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Major Proteins of the *Escherichia coli* Outer Cell Envelope Membrane as Bacteriophage Receptors

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Three *Escherichia coli* phages, TuIa, TuIb, and TuII*, were isolated from local sewage. We present evidence that they use the major outer membrane proteins Ia, Ib, and II*, respectively, as receptors. In all cases the proteins, under the experimental conditions used, required lipopolysaccharide to exhibit their receptor activity. For proteins Ia and II*, an approximately two- to eightfold molar excess of lipopolysaccharide (based on one diglucosamine unit) was necessary to reach maximal receptor activity. Lipopolysaccharide did not appear to possess phage-binding sites. It seemed that the lipopolysaccharide requirement reflected a protein-lipopolysaccharide interaction in vivo, and lipopolysaccharide may thus cause the specific localization of these proteins. Inactivation of phage TuII* by a protein II*-lipopolysaccharide complex was reversible as long as the complex was in solution. Precipitation of the complex with Mg^{2+} led to irreversible phage inactivation with an inactivation constant (37°C) $K = 7 \times 10^{-2}$ ml/min per μg. With phages TuIa and TuIb and their respective protein-lipopolysaccharide complexes, only irreversible inactivation was found at 37°C. The activity of the three proteins as phage receptors shows that part of them must be located at the cell's surface. In addition, the association of proteins Ia and Ib with the murein layer of the cell envelope makes this pair trans-membrane proteins.

The outer cell envelope membrane of *Escherichia coli* (and of most, if not all, gram-negative bacteria) contains a set of several major or abundant proteins with molecular weights between about 10,000 and 40,000 (7, 13, 20, 26, 41, 46). Although there is suggestive evidence for physiological functions of two of them (2, 30, 43), the main questions of more general biological interest that they pose are largely unanswered. For example, how are they translocated from the cytoplasm to the outer membrane, and what makes them specific for this and not the plasma membrane? How is their synthesis regulated? What are the structure-function relationships? One way to approach such problems obviously is to select various types of mutants of individual major proteins.

Outer membrane proteins can act as phage receptors; however, it has become clear that the same proteins physiologically serve other functions (6, 10, 15-18, 25, 34, 35, 45, 48). Therefore, since phages may have evolved toward existing membrane proteins, it is conceivable that one can find specific phages for most outer membrane proteins that are exposed to the environment, and such phages, of course, would be a powerful tool for the generation of mutants.

We have already reported on the isolation, from local sewage, of phage TuII*, which, according to the types of mutants resistant to it, appears to use one of the major outer membrane proteins as a receptor (9, 19). Mutants of membrane components, however, can be pleiotropic (3), and it is necessary, for such phage-receptor systems, to directly prove that a given protein does act as a specific receptor.

Here we present evidence for the protein in question being a receptor for phage TuII*, and we describe some properties of this phage-receptor interaction. We also describe two new phages that use two other major proteins as receptors.

MATERIALS AND METHODS

Abbreviations. Abbreviations used are: SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane. Genetic nomenclature is that of Bachmann et al. (5).

Media, growth conditions, strains, and phage assay. Cells were grown in a complete medium (antibiotic medium no. 3, Difco) at 30°C with forced aeration. For most experiments, strain P400 (44) from P. Reeves was used. As a source for protein Ib, we used a mutant resistant to phage TuIa and sensitive to phage TuIb; this mutant had been isolated in strain AB2847 (*aroB malA*), also a kind gift from Dr. Reeves.

Inactivation of phage was assayed by incubating

10^3 to 10^4 plaque-forming units in 2 ml of complete medium under the various conditions described in the text. Phage titer was determined by adding an appropriate volume (normally 0.1 ml) to 2.5 ml of soft agar containing 10^7 to 10^8 indicator cells (P400), which was poured over complete medium; further incubation was at 37°C. To determine reversible versus irreversible phage inactivation, the incubation mixture was diluted 10-fold into complete medium, shaken vigorously, left for 15 min at room temperature (or at 4°C if the first incubation was done at that temperature), and assayed as above.

Isolation of protein II* and lipopolysaccharide. The procedure described previously for the isolation of this protein from *E. coli* B/r (22) did not work so well with the *E. coli* K-12 strain P400 in that large losses of the protein occurred in step 2 (SDS-Mg^{2+} extraction of envelopes). The isolation was modified as follows. Envelopes were prepared as described (22). They were suspended (1 g [wet weight]/12.5 ml) with 4% dodecyltrimethylammonium bromide (Eastman) in 10 mM Tris-hydrochloride, pH 7.5, containing 0.05% mercaptoethanol. After centrifugation (40 min at 27,000 × *g*), extraction of the sedimented material was repeated and the supernatants were discarded. The sediment was suspended in water, acetone was added to a final concentration of 90%, and the sediment was recovered by centrifugation (10 min at 7,700 × *g*). It was washed two times with 90% acetone and lyophilized. This material was extracted (2 g/100 ml) with 3% SDS containing 10 mM Tris-hydrochloride (pH 7.5), 1 mM EDTA, and 0.1% mercaptoethanol. Centrifugation for 60 min at 39,000 × *g* yielded a clear supernatant from which pure protein II* was obtained by chromatography on Bio-Gel P150 as described (20).

Lipopolysaccharide was isolated from *E. coli* B/r according to Galanos et al. (11). Different preparations yielded almost identical analytical data. The ketodeoxyoctonate content (32) was 8.6%, or about 3 mol per mol of diglucosamine. As judged by gas chromatography (38), the material was free from phospholipid. Amino acid analyses of acid hydrolysates (6 N HCl, 24 h at 110°C) showed the presence of 0.22 to 0.25 μmol of glucosamine per mg of lipopolysaccharide (minimal chemical molecular weight, ~8,000 per diglucosamine) and that the preparations did not contain protein.

Receptor solubilization from ghosts. Ghosts (strain P400) were prepared as described (21). In brief, the procedure involves treatment of cells with Triton X-100 in 40% sucrose, and, successively, urea, trypsin, and lysozyme. Digestion with lysozyme was omitted in this work. Ghosts were extracted at room temperature (range, 20 to 26°C) by homogenizing 1 g (wet weight) of ghosts in 50 ml of 4% SDS or dodecyltrimethylammonium bromide, both containing 5 mM EDTA; the pH was adjusted to 7.5 by adding 0.2 M Tris-hydrochloride buffer, pH 9.0. After stirring for 15 min, insoluble material was removed by centrifugation (60 min, 60,000 × *g*) and extracted once more with 0.5 volume of the corresponding detergent. To the combined supernatants, acetone was added to 90% final concentration. The precipitated material was washed five times with 90% acetone and lyophilized.

Dimyristoyllecithin was from Sigma Chemical Co. (St. Louis, Mo.), and Triton X-100 was from Serva (Heidelberg, West Germany).

RESULTS

Proteins and isolation of phages. The proteins concerned are Ia, Ib, and II*. Protein II* (molecular weight, ~33,000; identical with Schnaitman's protein 3a [41]) has been shown to consist of a single polypeptide chain (13). Trypsin (used in ghost preparation; see above) removes part of protein II* and converts it to its tryptic fragment protein II (20; molecular weight, ~24,000), which remains with the ghost. Proteins Ia and Ib (molecular weight, ~37,000) are two proteins that are almost identical in primary structure. The chemical nature of the difference is not yet known, and this pair may arise by an in vivo modification process or may be due to the existence of two very similar genes (40). One of the two is identical with Rosenbusch's matrix protein (39), and their mixture is identical with Schnaitman's protein 1 (41).

Phage TuII* was isolated (19) by screening only 20 isolates of sewage phages for growth on wild-type *E. coli* K-12 and inability to form plaques on a *tolG* mutant (8) missing protein II*. We have also briefly mentioned the isolation of phage TuIb (then called TuI; 40). One phage among 40 isolated was found that could be propagated on *E. coli* K-12 strain P400 (harboring both proteins Ia and Ib) but not on *E. coli* B/r (possessing Ia but missing Ib). Phage TuIa was found in the following way. Selection for resistance to TuIb in strain P400 frequently yields mutants still producing Ia but not Ib (40). Sewage phages (61 isolates) were screened for growth on such a $TuIb^r$ mutant and inability to grow on strain P530 (missing both proteins Ia and Ib; 44), and isolate 61 was phage TuIa. Electron microscopically, all three phages are very similar to the T-even phages.

Sewage represents an enormous phage reservoir: phage titers were usually found to be 10^2 to 10^4 per ml, and on a single plate with about 100 plaques at least 10 different plaque types were easily discernible. From this fact and the specificity of the three phages to be described, it seems likely that specific phages can be found for the majority of recognizable outer membrane proteins.

Phage TuII* inactivation by cells and ghosts. Phage inactivation can be followed quantitatively by a kinetic assay if, under conditions of excess of receptor, this inactivation follows pseudo first-order kinetics (33, 49).

Phage inactivation can, in addition, be measured by a stoichiometric assay (excess of phage). The latter possibility cannot, at least at 37°C, be easily used with TuII* because at the phage concentrations required (10^8 to 10^{10}/ml), there is considerable phage inactivation in the absence of receptor. Concerning the former assay, there is one experimental restriction with TuII*: because of its short eclipse period (15 to 16 min at 37°C), actively metabolizing cells cannot, of course, be used for time intervals exceeding this latent period.

For these reasons, we used chloroform-killed cells for a kinetic assay of phage inactivation (Fig. 1). Identical results have been obtained with cells treated with chloramphenicol (200 μg/ml). Phage inactivation was irreversible and followed pseudo first-order kinetics. From several such experiments, the adsorption velocity constant was calculated according to $dp/dt = -k \cdot P \cdot C$ (where P is the concentration of phage remaining unattached at time t and C is the concentration of cells). It was found to be 5×10^{-10} to 5×10^{-9} ml/min, i.e., in the same order of magnitude as determined some 25 years ago for the *E. coli* phages T1, T2, T3, and T4 (33). Since such very rapid adsorption rates are most likely the maximum attainable theoretically (33), there is no reason to assume that the dead cells or those in the presence of chloramphenicol do not reflect the behavior of living cells.

The same experiments as shown in Fig. 1 were also carried out at 4°C, and no inactivation was found over a period of 1 h. This enabled us to look for the existence of a reversible step preceding the reaction causing irreversibility. Reversible phage inactivation could easily be demonstrated at 4°C simply by raising the concentration of cells (Fig. 1C).

Since functions of living cells are not required for irreversible phage inactivation, we could simplify the system by studying phage adsorption to ghosts. Ghosts are particles the size and shape of cells which consist almost exclusively of a derivative of the outer membrane (20, 21). As mentioned above, ghosts instead of protein II* contain its tryptic fragment protein II. Concerning irreversible and reversible phage inactivation, ghosts qualitatively and quantitatively behaved exactly as shown for cells in Fig. 1; i.e., there was no measurable loss of receptor activity during the ghost purification procedure.

We have not studied in any detail the nature of the irreversible phage inactivation, but we found that phage-cell association was very tight. Phages could not be removed (electron microscopy) from cells by several washings with water, 2% Triton X-100, 6 M urea, 1 M NaCl, or 0.2 M Tris-hydrochloride, pH 9. Treatment with 10 mM HCl removed phage heads; however, numerous phage tails were still associated with the cells.

Inactivation of phage TuII* by ghost extracts and isolated proteins II* and II. Extraction of ghosts at room temperature with dodecyltrimethylammonium bromide or SDS in the presence of EDTA solubilized much of the ghost protein (including protein II) together with lipopolysaccharide and phospholipid. The detergents could almost completely be removed from the extracted material by precipitation with acetone and extensive washing of the precipitate with acetone. Subsequent lyophilization yielded a mixture of protein (50% of dry weight), lipopolysaccharide, and phospholipid, and most of this material was soluble in 2 mM $NaHCO_3$ containing 0.3% Triton X-100.

Inactivation of phage TuII* with such extracts was found to be quite different from that described for cells or ghosts (Fig. 2). First, irreversible inactivation under the conditions used no longer occurred, and second, the rate of reversible inactivation was too fast (regardless of whether measured at 4 or 37°C) to be measurable with the simple methodology used. With the receptor in solution, therefore, this rate appeared to be diffusion controlled. Such an extract from a mutant resistant to the phage and missing protein II* was also tested, and no phage inactivation was found.

Protein II was then isolated from ghosts (12) and protein II* was isolated from cells (see Materials and Methods), and both were tested for their activities toward the phage. Protein II* turned out to be inactive, and protein II was very much less active in reversible phage inactivation than any of the extracts described above. For instance, 6 mg of protein II per ml in an assay mixture such as that used for the experiments of Fig. 2 achieved only 50% inactivation (30 min at 37°C). However, neither protein was, at least completely, soluble in the Triton-bicarbonate mixture used for the experiments with extracts from ghosts. After unsuccessfully trying a number of other nonionic detergents, we found that the best solubilizer for both proteins was lipopolysaccharide.

Lipopolysaccharide (10 mg/ml) was soluble in 2 mM $NaHCO_3$ containing 0.3% Triton X-100. (Lipopolysaccharide in such solution or suspended in water was completely inactive toward the phage.) Proteins II and II* at concentrations of 2 to 3 mg/ml dissolved in this lipopolysaccharide-Triton mixture as judged by turbidity.

The kinetics of phage inactivation with the

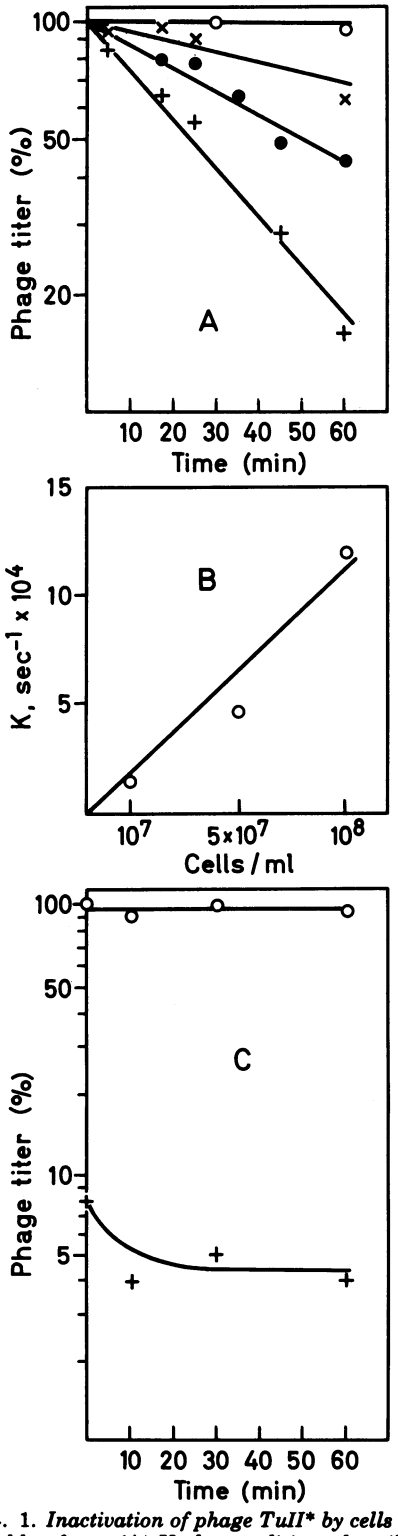

FIG. 1. *Inactivation of phage TuII* by cells killed with chloroform. (A) Under conditions described in Materials and Methods, 3 × 10³ phage (plaque-forming units) per ml were, at 37°C, exposed to: (×) 10⁷ cells/ml; (●) 2.5 × 10⁷ cells/ml; (+) 5 × 10⁷ cells/ml; (○) control without cells. The same data were obtained when samples were diluted after incubation. (B) From data such as those shown in (A), K was calculated according to* $P = P_0 e^{-Kt}$. *(C) 4 × 10³ phage/ml were exposed to 1.7 × 10¹⁰ cells/ml at 4°C. Symbols: (+) Phage measured by direct titration of the phage-cell mixtures; (○) phage titrated after 10-fold dilution of the same mixtures.*

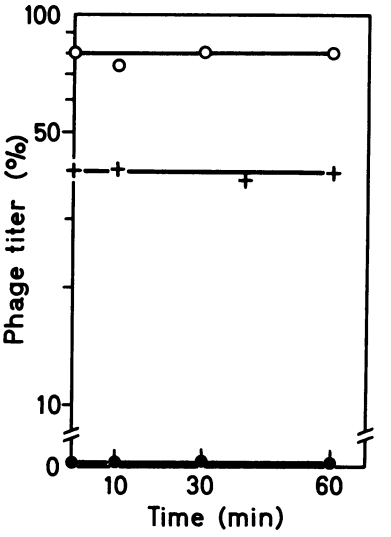

FIG. 2. *Inactivation of phage TuII* by solubilized receptor. A ghost extract obtained with dodecyl-trimethylammonium bromide (see Materials and Methods) was used. 6 × 10³ phage (plaque-forming units) per ml were incubated, at 37°C, with extract (corresponding to [○, ●] 1 mg of protein and [+] 50 μg of protein) dissolved in 2 mM $NaHCO_3$ containing 0.3% Triton X-100. Symbols: (●, +) Phage titrated directly; (○) phage titrated after 10-fold dilution.*

lipopolysaccharide-protein II*-Triton mixture was identical to that described in Fig. 2 for a ghost extract. There were rather large variations in the amount of protein required to achieve 50% phage inactivation, and between about 10 and 100 μg of protein per ml was needed for this activity. It was also found that lyophilized ghost extracts lost about 80% of their relevant activity during several weeks of storage in vacuo at room temperature. Preincubation of such extracts with dithiothreitol did not restore the original activity, and the cause of this "aging" process has remained obscure; as determined electrophoretically, it is not caused by proteolysis.

Reversible versus irreversible inactivation of phage TuII*. It was shown in the previous section that phage inactivation became reversi-

ble as soon as protein II* (or II) was, together with Triton and lipopolysaccharide, in solution. Cells killed with chloroform inactivated the phage irreversibly whether or not Triton (0.3%) was present; therefore, Triton does not appear to inhibit the reaction(s) leading from reversibility to irreversibility.

Protein II* together with lipopolysaccharide dissolved in Triton-bicarbonate could be precipitated with Mg^{2+}. At a 30- to 50-fold molar excess of lipopolysaccharide over protein (cf. Fig. 4), 20 mM $MgSO_4$ quantitatively precipitated both components. Reproducibly the highest activity of the protein was observed in such preparations, and only 0.5 to 1 μg of protein per ml was required for 50% phage inactivation (37°C, conditions of Fig. 1). This inactivation proved to be irreversible. It again followed pseudo first-order kinetics (cf. Fig. 1), and the protein-lipopolysaccharide complex had a rather high inactivation constant (37°C): calculation according to $\log Pt/P_0 = -k \cdot c \cdot t$ (P_0 and P_t are concentrations of plaque-forming units at times zero and t, respectively; c is the concentration of protein in micrograms per milliliter) yielded constant $K = 7 \times 10^{-2}$ ml/min per μg. Also, one "aged" protein batch (see above) from which 100 μg/ml in solution was needed to inactivate 50% phage showed the much higher activity just mentioned upon precipitation as protein-lipopolysaccharide complex with Mg^{2+}.

Irreversible phage inactivation may thus require Mg^{2+}, or the receptor needs to be contained at an extended surface, or both. Also, of course, the protein may undergo a conformational change upon precipitation together with lipopolysaccharide. To answer the question concerning the Mg^{2+} requirement, a lipopolysaccharide-protein complex had to be obtained in an insoluble state in the absence of Mg^{2+}.

Phospholipid dissolved in sodium cholate forms vesicles upon dilution of the solution (36). This cholate dilution method does not, however, lead to a more or less quantitative precipitation of lipopolysaccharide. Therefore, protein II* together with lipopolysaccharide and dimyristoyllecithin was subjected to cholate dilution. The component became insoluble and inactivated the phage irreversibly (Table 1). A drawback to these experiments consisted in a decreased activity of the protein when the phospholipid was present. There is no question, however, that Mg^{2+} is not required for irreversible phage inactivation.

Receptors for phages TuIa and TuIb. The characteristics of interactions of these phages with their receptors have not been studied in much detail, and our attention was restricted to the identity of these receptors.

Selection for resistance against phage TuIb (formerly called TuI; 40) yielded two types of mutants: one missing protein Ib and one, rarer, missing both proteins Ia and Ib (40). Selection for resistance against phage TuIa produced mutants missing protein Ia or missing both Ia and Ib. Few of these resistant mutants have been analyzed, and it is likely that additional classes can be recovered. In any case, the existing mutants suggested that TuIa and TuIb use proteins Ia and Ib, respectively, as receptors.

A mixture of isolated (22) proteins Ia and Ib suspended in 0.3% Triton X-100 did not inactivate the two phages, and the addition of lipopolysaccharide did not change this lack of activity. The isolation procedure of the proteins involves boiling in SDS, and we therefore tried more native protein preparations. Both proteins are tightly associated with the murein

TABLE 1. *Irreversible inactivation of phage TuII*[a]*

Concn (M) of components in solution			Detergent (concn, %)	Precipitated by:	Phage inactivation	
Protein II*	Lipopolysaccharide	Dimyristoyllecithin			Protein (μg/ml)	Percent inactivated
7.5×10^{-6}	3.5×10^{-4}		Triton (0.3)	$MgSO_4$ (10 mM)	1	87
7.5×10^{-6}	3.5×10^{-4}	10^{-3}	Triton (0.3)	45-fold dilution into $MgSO_4$ (10 mM)	5	20
7.5×10^{-6}	3.5×10^{-4}	10^{-3}	Cholate (1.3)	45-fold dilution into $MgSO_4$ (10 mM)	5	44
3.75×10^{-5}	1.75×10^{-3}	5×10^{-3}	Cholate (10)	500-fold dilution into EDTA (1 mM)	5	50
3.75×10^{-5}	1.75×10^{-3}	5×10^{-3}	Cholate (10)	500-fold dilution into $MgSO_4$ (10 mM)	5	70

[a] All precipitates were recovered by centrifugation and finely suspended in 1 mM *N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonate buffer, pH 7.4. Amounts of such suspensions to give the protein concentrations indicated were added to phage (4×10^3 plaque-forming units/ml), and samples were incubated for 30 min at 37°C. Phage inactivation was irreversible in all cases. Protein II* and dimyristoyllecithin without lipopolysaccharide is inactive toward phage TuII*.

layer of the cell envelope and complexes can be obtained (39) that essentially consist of proteins Ia, Ib, murein, and the lipoprotein that is covalently bound to murein (7). In our hands, such complexes also always contained residual lipopolysaccharide that could not be removed by repeated extractions with SDS at 60°C according to Rosenbusch (39). Also, some free lipoprotein was always found to remain associated with these complexes (Fig. 3). From appropriate mutants, such complexes can be prepared that contain only protein Ia or only protein Ib. The electrophoretic profiles of such complexes are shown in Fig. 3, and their activity toward the two phages is shown in Table 2. Clearly, complexes containing only protein Ia inactivated only phage TuIa, and complexes with protein Ib inactivated only phage TuIb. Proteins Ia and Ib could be removed from these complexes by treatment, at room temperature, with 0.5 M NaCl containing 2% SDS (31). Proteins Ia and Ib were solubilized this way. NaCl was removed by dialysis, and any remaining SDS was removed by precipitation of the protein with acetone and repeated washings with acetone. The resulting protein preparations were insoluble in the Triton-bicarbonate solution mentioned above and had very low activity toward both phages TuIa and TuIb. As in the case of protein II*, addition of lipopolysaccharide dissolved in Triton-bicarbonate led to solubilization of the proteins and appearance of activity with Ia toward phage TuIa and with Ib toward phage TuIb. Unlike the phage TuII*-protein II* system, protein Ia plus lipopolysaccharide in solution inactivated phage TuIa irreversibly (at 37°C), and there was an optimum for the lipopolysaccharide concentration (Fig. 4). For as yet unknown reasons, inactivation of phage TuIa by the lipopolysaccharide-protein Ia complex was rather sensitive to the concentration of Triton. At concentrations above

TABLE 2. *Inactivation of phages TuIa and TuIb by murein-protein complexes*[a]

Complexes		Percent inactivation of phage:	
From strain:	With protein:	TuIa	TuIb
P400	Ia and Ib	96	95
P400 TuIb^r	Ia	90	4
AB2847 TuIa^r	Ib	10	95

[a] Phages (4×10^3 plaque-forming units/ml) were incubated for 30 min at 37°C with amounts of complexes providing 100 μg of protein per ml in each case. The 10, 5, and 4% inactivations cannot be assayed reliably and may reflect no inactivation at all.

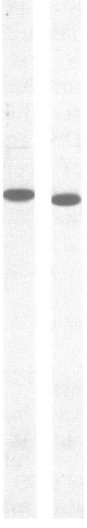

FIG. 3. *SDS-polyacrylamide gel electrophoresis of murein-protein I complexes. (Left) Complex with protein Ia from a phage TuIb^r mutant of strain P400; (right) complex with protein Ib from a TuIa^r mutant of strain AB2847 (see text). The faint band with the highest mobility represents outer membrane lipoprotein. Slab gel electrophoresis was performed as described (40).*

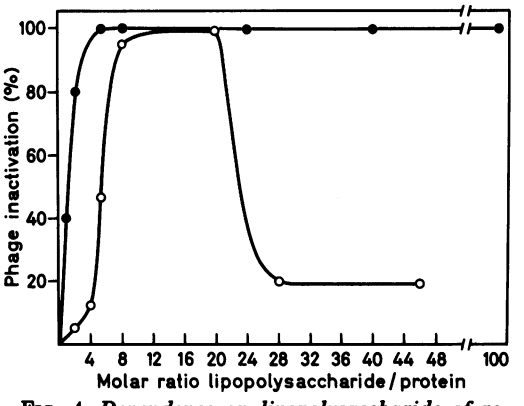

FIG. 4. *Dependence on lipopolysaccharide of receptor activity. To protein Ia (○) and protein II* (●), lipopolysaccharide dissolved in Triton-bicarbonate was added at the ratios indicated; the minimum chemical molecular weight was used for the latter (see text). Both protein-lipopolysaccharide complexes were used, for all data, at 10 μg of protein per ml in the phage inactivation mixture. Inactivation of phage TuIa (○) and TuII* (●) was for 30 min at 37°C and using 10^3 phage (plaque-forming units) per ml; phage were titrated without further dilution. The behavior of the proteins cannot be strictly compared, since phage TuII* inactivation under these conditions is reversible whereas TuIa inactivation is irreversible.*

0.03% in the inactivation mixture, the activity of the complex decreased and no phage inactivation occurred at 0.3%. Dependence of phage TuIb inactivation on the concentrations of lipopolysaccharide and Triton has not been tested.

DISCUSSION

The data reported do not by themselves show whether any one of the proteins or the lipopolysaccharide acts as phage receptors. Lipopolysaccharide in solution (Triton) or suspension is completely inactive against all three phages. The proteins (except protein II, which has low activity) without lipopolysaccharide are also inactive against these phages. Therefore, either lipopolysaccharide activates the proteins or the proteins activate lipopolysaccharide, the latter possibly involving some conformational change in the carbohydrate moiety of lipopolysaccharide. It is also possible that overlapping areas of both protein and lipopolysaccharide constitute a receptor site. There is, however, at least for the TuII*-protein II* and TuIb-protein Ib systems, little doubt that in each case the protein is the receptor. *Salmonella typhimurium* and *Proteus mirabilis* each possess a major outer membrane protein analogous to the *E. coli* protein II*, and neither organism can inactivate phage TuII*. However, *Salmonella-E. coli* and *Proteus-E. coli* hybrids harboring the structural gene for the *E. coli* protein II* produce this protein and do inactivate the phage (9). Therefore, outer membranes with three different lipopolysaccharides inactivate phage TuII* only if protein II* is present. In addition, a heptoseless *E. coli* K-12 lipopolysaccharide mutant (strain K2754 from K. Jann) possesses protein II* and is sensitive to phage TuII*. This particular lipopolysaccharide mutant of the deep rough type is missing protein Ia and produces protein Ib. It is sensitive to phage TuIb and resistant to phage TuIa. All these facts show that rather drastic differences in the lipopolysaccharide carbohydrate moiety are not recognized by the two phages.

It is also very likely that for TuIa only protein Ia serves as a receptor. It has been shown that for receptor-active protein-lipopolysaccharide complexes there is an optimum for the lipopolysaccharide concentration at a 10- to 20-fold molar excess of the latter over protein, and this fact certainly points more to an effect of lipopolysaccharide on protein than vice versa. Also, among some 30 different TuIa^r mutants, heptoseless lipopolysaccharide mutants have not been found.

Experiments on the presumed function of protein II* as a receptor in F-mediated conjugation have shown that lipopolysaccharide is required for this function (43). The same requirement has now been demonstrated for proteins Ia, Ib, and II* to act as phage receptors. Such a lipopolysaccharide requirement has also been found by van Alphen et al. (2) for the activity of protein II* (their protein d) as an inhibitor in conjugation and as a phage receptor. Present studies in this laboratory strongly indicate that the binding of these proteins to lipopolysaccharide is specific, and that these interactions very likely reflect such an interaction in the outer membrane in vivo. It is known that lipopolysaccharide is located only at the outer surface of the outer membrane (28). The function of the proteins mentioned as phage receptors implies, of course, that parts of them must also be localized at this surface. In addition, we showed earlier that the pair Ia-Ib and II* is packed very densely in the outer membrane (14). It therefore would appear almost certain that a protein-lipopolysaccharide interaction exists in vivo. In this context it is of interest to note that it has been found in *E. coli* (24) and in *S. typhimurium* (4) that heptoseless lipopolysaccharide mutants possess greatly decreased amounts of the major proteins in question. On the basis of other observations, we have suggested a feedback control for the synthesis of protein II* where intracellular protein might inhibit its rate of synthesis (9). If anchorage of such a protein in the outer membrane is achieved by specific binding to lipopolysaccharide, then the behavior of the lipopolysaccharide-defective mutants would easily be understandable. It is, of course, also quite conceivable that protein-lipopolysaccharide interaction occurs already during synthesis of these macromolecules, and such binding may then be important for their translocation from the site of synthesis to the outer membrane.

The amount of lipopolysaccharide required for proteins Ia and II* to be become fully active as phage receptors in vitro need not reflect, if existent, the true stoichiometry in vivo. It nevertheless is of interest to note that the ratios of lipopolysaccharide to protein found do not constitute an impossibility in vivo: Mühlradt et al. (29) have determined that one cell of *S. typhimurium* bears about 10^6 molecules (based on one diglucosamine unit) of lipopolysaccharide, and there are about 10^5 copies of proteins Ia-Ib and II* per cell in *E. coli* (20, 39).

Proteins Ia and Ib are tightly associated with the murein layer (39). There is no reason to assume that this association is an artifact of preparation; therefore, the phage receptor function shows that the pair should be trans-mem-

brane proteins. This fact is consistent with the evidence suggesting that these proteins may form a hydrophilic pore through the outer membrane (30). The exposure of all proteins discussed here on the cell surface also agrees well with relevant data recently published by Kamio and Nikaido (23) for *S. typhimurium* and *E. coli* B.

The existence of several phages using major outer membrane proteins as receptors poses an interesting evolutionary question. In addition to the phages described here, protein Ia or Ib very likely serves as receptor for the lambdoid phage PA-2 (42), and Ib may also be a receptor of phage MeI (47). Protein II* also serves as receptor for phage K3 (2). Much evidence has now accumulated that these receptor functions are incidental in that the relevant proteins play other physiological roles. Thus, even very different phages (TuIa-TuIb versus PA-2) appear to have evolved toward the same outer membrane proteins, and such phages can sharply discriminate between very similar proteins (Ia-Ib). Taking into account, in addition, the existence of a whole series of host-range mutants of phage K3 (27), one may be tempted to consider all these phage-receptor systems as somewhat analogous to the possibility of a mutational origin of antibody diversity (see reference 1). A structural characterization of the binding sites of the phage and the protein may thus be rather interesting. A final interesting aspect of the phage-receptor systems concerns the reversible and irreversible inactivation of phage TuII*. Irreversibility can simply be "reconstituted" by Mg^{2+}-dependent precipitation of a protein-lipopolysaccharide mixture, and Mg^{2+} is not required for the establishment of irreversibility. It may be that all that is needed for irreversibility is to have the receptor complex on an extended surface, perhaps to allow a second tail component such as a tail pin to interact with this surface.

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