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Inactivation of Bacteriophages by Protein E, a New Major Membrane Protein Isolated from an *Escherichia coli* Mutant

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Pure protein E, obtained after diethylaminoethyl-cellulose chromatography of ethylenediaminetetraacetic acid-Triton X-100-solubilized outer membrane proteins of *Escherichia coli* strain JF694, inactivated bacteriophage K3. Lipopolysaccharide enhanced bacteriophage inactivation. Antibody prepared against purified protein E protected bacteriophage K3 from inactivation by protein E. Bacteriophage K3 used a major outer membrane protein, protein II*, as part of its receptor. We conclude that proteins E and II* have a common region which interacts with bacteriophage K3. Protein E also inactivated two recently described bacteriophages, TC45 and TC23, that use protein E as at least part of their receptor.

The outer membrane of *Escherichia coli* strains contains several peptidoglycan-associated proteins with apparent molecular weights between 36,000 and 40,000. These proteins include proteins Ia, Ib (27), and 2 (29) and the recently reported proteins E (11), Ic (16), and e (34). Other nomenclatures for these proteins have been summarized by Henning and Haller (15), Lugtenburg et al. (21), and, more recently, Bassford et al. (2). These proteins share some common features, both chemical and functional: (i) all are bound to peptidoglycan in a form stable at 60°C in buffer containing 2% sodium dodecyl sulfate (SDS); (ii) all have similar amino acid composition, with alanine as N-terminal acid; (iii) all form transmembrane channels for passive transport of small hydrophilic compounds; and (iv) all but protein 2 serve as receptors for specific bacteriophages. At least four genetic loci, *tolF* (9), *ompB* (26), *par* (2) or *meoA* (35), and *nmpA* (12), have been described that control the synthesis, processing, or translocation of these related outer membrane proteins.

The major outer membrane proteins of *E. coli* serve as in vivo specific receptor sites for certain bacteriophages. In addition, purified major outer membrane proteins Ia, Ib, and II* have been shown to specifically inactivate bacteriophages TuIa, TuIb, and TuII*, respectively (8).

Protein E, absent in wild-type strains of *E. coli*, is found in a spontaneous fast-growing mutant isolated from a slow-growing *tolF par* double mutant. The phenotypic expression of protein E is under the control of a gene that we call *nmpA*. The appearance of protein E in the outer membrane restores both the antibiotic sensitivity lost as a result of *tolF* mutation (11) and the rapid ur' ike of small hydrophilic molecules lost in *tolI par* double mutants (Foulds and Chai, unpublished data). This suggests that protein E functions as a pore in the outer membrane. Preparations containing purified protein E contain only a single polypeptide as detected by diethylaminoethyl (DEAE)-cellulose chromatography or electrophoresis in polyacrylamide gels containing SDS. The preparation contains only a single N-terminal amino acid (alanine). The purified protein forms a single precipitin band on Ouchterlony double-diffusion plates, using antibody prepared against protein I. Gas-liquid chromatography studied on purified protein E demonstrated a negligible amount of carbohydrate (one molecule of glucose per four molecules of protein E; Chai and Foulds, manuscript in preparation). Several lines of evidence show that protein E is similar, if not identical, to proteins Ic and e. For example, (i) these proteins all migrate at an identical position after electrophoresis in polyacrylamide gels containing SDS; (ii) they have similar chemical compositions and physiological functions; (iii) only strains which contain any one of these three proteins are sensitive to bacteriophages TC45 and TC23 (these bacteriophages were isolated specifically with protein E as at least part of their receptor [7]); (iv) the mutations leading to the appearance of one of these proteins all were located between *uncA* and *rbsK* on the *E. coli* linkage map described by Bachmann et al. (1, 12); and (v) these mutations were all isolated in strains whose outer membranes were lacking detectable amounts of the major outer membrane proteins

Ia and Ib. Here we show that purified protein E can inactivate bacteriophage TC45 or TC23 and that this inactivation is enhanced by added lipopolysaccharide (LPS).

Although bacteriophages K3 (22, 33) and TC45 adsorb to outer membrane proteins II* and E, respectively, we show here that purified protein E in vitro can inactivate either bacteriophage TC45 or K3. This suggests that portions of outer membrane proteins E and II* have similar structures.

MATERIALS AND METHODS

Microorganisms and media. *E. coli* K-12 strains and bacteriophages are described in Table 1. Strain JF568 was used as the host for the growth of bacteriophages TuIa, TuIb, and TuII*; strain P400 was used as the host for the growth of bacteriophage K3. To prepare bacteriophage stocks, L-broth medium (18) containing 2.5 mM $CaCl_2$ and solidified with 0.75% agar was used. Bacteriophages TC45 and TC23 lysates were prepared on strain JF694 as previously described (7). Tryptone agar overlaid with tryptone soft agar containing appropriate host bacteria was used to determine bacteriophage titer (7). Proteose peptone-beef extract medium has been described (10).

Isolation of outer membrane proteins. Strain JF694 was grown at 37°C with aeration in 50 liters of proteose peptone-beef extract in a fermentor to an absorbance at 600 nm of about 1.5. Cells were harvested by centrifugation and washed once with saline. Solubilization of outer membrane proteins was accomplished by first extracting cell envelope materials, prepared as previously described (28), with 10 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, pH 7.3, containing 2% Triton X-100. After centrifugation (200,000 × *g*, 60 min) the sedimented material containing primarily outer membrane materials was extracted with HEPES buffer containing 2% Triton X-100 and 5 mM ethylenediaminetetraacetic acid as described by Schnaitman (28). Protein E was isolated from the solubilized outer membrane proteins by DEAE-cellulose chromatography (28).

TABLE 1. *Bacterial strains and bacteriophages*

Bacteria/phage	Description[a]	Source or reference
Bacterial strain		
JF568	*aroA357 ilv-277 metB65 his-53 purE41 proC24 cyc-1 xyl-14 lacY29 rpsL77 tsx-63* λ⁻	5
JF694	JF568 *aroA*⁺ *tolF4 par nmpA1*	11
P400	F− *thi argE proA thr leu mtl xyl ara galK lacY rpsL supE non* λ⁻	30
Bacteriophage	Receptor[b]	
TC45	Protein E	7
TC23	Protein E	7
K3	Protein II*	33
TuII*	Protein II*	8
TuIa	Protein Ia	8
TuIb	Protein Ib	8
PA-2	Protein Ib	29

[a] Genetic nomenclature as described by Bachmann et al. (1), except that *nmpA* is described separately (12).

[b] The major outer membrane protein required for growth of the bacteriophage.

SDS-gel electrophoresis and protein estimation. Membrane proteins were dissolved in 0.06 M tris(hydroxymethyl)aminomethane buffer (pH 6.8) containing 1.25% SDS and 12.5% glycerol and heated at 100°C for 5 min, followed by electrophoresis according to the method described by Lugtenburg et al. (20). Gels were stained in Coomassie brilliant blue G and destained in 10% acetic acid. For determination of relative protein content in each protein band, each lane of slab gels was cut carefully and scanned at 637 nm, using a Gilford spectrophotometer equipped with a linear transport apparatus and recorder. Peak area was measured as previously described (5).

Preparation of LPS. LPS was prepared from strain *E. coli* J-5 by the method of Galanos et al. (13).

Immunological techniques. For antisera preparation, 0.5 ml (2 mg/ml) of protein E (column 2, see below) obtained after DEAE-cellulose chromatography of Triton-EDTA-solubilized cell envelope materials or 0.4 ml (2×10^{10} plaque-forming units [PFU]) of bacteriophage TC45 purified by equilibrium centrifugation in CsCl was mixed with an equal volume of complete Freund adjuvant (Difco) and injected intradermally into rabbits, followed by two additional boosters according to the procedure described by Meagher and Ornston (23). Purification of antibodies directed against bacteriophage TC45 was accomplished after first mixing 2.0 ml of a suspension of bacteriophage TC45 (3×10^{10} PFU) with 3.0 ml of antiphage serum. The precipitate which formed after incubation for 20 min at room temperature was washed three times with saline (0.5%) to remove nonspecific antibodies and other impurities. The bound specific antibodies were then solubilized by two extractions of the precipitate with 15% NaCl, and the suspension was centrifuged to remove bacteriophage. This was followed by dialysis of the supernatant solution to remove salt (17). Immunodiffusion experiments were conducted by the Ouchterlony double-diffusion technique (31).

Bacteriophage inactivation. To demonstrate inactivation of bacteriophage by purified protein E, 40 μg of protein E, 30 μg of LPS, and 10 μl of $MgSO_4$ (0.1 M) were first mixed with tryptone broth in a final volume of 80 μl. Next, 20 μl of diluted bacteriophage lysate (2,000 PFU) was added and, after a 2-h incubation at 37°C, 1 drop of midlog-phase host culture containing about 5×10^7 cells and 2.5 ml of soft tryptone agar were added, and the entire mixture was overlaid on fresh tryptone agar to determine the viable bacteriophage.

Reaction of bacteriophages with antibodies. To demonstrate the inactivation of bacteriophages by antiphage TC45 sera, 0.2 ml of 10^{-1} or 10^{-2} diluted antisera was added to 50 μl of a diluted bacteriophage lysate containing about 2,000 PFU, and the mixture was incubated at 37°C for 15 min. The titer of surviv-

ing bacteriophage was determined by the soft-agar overlay technique.

To demonstrate the protection by specific antisera of bacteriophage K3 from inactivation by protein E, 10 μl of antiprotein E sera was first mixed with 100 μl of protein E (0.4 mg/ml). After 15 min of incubation at 37°C, 40 μl of diluted bacteriophage K3 lysate containing about 2,000 PFU, 30 μl of LPS (1 mg/ml), and 20 μl of $MgSO_4$ (0.1 M) were added to the mixture, and the number of viable bacteriophage was determined. The bacteriophage titer obtained was compared with that from similar mixtures containing protein E and either preimmune serum or no serum.

Chemical analysis. Protein concentration was determined by the method of Lowry et al. (19). The amount of 2-keto-3-deoxyoctonate was measured as described by Osborn et al. to determine the LPS content (24).

RESULTS

Purity of proteins E and II*. Triton-EDTA-solubilized outer membrane material prepared from strain JF694 contained mainly proteins E and II*. After DEAE-cellulose chromatography, the ethanol precipitates of pooled protein fractions were examined to determine the content of protein and LPS, and the results are shown in Table 2. The level of other proteins in the pooled fractions containing protein E or II* was determined from densitometer tracings of stained polyacrylamide gels after electrophoresis.

Protein E used in this study was prepared in a batch (3,000 mg of EDTA-Triton-extracted outer membrane proteins chromatographed on a 2.5- by 100-cm column) such that this preparation not only contained significant amounts of LPS, but also 1.5% of the total protein was protein II*. These data are summarized in Table 2 (column 1 preparation). A portion of this fraction of protein E (column 1) was stored at 2°C.

Protein II*, which was eluted from column 1 at a lower NaCl concentration than protein E, contained large quantities of LPS but no detectable protein E (Table 2).

A portion of protein E (column 1) material was rechromatographed on a second DEAE-cellulose column under conditions identical to those with the first column. After ethanol precipitation the purity of protein E (column 2) was markedly increased. It contained no detectable protein II* or other proteins, and the molar ratio of LPS to protein (assuming one polysaccharide chain for each LPS molecule) had been reduced from 1.40 to 0.3 as compared with protein E (column 1).

Protein E inactivates bacteriophage K3. Purified protein E eluted from column 2, containing no protein II*, demonstrated an ability to neutralize bacteriophage K3. Purified protein II*, obtained from column 1, contained a substantial amount of LPS. This protein II* preparation was also able to inactivate bacteriophage K3. Based on equivalent quantity of proteins used under optimum reaction conditions, the capacity of protein E preparations to inactivate bacteriophage K3 was apparently higher than similar protein II* preparations. The bacteriophage K3-inactivating capacity of column 1 protein E, column 2 protein E, and protein II* was determined, and the results are summarized in Table 3. These results suggest that the capacity of protein E preparations to inactivate bacteriophage K3 is not due to a low level of contamination by protein II* (see below).

Our first experiment showed that protein E (column 2) containing no detectable protein II* inactivated bacteriophage K3; however, the inactivation capacity of this protein E preparation was lost after storage at −20°C for 1 week. Most experiments used protein E (column 1), so that inactivation of bacteriophage K3 by this preparation may have been due to the 1.5% protein II* that contaminated the protein E (column 1) preparation. To test this hypothesis, we mixed protein E (column 2), inactivated after storage at −20°C, and a biologically active preparation of protein II* in a ratio of 98.5:1.5 to obtain a mixture similar to protein E (column 1). The ability of this mixture to inactivate bacteriophage K3 was then determined. The results, summarized in Fig. 1 (lower curve), showed that the capacity of this mixture to inactivate bacte-

TABLE 2. *Purity of proteins E or II* precipitated with ethanol after DEAE-cellulose chromatography*

Protein	DEAE-cellulose column	Concn (mg/ml)				% of total protein[a]			
		Protein E	Protein II*	Other proteins	LPS	Protein E	Protein II*	Other proteins	LPS
Protein E	1	6.0	0.09	<0.05	1.5	97.7	1.5	<0.8	19
Protein E	2	11.2	<0.005	<0.05	0.6	99.5	<0.04	<0.4	5
Protein II*	1	<0.001	3.3	0.08[b]	2.5	<0.03	97.6	0.24	42

[a] Percentage of individual protein(s) relative to the total protein in the ethanol precipitates except LPS, where the numbers represent the percentage of the total weight of ethanol precipitates.

[b] The protein II* preparation contained a visible protein band which migrated after electrophoresis more rapidly than II*. This band is probably a degradation product of protein II*.

TABLE 3. *Inactivation of bacteriophage K3 by protein E or II**

Protein (40 μg)	LPS (30 μg)	Surviving bacteriophage (N/N_0)[a]
Protein E (column 1)	−	0.11
Protein E (column 1)	+	0.002
Protein E (column 2)	−	0.40
Protein E (column 2)	+	0.004
Protein II* (column 1)	−	0.01
Protein II* (column 1)	+	0.009
None	+	0.94

[a] N_0, Initial number of bacteriophage K3; N, final number of bacteriophage K3.

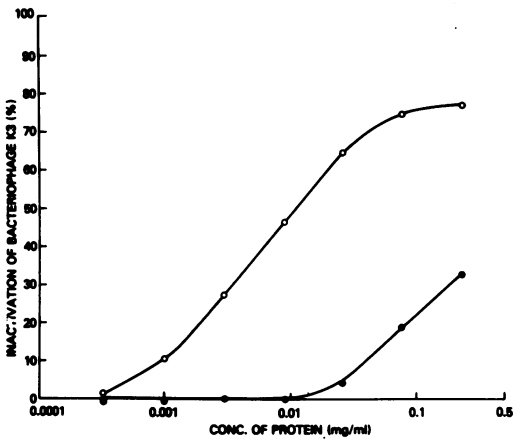

FIG. 1. *Inactivation of bacteriophage K3 by protein E. Protein E at different concentrations was added with bacteriophage K3 and $MgSO_4$ (10 mM). After incubation, the number of surviving bacteriophages was determined as described in the text. LPS was not added. Symbols:* (○) *protein E collected after column 1 chromatography;* (●) *protein mixture by mixing 98.5 parts of protein E (collected after column 2 and inactivated after storage at −20°C) and 1.5 parts of protein II*.*

riophage K3 was much lower than pooled protein E (column 1). These results indicated that the inactivation of bacteriophage K3 was due to protein E and not to the low level of protein II* in the protein E (column 1) preparation.

Role of LPS in the inactivation of bacteriophage K3 by protein E. LPS was shown to enhance the ability of protein E to inactivate bacteriophage K3. LPS itself did not inactivate bacteriophage K3 (Table 4). The bacteriophage inactivation by protein E became greater as the concentration of added LPS was increased. The extent of bacteriophage K3 inactivation increased 50-fold when 30 μg of LPS was mixed with 40 μg of protein E (column 1) (Table 4). The extent of bacteriophage K3 inactivation by protein E (column 2) containing less LPS (Table 2) was increased more than 100-fold by added LPS (Table 3). Assuming three polysaccharide units per molecule of LPS and a molecular weight of approximately 40,000 for protein E, the molar ratio of LPS to protein in protein E (column 1) preparations and purified protein II* would be 0.5 and 1.2, respectively. The highest bacteriophage K3 inactivation capacity of protein E was demonstrated where the ratio of LPS to protein E was 2.0 (or 6.0 based on one polysaccharide unit). This LPS-protein ratio is in agreement with that reported by Datta et al. (8) for the inactivation of bacteriophage TuIa, TuIb, and TuII* by proteins Ia, Ib, and II*, respectively.

The protein E preparation (column 1) containing 19% LPS (wt/wt) was stable at 2°C for more than 6 weeks, whereas the column 2 preparation of protein E containing 5% LPS lost all bacteriophage K3-inactivating activity within 1 week at −20°C. This indicates that LPS may stabilize the bacteriophage-inactivating capacity during storage.

Pooled protein E (column 1) effectively neutralized bacteriophage K3. Without additional LPS, the ability of protein E (column 1) to inactivate bacteriophage K3 increased with the concentration of protein E added in the reaction mixture up to a protein E concentration of 0.2 mg/ml (Fig. 1, upper curve). Above this concentration the ability of protein E to inactivate bacteriophage K3 did not increase. This may reflect a tendency of protein E to aggregate at higher concentrations.

Rate of inactivation of bacteriophage K3 by protein E. Under our experimental conditions, after a 30-min incubation at 37°C about 95% of the added bacteriophage K3 was inactivated (Fig. 2). After a 2-h incubation more than 99.8% bacteriophage particles were inactivated. When protein E was omitted, no change in viability of bacteriophage K3 after a 2-h incubation at 37°C was observed.

TABLE 4. *Effect of LPS on activity of protein E to inactivate bacteriophage K3*

Protein E (μg)	LPS (μg)	Surviving bacteriophage (N/N_0)[a]
0	0	1.00
0	100	0.87
40	0	0.11
40	1	0.11
40	3	0.11
40	10	0.09
40	30	0.002
40	100	0.01
40	300	0.07

[a] See footnote, Table 3.

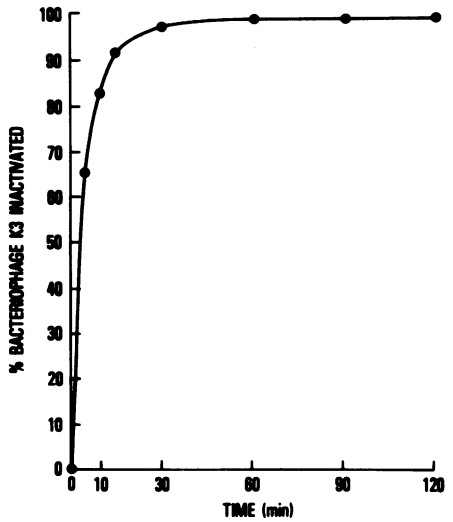

FIG. 2. *Inactivation of bacteriophage K3. A 0.5-ml amount of tryptone broth containing 0.2 mg of protein E, 0.15 mg of LPS, 4 × 10⁴ phage K3, and 10 mM $MgSO_4$ was incubated at 37°C. Samples were taken as indicated to determine the viable phage.*

The bacteriophage inactivation constant, k, was calculated by using the formula, $\log P_t/P_0 = kct$, where P_0 and P_t represent the number of PFU at times 0 and t, and c is the concentration of protein (micrograms per milliliter). According to this equation, the inactivation constant k calculated for protein E (column 1) was 5×10^{-3} ml/min per μg. Bacteriophage inactivation was apparently irreversible, since a further 10-fold dilution of protein-phage mixture did not alter the bacteriophage infectivity.

Protection of bacteriophage K3 by antiprotein E sera. An Ouchterlony double-diffusion experiment showed a single sharp immunological precipitin band between antiprotein E serum and pure protein E. When 40 μg of protein E (column 1) in 0.1 ml was incubated for 15 min with 10 μl of specific antibody and 2,000 PFU of bacteriophage K3 was added, the bacteriophage titer was not reduced (Table 5). However, the same antisera failed to protect bacteriophage K3 from the inactivation by protein II*. This showed that it was protein E (column 1) and not the 1.5% contaminating protein II* present in this preparation which was responsible for the inactivation of bacteriophage K3. It also showed that antiprotein E sera did not alter the ability of protein II* to inactivate bacteriophage K3.

Inactivation of various bacteriophages by protein E. The ability of protein E to inactivate various bacteriophages was determined (Table 6). Each of these bacteriophages uses an individual major outer membrane protein (E, Ia, Ib, or II*) as at least a part of its receptor (Table 1). Protein E inactivated bacteriophages K3, TC45, and TC23. LPS enhanced this inactivation. Protein E did not inactivate bacteriophages TuIa, TuIb, and Pa-2. The inactivation of bacteriophage TuII* by protein E was marginal but reproducible. It was not enhanced by LPS.

Since protein E effectively inactivated bacteriophage K3, we expected that it would also inactivate bacteriophage TuII* because both of these bacteriophages use protein II* as at least part of their receptor (8, 33). The data in Table 6 show that protein E inactivated bacteriophage K3 much more effectively than TuII*. This suggests that either (i) these bacteriophages interact with different sites on protein II* in vivo and protein E in vitro contains a site similar to one of them, or (ii) these bacteriophages interact with the same site in protein II* in vivo and protein E in vitro contains a similar site but the structural requirements for receptor function are more stringent for bacteriophage TuII* than for bacteriophage K3.

We have reported previously that bacteriophage TC45 was not inactivated by protein E even in the presence of added LPS (7). These experiments were performed with a preparation of protein E similar to that described here from column 2 and stored at −20°C. These conditions

TABLE 5. *Protection of bacteriophage K3 by antiprotein E sera from inactivation by protein E or II**

Protein (40 μg)	Antiprotein E sera (μl)	% Phage K3 protected
None	10	100
Protein E	0	0
Protein E	10	105
Protein E	2	66
Protein II*	0	2
Protein II*	10	6
Protein II*	2	5

TABLE 6. *Inactivation of various bacteriophages by protein E*

Bacteriophage	LPS (100 μg)	Surviving bacteriophage (N/N_0)[a]	
		Protein E (40 μg)	LPS (100 μg) + protein E (40 μg)
TC45	1.00	0.30	0.06
TC23	1.00	0.24	0.04
K3	1.00	0.11	<0.02
TuII*	1.00	0.48	0.46
TuIa	1.00	1.00	1.00
TuIb	1.00	1.00	1.00
PA-2	1.00	1.00	1.00

[a] See footnote, Table 3; 1.00 = >0.90.

(low level of LPS and low temperature) result in the loss of the ability of protein E to inactivate bacteriophages K3 and TC45.

Antigenic relationship among bacteriophages. Antisera prepared with bacteriophage TC45 as an antigen were examined for their ability to inactivate other bacteriophages. The results (Table 7) indicate that anti-TC45 sera inactivated bacteriophages TC45, TC23, K3, and TuIa effectively.

After removing the nonspecific antibodies and other impurities, purified antiphage TC45 antibodies still showed significant activity against bacteriophages TC23 and K3, although K3 was somewhat less affected, but had completely lost the capacity to inactivate bacteriophage TuIa (Table 7). Evidently bacteriophage TuIa inactivation by crude antisera was due to the reaction of a nonspecific antibody in the anti-TC45 sera. The results demonstrated that three phages, K3, TC45, and TC23, all contained a common antigenic determinant(s).

DISCUSSION

Outer membrane protein E is distinct from protein II* both in chemical properties and physiological functions. Protein E is a peptidoglycan-associated protein with a molecular weight of about 39,000 that forms transmembrane channels for the passive transport of various small hydrophylic molecules (11; Foulds and Chai, unpublished data). Protein II*, which is heat modifiable and is partially protease sensitive, has an apparent molecular weight of about 33,000 after heating at 100°C in SDS-containing solutions. It participates in conjugation and colicin L tolerance (10, 30) and serves as at least part of the receptor for bacteriophages K3 and TuII*. The ability of protein E to inactivate bacteriophage K3 indicates that a region in protein E may be similar to one in protein II*. The receptor site for bacteriophage K3 might reside in the peptide region. Another possibility is that the bacteriophage receptor could be located on a carbohydrate moiety; e.g., protein II* contains one or two sugars (6, 28). The common region could also be a site of interaction with LPS.

The bacteriophage K3 inactivation is caused by protein E but not by protein II*, which contaminated some protein E preparations. Three lines of evidence support this conclusion: (i) purified protein E containing no detectable protein II* effectively inactivates bacteriophage K3; (ii) a mixture of purified protein E which had lost its bacteriophage K3-inactivating activity and protein II*, compositionally identical to protein E preparation (column 1), has a much lower capacity to inactivate the bacteriophage K3 than the active protein E (column 1) preparation itself; and (iii) antiprotein E serum only protects the inactivation of bacteriophage K3 by protein E but not by protein II*.

In their studies of bacteriophage receptor activity of proteins II*, Ia, and Ib, Datta et al. (8) report that the proteins or LPS alone show no capacity to inactivate bacteriophages. The inactivating capacity can only be demonstrated when proteins and LPS are mixed.

We have shown in this report that protein E can inactivate bacteriophage K3. Since we were unable to obtain protein E completely free of LPS, we are unable to conclude that protein E alone is sufficient for this inactivation. Nevertheless, our most purified protein E preparation contains an amount of LPS-protein E (molar ratio of 0.3, assuming LPS contains a single polysaccharide chain) which is less than the minimal level of LPS required for the bacteriophage inactivation by membrane proteins reported by Datta et al. (8).

The function of LPS in the bacteriophage inactivation by receptor protein is not known. LPS may serve as a solvent to prevent the aggregation of functional protein. LPS apparently stabilizes protein E during storage at low temperatures. LPS and protein E at the optimum ratio may form micellular structures that provide the appropriate orientation for the inactivation of bacteriophages in vitro and maintain this biological activity during cold storage. Also, the possibility cannot be ruled out that LPS itself might be a part of the bacteriophage receptor, or it could serve in a synergistic capacity for receptor activity.

The major outer membrane proteins in *E. coli* strains are exposed on the outer surface of the membrane, because these proteins serve as receptors for various bacteriophages, bacteriocins,

TABLE 7. *Inactivation of various bacteriophages with crude or purified antiphage TC45 serum*

Antiserum dilution	% Bacteriophage inactivated						
	TC45	TC23	K3	TuIa	TuIb	PA-2C	TuII*
Crude, 10^{-1}	100	100	93	98	29	15	0
Crude, 10^{-2}	100	100	58	45	0	0	0
Crude, 10^{-3}	98	99	18	5	0	0	0
Purified, 10^{-1}	73	82	33	0	0	0	0

and some nutrients. Most multifunctional receptor proteins contain different active sites for different substances that do not interfere with each other in receptor activity. The *lamB* protein serves as a receptor for bacteriophage λ (25) and is involved in the diffusion of maltose or maltotriose across the outer membrane barrier (32). The *tsx* protein serves as a receptor for bacteriophage T6 and functions in nucleotide transport (14). Certain receptor proteins might contain a closely related cluster of receptor sites or possibly even a single site shared by different substances as a receptor. In such cases the absorption of one substance will interfere in the absorption of the other(s). For example, a single receptor protein is involved in the binding of bacteriophage BF23, vitamin B_{12}, and group E colicins. These substances mutually compete for the binding to the same outer membrane protein. *bfe* mutant strains, missing this protein, are defective in all these functions (3, 4). The function of protein E in promoting the diffusion of small hydrophilic molecules and as the receptor site for both bacteriophages TC45 and TC23 suggests that protein E belongs to the second group.

The receptor site for bacteriophage K3 in protein E is probably different from that for bacteriophages TC45 and TC23. Bacteriophage P1-mediated *nmpA* (protein E^+) *ompA* (protein II^{*-}) transductants were resistant to bacteriophage K3 but were sensitive to bacteriophage TC45 (unpublished data). This indicates that the receptor on protein E for bacteriophages TC45 and TC23 is exposed on the outer surface of the cells, whereas the receptor for bacteriophage K3 is exposed only after solubilization.

Bacteriophages K3 (22), TC45, and TC23 (7) are structurally similar to T-even bacteriophages. Bacteriophages TC45 and TC23 both specifically use protein E at least as a part of their receptors, but they are different in plaque morphology, infectivity, and bacteriophage density. All three bacteriophages, K3, TC45, and TC23, have at least a common or related antigenic determinant that stimulates the formation of a common antibody. A more definite conclusion on the related antigenic determinant(s) among these bacteriophages cannot be made until reciprocal studies are accomplished to establish the relationship between these bacteriophages and antibodies prepared with bacteriophages other than TC45.

This is the first report that two different outer membrane proteins prepared from a single *E. coli* strain can inactivate the same bacteriophage. It also is the first demonstration that protein E in vitro can inactivate distinct bacteriophages that use different proteins as receptors. We conclude that protein E in vivo has a hidden bacteriophage K3 receptor that is not exposed to the external medium. It will be interesting to determine if portions of proteins E and II* do, in fact, have similar structures. This information will provide further understanding of the relation of protein E to other major outer membrane proteins. For example, there may have been a common precursor for major outer membrane proteins, a single gene which has been duplicated and then altered by mutation leading to the production of different major outer membrane proteins. One could imagine that gene duplication could give a strain a selective advantage since peptidoglycan-associated proteins play an important role in diffusion of metabolites through the outer membrane barrier.

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