at 37°C. Adsorption of T5*oad* was slower with dilution at 37°C than with dilution at 0°C; no difference was observed for T5*st* (Fig. 8).

Support for the idea that the *oad* mutation affected the receptor binding protein came from the finding that the *oad* mutation could not be crossed into BF23, which uses another receptor, the BtuB protein (2, 6). For this purpose we crossed T5*oad* with the amber phages BF23*amN*5 and BF23*amN*4, selected for BF23 wild-type phages, and tested for the Oad phenotype. Of 70 single plaques from the T5*oad* × BF23*amN*5 cross and 96 plaques from the T5*oad* × BF23*amN*4 cross, none showed the Oad phenotype. In a control experiment we tested for double crossover in that genetic region by crossing T5*oadamN*5 with T5*amN*4-2 (genotypically *oad*$^+$) and selecting for nonamber recombinants. About 20% of these recombinants turned out to be *oad* mutants.

Attempts to identify the tail protein affected by the *oad* mutation failed. Purified tails of T5*stamN*5 and T5*oadamN*5 showed exactly the same protein patterns in SDS-PAGE (Fig. 9). Antibody blotting of SDS gels with T5 antiserum (21) and O'Farrell gels (16) also did not reveal any significant difference beween T5*st* and T5*oad* (data not shown). Furthermore, purified tails from ten independently isolated wild-type revertants from T5*oadamN*5, with respect to the *oad* mutation, were analyzed in SDS-PAGE. Among all of the revertants, no tail protein was observed to run in a different position on the gel (data not shown).

DISCUSSION

The interaction between the LTF of T5 and the O antigen of *E. coli* F results in accelerated adsorption (8, 9). This is probably due to a prolonged phase of surface attachment (8, 9, 19) during which the phage can irreversibly bind to its FhuA receptor (4, 5, 8). The interaction of LTF with the O antigen

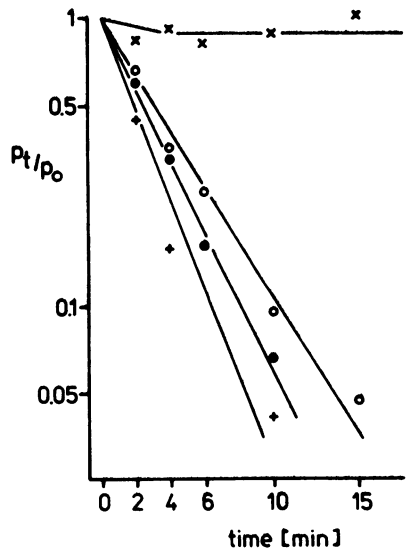

FIG. 3. Adsorption of T5 amber mutants to *E. coli* F/21-1. Symbols: ×, T5*amN*4; ○, T5*D*17*am*34d; ●, T5*D*18*am*5; +, T5*D*16*am*34a. p_t/p_o, Number of unadsorbed phages per input phage numbers.

TABLE 2. Effect of *oad* mutation on adsorption rate and plating efficiency

Strains	Mannose content (% of LPS dry weight)	K_{ads} ([ml · min^{-1}] × 10^{-9})[a]			Plating efficiency		
		T5*st*	T5*oad*	T5hd-2	T5*st*	T5*oad*	T5hd-2
F	9.0	1.6	1.6	0.08	1	1	1
F/21-3	3.6	0.9	0.023	0.16	0.9–1	0.2–0.7	1
F/21-4	0.8	0.38	<0.002	0.22	0.5–0.7	0.03–0.1	1
F/21-1	0.2	0.18	<0.002	0.24	0.4–0.65	0.004–0.01	1

[a] K_{ads}; Adsorption constant.

is nonessential, since T5 phages lacking LTF are viable and not reduced in plating efficiency (18).

The T5 mutant described in this communication shows the unusual property of being dependent in its infection on the amount of O antigen at the cell surface. This may imply that the mutation does not affect the LTF. However, from the results shown in Table 2, it follows that the possession of LTF is a disadvantage for T5*st* when infecting O antigen-deficient *E. coli* strains. Thus, the *oad* mutation may be located in LTF, amplifying the otherwise small effects on plating efficiency of T5. Since the data show that LTF of T5*oad* have the properties of wild-type LTF and since the structural gene for LTF is different from the gene affected by the *oad* mutation, it is evident that LTF of T5*oad* are wild type. From this evidence and from the O antigen dependency of T5*oad* it is most probable that T5*oad* is dependent on the possession of LTF. This has not been demonstrated directly by isolating T5*oad* lacking LTF. Instead, we tested the inactivation by an anti-T5 serum, which preferentially inactivated LTF$^+$ phages. The inactivation of LTF$^+$ phages at concentrations at which no inactivation of LTF$^-$ phages occurred was probably due to clumping of the phages and sticking together of LTF and thus prevented interaction of the tip of the tail with the FhuA receptor. T5*oad* was already inactivated at antiserum concentrations at which clumping of the phages should not occur. We conclude that inactivation of T5*oad* at low antiserum concentrations was effected by binding of one antibody to one LTF, which prevented binding of LTF to the O antigen.

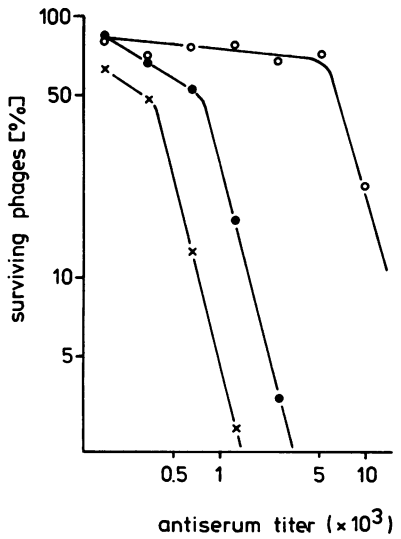

FIG. 4. Inactivation of T5 phages by anti-T5 serum. Incubation was performed at 37°C for 1 h. Symbols: ○, T5*hd*-2; ●, T5*st*; ×, T5*oad*.

Since binding of LTF of T5*oad* seems not to be affected, we think that the *oad* mutation influences the interaction of the phage with the FhuA receptor protein. Evidence came from the fact that the mutation could not be crossed into BF23 (a T5 relative, which uses the BtuB protein as the receptor) (2, 6) and from inhibition studies with ferrichrome.

Ferrichrome is known to compete with T5 for binding to the FhuA protein (3, 7). Under conditions at which no transport of ferrichrome occurs (e.g., *tonB* cells) and binding to the O antigen is prohibited (LTF$^-$ phages or O antigen-negative cells), adsorption is inhibited to 50% by 0.01 μM ferrichrome (7, 8). The interaction of LTF with O antigen results in a drastic reduction of inhibition by ferrichrome. About 20 μM ferrichrome is needed for 50% inhibition (8). There is no inhibition, even with 100 μM ferrichrome, when the adsorption of T5*st* to *E. coli* F (tonB$^+$) is tested (this study). Under the same conditions, 50% inhibition of T5*oad* adsorption was observed with 1 μM ferrichrome (Fig. 7). The poor inhibition of T5*st* may be explained by the prolonged phase of surface attachment on *E. coli* F. As soon as a receptor occupied by ferrichrome becomes accessible after ferrichrome has been transported into the cell, the phage attached to the cell surface can bind to the receptor. The lag between reversible binding to the O antigen and irreversible binding to the receptor appears to be very short for T5*st* (8). However, on some *E. coli* strains of the O9 serotype (the same serotype as *E. coli* F) (9) with reduced amounts of FhuA protein in the outer membrane, such a lag could be demonstrated (K. Heller, unpublished results). For T5*oad* this lag appears to be prolonged (Fig. 8), resulting in a better inhibition by ferrichrome; accessible receptors are occupied by ferrichrome before the phage is able to bind. This explanation implies that the *oad* mutation reduces the affinity of the phage for the receptor. Thus, successful binding can only be achieved in the presence of O antigen in which

TABLE 3. Mapping of the structural gene for the LTF

Phage crosses[a]	No. of recombinant phages	
	Tested[b]	Showing hd phenotype
T5*hd*-1*amD*18 × T5*D*16*am*34a	20	20
T5*hd*-1*amD*18 × T5*D*17*am*34d	20	19
T5*hd*-1*amD*18 × T5*amN*4	20	8
T5*hd*-1*amD*18 × T5*stamN*5	20	5

[a] T5*hd*-1 does not synthesize LTF (21).

[b] Single plaques of nonamber recombinants were picked and tested for adsorption to *E. coli* F and F/21-1.

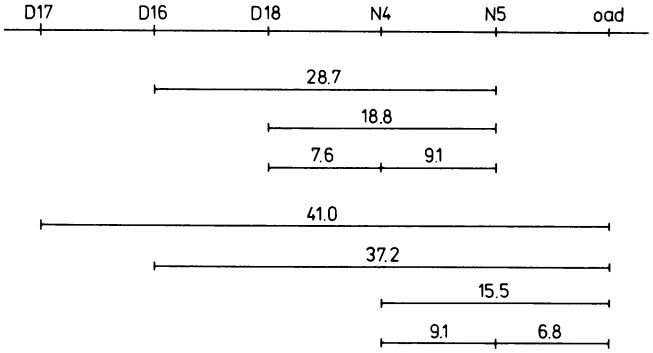

FIG. 5. Mapping of the genes *N5* and *oad*. The numbers above the lines show the values for percentage recombination as obtained from two-factor crosses. p_t/p_0, Number of unadsorbed phages per input phage numbers.

binding to the receptor is stabilized by the additional interaction of LTF with LPS.

This rather simple explanation does not take into account the very high ferrichrome concentrations needed for inhibition of T5*st* adsorption to *E. coli* F. We think it is more likely that there are either two sites on the FhuA protein capable of binding T5*st* irreversibly or that the FhuA protein exists in two different conformations at the cell surface. Phage binding to one of these sites or conformations can be blocked by ferrichrome under certain conditions (e.g., in *tonB* cells). The second site or conformation would be independent of ferrichrome but accessible only to phage already bound to LPS. For T5*oad* this second site would not be accessible. As a consequence, the receptor density on the cell surface would be reduced for T5*oad*.

The effect of receptor density has been extensively discussed by M. Schwartz (19) for the adsorption of phage

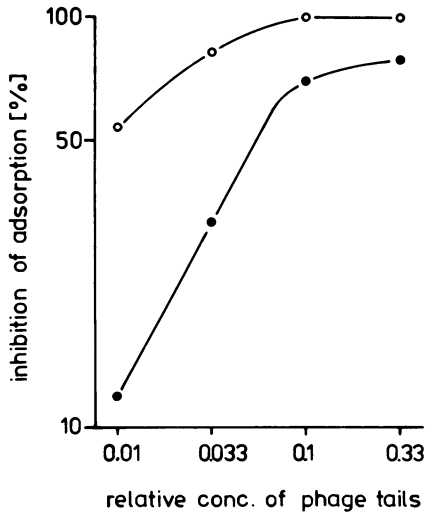

FIG. 6. Inhibition of T5 adsorption to *E. coli* F/21-1 isolated phage tails. A 10-μl amount of phage tails was incubated with 20 μl of F/21-1 cells (4×10^9/ml) at 37°C for 10 min. After addition of T5*st* (10^7/ml) and further incubation at 37°C for 15 min, adsorption was stopped by 500-fold dilution into ice-cold PBS. ○, Tails from T5*stamN5*; ●, tails from T5*oadamN5*. The concentrations of the tails were adjusted from scans of SDS-PAGE.

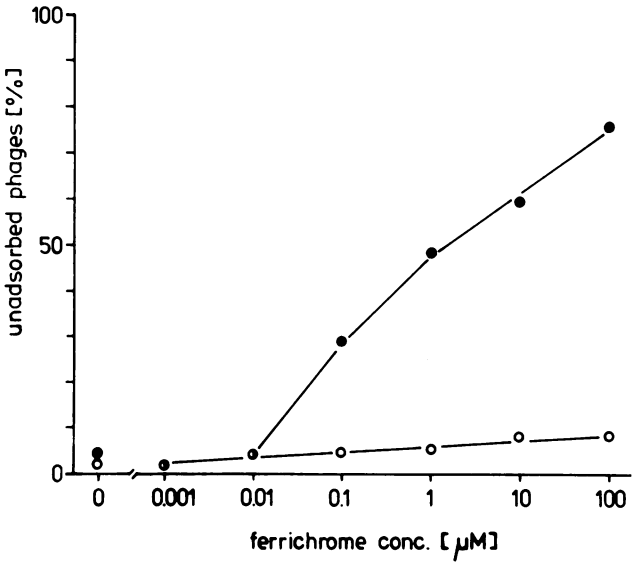

FIG. 7. Inhibition of T5*st* and T5*oad* adsorption by ferrichrome. The inhibition of adsorption of T5*st* (○) and of T5*oad* (●) to *E. coli* F by different concentrations of ferrichrome was measured.

lambda to its LamB receptor. This system offers the advantage that the amount of LamB at the cell surface can be adjusted by medium variations over a very wide range (19). Adsorption kinetics revealed that the adsorption constant was nearly proportional to the receptor density at very low densities. At high densities, a 20-fold increase in the amount of receptor resulted only in a 2-fold increase of the adsorption constant (19). The high probability of irreversible binding of lambda at high receptor densities was attributed to the retention effect discussed by Silhavy et al. (20). This effect leads to a prolongation of the phase at which the phage remains in the vicinity of the bacterial surface.

Applied to the adsorption of T5, this could mean that a reduction of the receptor density to very few receptors per cell may be without any effect on the adsorption constant, because the phage remains attached to the bacterial surface for a considerably long time by reversible binding to the O antigen. If this time is reduced by reducing the amount of O

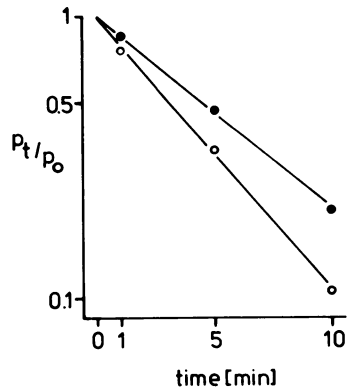

FIG. 8. Adsorption of T5*oad* to *E. coli* F. T5*oad* (2×10^7/ml) was incubated together with *E. coli* F (1.5×10^8/ml) at 37°C. Adsorption was stopped by 500-fold dilution into ice-cold PBS (○) or PBS at 37°C (●).

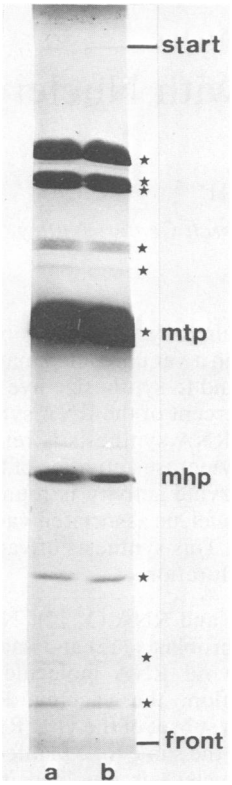

FIG. 9. Protein composition of purified phage tails. Purified tails from T5*stamN5* (a) and T5*oadamN5* (b) were subjected to SDS-PAGE. Tail proteins are indicated by an asterisk. The positions of major tail protein (mtp) and of residual major head protein (mhp) are shown.

antigen at the cell surface, the probability of irreversible binding to one of the few receptors will be reduced concomitantly. Support for this idea comes from adsorption kinetics of T5 to *E. coli* O9 strains with reduced amounts of FhuA protein; the adsorption constant of T5*hd*-2 (LTF^-) is reduced by a factor of six, whereas the adsorption constant for T5*st* (LTF^+) remains unaltered (K. Heller, unpublished results).

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