JOURNAL OF BACTERIOLOGY, Aug. 1977, p. 608-622
Vol. 131, No. 2

Printed in U.S.A.

Outer Membrane Proteins of *Escherichia coli*
VI. Protein Alteration in Bacteriophage-Resistant Mutants

PHILIP J. BASSFORD, JR., DANA L. DIEDRICH, CARL L. SCHNAITMAN,* AND PETER REEVES

Department of Microbiology, University of Virginia Medical School, Charlottesville, Virginia 22901, and Department of Microbiology, The University of Adelaide, Adelaide, South Australia 5001*

Received for publication 26 May 1977

Protein 1 was shown to be the receptor for phage PA-2 by the observations that the purified protein inactivates the phage, mutants lacking the protein are resistant to the phage, and mutants selected for PA-2 resistance have altered protein. Protein 1 appears as two bands (1a and 1b) on high-resolution polyacrylamide gels. The most abundant classes of mutants (ParI and ParII) selected for PA-2 resistance were found to lack band 1b. The mutations responsible for the ParI and ParII phenotypes were mapped at a locus termed *par*, which is near *nalA* on the *Escherichia coli* chromosome. The cyanogen bromide peptides of proteins 1a and 1b are similar, suggesting that these bands represent modified forms of the same polypeptide. Strains carrying the *tolF* mutation produce only band 1b. When a *par tolF* double mutant was constructed, this strain produced only band 1a. These results suggest that genes at the *par* and *tolF* loci are involved in modification of protein 1, or regulation of such modification, and are not structural genes for protein 1.

Escherichia coli lysogenic for the lambdoid phage PA-2 produces a new, major outer membrane protein ("protein 2") that is not produced by nonlysogenic cells (21). When wild-type lysogenic cells are grown under conditions of low catabolite repression, protein 2 almost entirely replaces protein 1, which is the major outer membrane protein of nonlysogenic cells. Proteins 1 and 2 are very similar in structure and presumably in function. They are, however, distinctly different polypeptides (8).

This unusual form of lysogenic conversion, in which a protein coded for or directed by a prophage replaces a normal protein on the cell surface, suggested that the phage receptor might be the protein that is replaced during the lysogenic state. Preliminary results on the rate of adsorption of phage to lysogenic and nonlysogenic cells (21) indicated that this assumption might be correct. Lysogenic cells grown under conditions where they made little protein 1 exhibited a reduced rate of phage adsorption. We were unable to test this directly at that time, first because no mutants lacking protein 1 were available, and second because the high lysogenization frequency of phage PA-2 prevented the use of wild-type phage for the selection of resistant bacterial mutants. These problems have now been overcome, both by the isolation of mutants lacking protein 1 and by the isolation and construction of clear-plaque or virulent phage having the PA-2 host range that may be used to select resistant bacterial mutants. This report describes in detail evidence that protein 1 is a component of the receptor for phage PA-2 and some of the properties of PA-2-resistant mutants.

MATERIALS AND METHODS

Bacteria and bacteriophage. Bacterial strains for this study are described in Table 1, and the bacteriophage are described in Table 2. Cultures of *E. coli* used for isolation of outer membrane protein for phage neutralization or for isolation of proteins 1a and 1b were grown on minimal medium with either [^{3}H]- or [^{14}C]leucine and 0.5% lactic acid as the carbon source, as previously described (23). Cultures grown for electrophoretic analysis of outer membrane proteins were grown on the medium described above with either lactic acid or 0.5% glucose as carbon source, or on various complex media as indicated in Results. Minimal medium for plates was medium A of Davis and Mingioli (7) supplemented with appropriate amino acids, vitamins, and carbon sources as required. Bacteriophage were assayed and propagated on Luria broth (LB) as previously described (21), with the exception that 0.05% maltose was added to the seed culture medium and to the soft agar overlay in the case of hy7.

Construction of hybrid phage. An overnight shaken culture of W1485F$^-$ in LB was diluted 1:25 in tryptone broth (16) and shaken for 1 h at 37°C. To 2 ml of this culture 0.5 ml of tryptone broth containing 8×10^8 plaque-forming units (PFU) of each phage to be crossed was added, and this was allowed to stand for 20 min at 37°C. This mixture was diluted 1:100

TABLE 1. *Bacterial strains*

Strain	Genotype or phenotype[a]	Source or derivation
Escherichia coli K-12 strains:		
W1485F⁻	As W1485 (CGSC 5024), except F⁻	(21)
PB101	As W1485F⁻, except *par-1*; EMS-induced ParI mutant	This study
PB102	As W1485F⁻, except *par-2*; spontaneous ParI mutant	This study
PB103	As W1485F⁻, except *par-3*; spontaneous ParII mutant	This study
PB104	As W1485F⁻, except *par-4*; EMS-induced ParIII mutant	This study
PB105	*nalA* Par⁺	From W1485F⁻ by P1*kc* transduction with a *nalA* derivative of PB101
PB106	*nalA par-1* (ParI)	As PB105
PB107	*nalA par-3* (ParII)	From W1485F⁻ by P1*kc* transduction with a *nalA* derivative of PB103
PB108	EMS-induced TolIV mutant in W1485F⁻	This study
PB109	EMS-induced TolXIV mutant in W1485F⁻	This study
AB1133	F⁻ *thi argE his proA thr leu mtl xyl ara galK lacY rpsL supE*	CGSC
P210	As AB1133, except TolIb	(6)
P460	As AB1133, except *his*⁺ *non ompA*	(22)
P530	As AB1133, except TolXIV	(6)
P692	As AB1133, except TolIV	(6)
P783	Δ*lamB*	W. Woods
JF568 *tolF4*	*ilv-277 metB65 his-53 purE41 proC24 cyc-1 xyl-14 lacY29 rpsL77 tsx63 tolF4*	J. Foulds
PB1002	*trp*(Am) *lacZ*(Am) *val*ʳ *supD74*(Ts)	From MX364 of M. Oeschger by conjugation with MX419 of M. Oeschger
Lin221	CGSC 4861; Δ*phoA8 glpT6 relA1 tonA22*	CGSC
DF2000	HfrC *thi pgi zwf*	R. Kadner
M226	CGSC 4997; *trp-50 galU106 rel-1 rpsL150*	CGSC
D31m3	CGSC 5166; *ampA1 rpsL174 his-51 trp-30 galU50 lac-28 pro-23 tsx-81*	CGSC
E. coli B strains:		
B(Hill)	ATCC 23225; wild type	ATCC
BE	Wild type	J. Rosenbusch
B837	r_B^- m_B^+ *gal met* λ-sensitive (24)	R. Benzinger

[a] Abbreviations are those of Bachmann et al. (2). CGSC, *E. coli* Genetic Stock Center, Yale University. ATCC, American Type Culture Collection.

with prewarmed tryptone broth and shaken at 37°C for 90 min. Chloroform was added, and dilutions of this culture were plated on appropriate selective strains (Table 2).

Genetic techniques. Interrupted mating and phage P1*kc* transduction were as described by Miller (16). Spontaneous *nalA* mutants were selected for resistance to 20 μg of nalidixic acid per ml, and the delayed selection technique of Hane and Wood (12) was used to select *nalA* transductants. Acquisition of *glpT*⁺ after transduction was selected for by growth on minimal plates supplemented with 0.4% α-glycerol phosphate as sole carbon source.

Selection of mutants. Mutagenesis with ethyl methane sulfonate (EMS), 2-aminopurine (2-AP), or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was carried out as described by Miller (16). Phage PA-2-resistant mutants were selected by mixing 0.1 ml of phage suspension containing 2×10^{11} PFU of either PA-2*c* or hy2 with 0.1 ml of a mid-log-phase culture of bacteria. This was incubated for 10 min at 37°C, added to soft agar, and plated on LB. Resistant clones were picked, restreaked several times, and tested for phage sensitivity. TolIV and TolXIV were selected in the same fashion from EMS-mutagenized cultures, except that 0.1 ml of colicin E2 (a 1:25 dilution of a stock solution with a titer of 10^5 killing units) was added to the phage-bacteria mixture. Resistant clones were then tested for sensitivity to colicins E1, E2, E3, and K and phage BF23.

TABLE 2. *Bacteriophage strains*

Strain	Genotype[a]	Growth or plaque morphology on:				Source
		W1485F⁻	W1485F⁻·PA-2	P530	P783	
PA-2	Wild type	Small, turbid	NP[b]	NP	Small, turbid	(21)
PA-2*c*-1	PA-2*c*	Small, clear	NP	NP	Small, clear	NTG mutagenesis of W1485F⁻·PA-2
hy2	λ*vir* h^{PA-2}	Large, clear	Large,[c] clear	NP	Large, clear	PA-2 × λ*vir*, selected on P783·PA-2
hy7	PA-2h^{λ}	Small,[d] turbid	NP	Small, turbid	NP	PA-2 × λ, selected on P530·λ
hy8	PA-2*c* h^{λ}	Small, clear	NP	Small, clear	NP	PA-2*c*-1 × λ, clear plaque selected on P530

[a] Genetic symbols: *c*, clear plaque; *vir*, virulent; *h*, host range.

[b] NP, No plaques.

[c] When plated on a PA-2 lysogen on LB without glucose, hy2 yields only 10% of the plaques observed on a nonlysogenic host due to the presence of protein 2. The difference in plating efficiency on lysogenic and nonlysogenic hosts is abolished by the addition of glucose to the medium used for the seed culture and to the soft agar.

[d] Plated on LB + 0.05% maltose. Plaques are very small when maltose is omitted.

The phage mutant PA-2*c-1* was selected by mutagenizing a culture lysogenic for wild-type PA-2 with NTG. After outgrowth of the mutagenized culture, the culture supernatant fluid was screened for phages forming clear plaques.

Colicin sensitivity was determined by spot testing (17), and phage sensitivity was determined by cross-streaking, by reduction in plating efficiency, or by the multiple-spot test (25).

Envelope isolation and fractionation. Mid-log-phase cultures were broken by passage through a French pressure cell; the envelope was isolated by centrifugation, and cytoplasmic membrane proteins were removed by extraction with Triton X-100 (20). The Triton-extracted envelope was dissolved directly in the appropriate solubilizing solution when samples were to be analyzed by sodium dodecyl sulfate (SDS)-gel electrophoresis. Isolation of envelope fractions for phage neutralization involved differential extraction with SDS plus Mg^{2+} followed by extraction of protein 1 by Triton X-100 plus ethylenediaminetetraacetic acid (EDTA), as previously described (8). When protein 1 was to be separated into 1a and 1b components, the same procedure was followed except that the Triton-EDTA-solubilized protein was precipitated with ethanol and dissolved in tris(hydroxymethyl)aminomethane(Tris)-Triton-EDTA buffer (6), and 46 mg of the protein was applied to a 100-ml column of quaternary aminoethyl (QAE) Sephadex A-50 equilibrated in the same buffer. This column was washed with 200 ml of the same buffer, followed by 600 ml of a salt gradient of from 0 to 0.2 M NaCl in the same buffer. Peak fractions enriched for protein 1b were identified by SDS-gel electrophoresis (thin-slab system) and were pooled and precipitated with ethanol. These were dissolved as above and applied to a 50-ml QAE-Sephadex column. This was eluted with 100 ml of buffer followed by a 300-ml salt gradient as above. The peak fraction of protein 1b was again precipitated, and it yielded 8 mg of protein.

Phage neutralization by isolated envelope fractions. A mixture consisting of 0.1 ml of various dilutions of envelope fractions, 0.2 ml of LB containing 2.5 mg of bovine serum albumin per ml, and 0.6 ml of LB was warmed to 37°C. To this was added 0.1 ml of LB containing 10^8 PFU of PA-2*c*, and this was incubated with gentle mixing for 60 min at 37°C. The tubes were then chilled on ice, diluted, and plated to determine residual viable phage. Controls consisting of the various buffers and detergent solutions in which the envelope fractions were suspended or dissolved showed no phage inactivation.

SDS-polyacrylamide gel electrophoresis. Samples for analysis in 5-mm cylindrical gels were prepared by boiling in an SDS-EDTA solution, as previously described (20), followed by electrophoresis in the alkaline phosphate-buffered system of Bragg and Hou (20). Samples were also analyzed in a high-resolution thin-slab system. The thin-slab system was the discontinuous buffer system of Laemmli (13) and incorporated modifications suggested by Ames (1) and Lugtenberg et al. (14). The thin-slab gels were 0.75 mm thick and 30 cm long, contained 11.5% acrylamide, 0.21% bisacrylamide, 0.1% SDS, and 3.0% urea, and were prepared in 0.375 M Tris adjusted to pH 8.8 with hydrochloride. The gel was polymerized by the addition of 40 μl of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and 15 mg of ammonium persulfate per 100 ml of acrylamide solution. The stacking gel contained 2.5% acrylamide, 0.625% bisacrylamide, 0.1% SDS, and 10 μg of riboflavin per ml in 0.125 M Tris adjusted to pH 6.8 with hydrochloride. TEMED (25 μl/100 ml of solution) was added, and the stacking gel was photopolymerized. The electrophoresis buffer (final pH, 8.3) contained 3.08 g of Tris, 14.4 g of glycine, and 1.0 g of SDS per liter. Samples were dissolved at a concen-

tration of 1 to 3 mg/ml in a solution containing 12.5% glycerol, 1.25% SDS, and 1.25% 2-mercaptoethanol in 0.0625 M Tris buffer adjusted to pH 6.8 with hydrochloride. Samples were boiled for 5 min prior to electrophoresis. Electrophoresis was carried out at a constant voltage of 60 V until the samples entered the running gel, followed by 170 V for 14 h. Gels were stained by the method of Fairbanks et al. (9).

RESULTS

Neutralization of phage PA-2 by isolated envelope fractions. When the envelope of *E. coli* is extracted sequentially with Triton X-100, SDS plus Mg^{2+}, and Triton X-100 plus EDTA by a scheme previously described for purification of protein 1 (8), those fractions that are enriched for protein 1 exhibit phage neutralization activity (Fig. 1 and 2). Within the limitations of the neutralization assay, phage neutralization activity appears fully conserved in the pooled protein 1 fraction obtained after chromatography of the solubilized Triton-EDTA-solubilized material on diethylaminoethyl (DEAE)-cellulose. Examination of this pooled protein 1 fraction by gel electrophoresis (data not shown) indicated that it consisted almost entirely of protein 1 with a minor contamination by protein 3a.

Our previous studies of this DEAE-cellulose chromatography system (18) indicate that free lipopolysaccharide (LPS) is not retained on the column. However, protein 1, after DEAE-cellulose chromatography, does still contain significant amounts of phosphate, indicative of contamination by LPS. Thus, while these data tend to rule out LPS alone as the receptor for PA-2, they do not eliminate the possibility that the receptor is a complex of protein 1 and LPS.

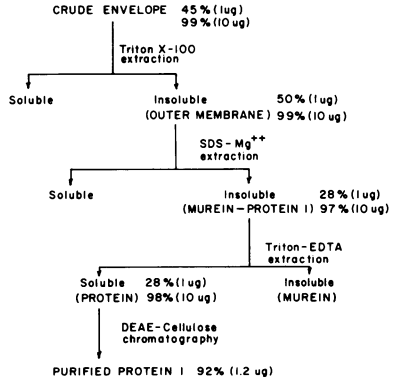


FIG. 1. *Flow scheme illustrating steps in the isolation of protein 1 from the crude envelope fraction and phage neutralization activity of various fractions enriched for protein 1. Percent figures indicate percentage of inactivation of phage, and numbers in brackets indicate amount of protein in the inactivation assay as described in Materials and Methods. Details of the fractionation are as described in (8) and in the text.*

Phage PA-2 sensitivity of mutant strains with cell wall defects. Table 3 shows the sensitivity of a number of *E. coli* strains with cell wall defects to PA-2. These mutant strains were all selected for resistance to colicins or to lethal zygosis. Strain P460, which carries a mutation in *ompA* and produces no protein 3a (15), was sensitive to PA-2, and in fact showed a slightly higher plating efficiency for the phage than did the parent strain AB1133. Two strains that produce reduced amounts of protein 1 (P210, which has the phenotype TolIb, and JF568 *tolF4*) were still phage sensitive. However, strains P530 (TolXIV) and P692 (TolIV),

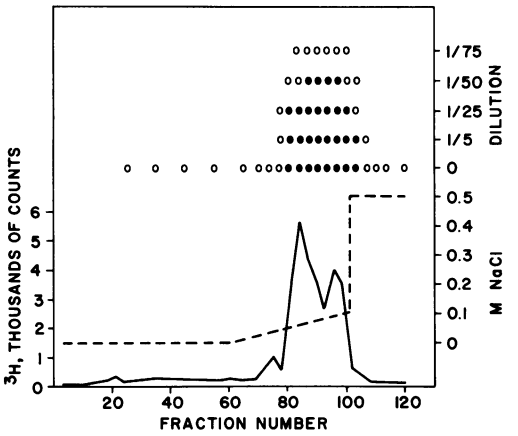


FIG. 2. *Coelution of phage neutralization activity and protein (solid line) from a DEAE-cellulose column. The circles shown in the upper portion of the figure indicate the various dilutions of the column fractions assayed for phage neutralization, and the filled circles indicate greater than 50% inactivation of the phage. The dashed line indicates the NaCl gradient. The sample applied to the column was the Triton-EDTA-solubilized protein from the SDS-Mg^{2+}-insoluble fraction as illustrated in Fig. 1.*

TABLE 3. *Sensitivity of various strains to phage PA-2*

Strain	Phenotype	PA-2 sensitivity[a]
AB1133	Wild type	S
P210	TolIb; low protein 1	S
P460	OmpA; no protein 3a	S
JF568 *tolF4*	TolF; low protein 1	S
P530	TolXIV; no protein 1	R
P692	TolIV; no protein 1	R

[a] S, Plating efficiency with respect to wild type of greater than 0.75. R, Plating efficiency of less than 0.01.

which we have found to produce no protein 1, were resistant to the phage.

Spontaneous mutants resistant to phage PA-2. The inability to obtain mutants resistant to PA-2 because of the high frequency of lysogeny by the wild-type phage was overcome by the isolation of a clear-plaque mutant, phage PA-2*c*, and by the construction of a hybrid of PA-2 and λ*vir*, hy2, which has a Vir phenotype but a PA-2 host range (Table 2). We have used PA-2*c* and hy2 interchangeably for the selection of resistant mutants, since both seem to give the same mutant classes in the same frequency.

We first examined spontaneous PA-2-resistant mutants selected in W1485F⁻. Ten such mutants were examined in detail, and seven were found to fall in a phenotypic class we designate as ParI, whereas three fell in a related phenotypic class we designate as ParII. When ParI and ParII outer membrane proteins were compared with those of the parent by the alkaline Bragg-Hou gel system, it was found that the amount of protein 1 was greatly reduced in the mutants. As will be described later, the extent of reduction of protein 1 was strongly influenced by the growth medium. The ParI and ParII phenotypes differ in two respects: first, when both classes of mutants are lysogenized by hy7, the outer membranes of the ParI mutants contained an amount of protein 2 comparable to that produced by the wild-type parent, whereas the outer membrane of the ParII mutants contained no detectable protein 2 (Fig. 3); second, the ParI mutants exhibited tight resistance to phage PA-2, whereas the ParII mutants were all slightly leaky. Since both of these classes are resistant when tested by cross-streaking and will not plaque hy2 or PA-2*c*, the tightness of resistance was tested: lawns of hy7 lysogens of ParI and ParII mutants were spotted with a broth suspension of hy2 at a concentration of 10^6 PFU/ml. No plaques were observed with lysogens of ParI mutants, but large clear plaques of λ*vir* were observed with lysogens of ParII mutants, indicating that a few hy2 phage had managed to infect the mutant cells where they recombined with hy7 to regenerate λ*vir*.

Both ParI and ParII mutants grew the same as the parent strain on both minimal and complex liquid medium and exhibited normal growth and colony morphology on LB agar, lactose eosin-methylene blue agar, and MacConkey agar. The ParI and ParII mutants have the same sensitivity towards colicins E3 and K as the parent, and by cross-streaking appear to be equally sensitive to phages T4, T6, λ*c*, φ80*vir*, and BF23.

More extensive testing of phage and colicin sensitivities of ParI mutants has been done. Sensitivity of the ParI mutants was identical to that of the parent strain towards colicins E1, E2, K, L, A, S4, and X of group A and colicins B, D, Ia, Ib, and M of group B. The sensitivity of the ParI strain PB106 and the isogenic Par⁺ strain PB105 towards a large number of phages was tested by the multiple-syringe spot test technique (25). Both of these strains and the parent strain W1485F⁻ exhibited identical sensitivity to T2, T5, K10, H3, H1, Oxl, K9, K3, K4, K5, Ac3, Ox3, Ml, Ox2, Ox4, K2, K20, K21, K29, Ox5, K16, F27, K17, E11, φ1, and K15. The ParI mutant differed from Par⁺ strains in that it was resistant to E7 and gave normal sensitivity towards phages H8, K18, and K31, while Par⁺ strains gave turbid plaques with these latter three phage. Both the ParI mutant and Par⁺ strains were resistant to phages T3, E4, and C21 and gave turbid plaques with phages A, K19, W31, and H. A number of spontaneous Par mutants (phenotype not identified) were isolated in strain AB1133, and these were specifically resistant to phage E7.

EMS-induced PA-2 resistant mutants. To obtain more diverse phenotypes, we examined EMS-induced mutants. This mutagen was found to increase the frequency of mutation to

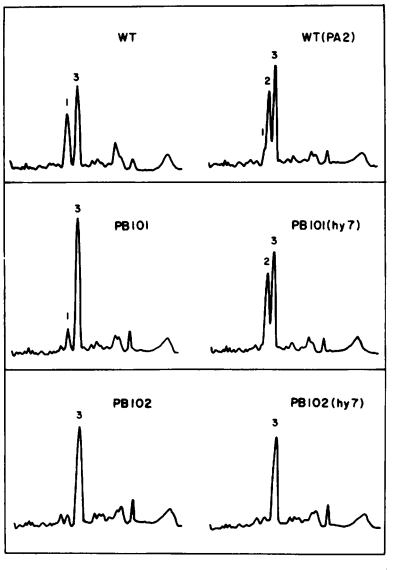


FIG. 3. *Scans of stained alkaline (Bragg-Hou) SDS-tube gels of the outer membrane proteins of the wild-type (W1485F⁻), ParI (PB101), or ParII (PB102) mutants and the corresponding strains lysogenic for either PA-2 or hy7. The cultures were grown on Trypticase soy broth.*

PA-2 resistance more than 100-fold. We examined in detail nine such mutants selected in W1485F⁻. Of these, one was missing protein 1 completely and was resistant to λc as well as PA-2. This strain was unstable and was lost on transfer. Of the remainder, four were ParI and two were ParII. Two other mutants were of a class we designated as ParIII. These mutants are slightly mucoid, although still sensitive to T7 and hence not *lon*. Some inhibition of growth was noted when these mutants were streaked against hy2 or PA-2*c*, although the latter phage gave no plaques even when plates were seeded with as many as 10^{10} PFU. ParIII mutants exhibit a normal outer membrane profile when examined by gel electrophoresis with the Bragg-Hou system, and they produced normal amounts of protein 2 when lysogenized with hy7. Unlike ParI and ParII mutants, ParIII mutants exhibit no cotransduction with *nalA* (Table 4).

We attempted to isolate amber mutants resistant to PA-2. Strain PB1002, which carries a temperature-sensitive *supD* mutation, was mutagenized with 2-AP, and mutants resistant to PA-2*c* were selected at 42°C. These were then screened for sensitivity to PA-2*c* at 20°C. Of more than 200 such mutants screened, only 1 was found to be temperature sensitive. This mutant was of the ParI class and was shown to be an amber mutant by transfer of the mutant allele to Sup⁺ and temperature-independent *supD* strains. We do not know the reason for the low recovery of amber mutations by this technique, since a similar isolation scheme yielded large numbers of amber mutations in *tonB* and *bfe*.

Genetic mapping of the *par* locus. Preliminary genetic analyses involving crosses of Par mutants isolated in various Hfr strains indicated a close linkage of ParI and ParII mutations to *nalA*. Table 4 shows a more detailed genetic analysis by P1*kc* transduction. Both ParI and ParII mutations exhibited similar (although not identical) cotransduction frequencies with *nalA*. The reduced cotransduction frequency with *glpT* indicates that the loci of these mutations are clockwise from *nalA* at approximately minute 47.5 of the recent *E. coli* K-12 linkage map (2). We term the locus of these mutations *par* for PA-2 resistance. Figure 4 summarizes the relative arrangement of the *par* locus with respect to *nalA* and *glpT*. The ParIII mutation is apparently not closely linked to *nalA*.

Further characterization of ParI and ParII

TABLE 4. *Genetic analysis of Par mutants by P1kc transduction*

Cross no.	Donor	Recipient	Selected marker (no. scored)	Recombinant class	Cotransduction frequency (%)
1a	PB101 *nalA* (ParI)	Lin221 *glpT*	*glpT*⁺ (300)	*nalA* Par	12.0
				nalA Par⁺	37.0
				nal⁺ Par	0
				nal⁺ Par⁺	51.0
1b	PB101 *nalA* (ParI)	Lin221 *glpT*	*nalA* (400)	Par *glpT*	6.0
				Par *glpT*⁺	20.8
				Par: *glpT*	24.4
				Par⁺ *glpT*⁺	48.8
2	Lin221 *glpT nalA*	PB101 (ParI)	*nalA* (149)	Par *glpT*	23.5
				Par *glpT*⁺	36.2
				Par⁺ *glpT*	24.2
				Par⁺ *glpT*⁺	16.1
3a	PB102 *nalA* (ParII)	Lin221 *glpT*	*glpT*⁺ (226)	*nalA* Par	4.4
				nalA Par⁺	37.2
				nal⁺ Par	0
				nal⁺ Par⁺	58.4
3b	PB102 *nalA* (ParII)	Lin221 *glpT*	*nalA* (208)	Par *glpT*	20.2
				Par *glpT*⁺	7.7
				Par⁺ *glpT*	37.5
				Par⁺ *glpT*⁺	34.6
4	Lin221 *glpT nalA*	PB102 (ParII)	*nalA* (247)	Par *glpT*	38.8
				Par *glpT*⁺	31.6
				Par⁺ *glpT*	15.8
				Par⁺ *glpT*⁺	13.8
5	Lin221 *glpT nalA*	PB104 (ParIII)	*nalA* (98)	Par *glpT*	40.8
				Par *glpT*⁺	59.2
				Par⁺ *glpT*	0
				Par⁺ *glpT*⁺	0

mutants. The amount of protein 1 produced by ParI and ParII mutants depends strongly upon the growth medium. When these mutants are grown on nutrient broth, the amount of protein 1 in the outer membrane as determined by scans of Bragg-Hou gels is 70 to 90% of that produced by the parent or isogenic Par$^+$ transductants. However, when grown on Trypticase soy broth, ParI and ParII mutants produced almost no detectable protein 1, and when the cultures were grown on minimal medium with lactate or glucose as the sole carbon source, the amount of protein 1 was about 30% of that produced by Par$^+$ strains.

The reason for this variation is apparent when the outer membrane proteins of ParI, ParII, and Par$^+$ strains are compared by a high-resolution thin-slab gel system. Protein 1 from a Par$^+$ strain grown on lactate-minimal medium is resolved into two closely spaced bands (Fig. 5). These are designated as bands 1a and 1b and correspond to bands Ia and Ib reported by Schmitges and Henning (19). In agreement with the results of Schmitges and Henning (19), band 1b is absent from outer membranes of *E. coli* BE and all of the other *E. coli* B derivatives and strains in our collection. Protein 1b is also missing from outer membranes of ParI and ParII mutants.

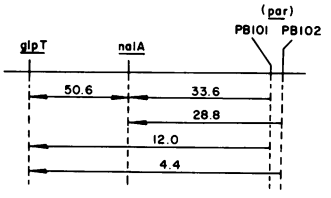


FIG. 4. *Scheme illustrating the relationship of the par locus to nalA and glpT as determined by transduction with P1kc. The numbers indicate percentage of cotransduction (Table 4), and the arrowheads indicate the selected markers.*

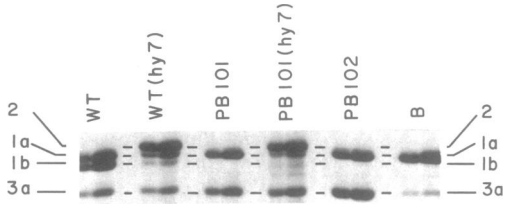


FIG. 5. *High-resolution slab gels (Laemmli buffer system) of the outer membrane proteins of various strains grown on lactate-minimal medium. Only the major protein region of the gel is shown. In this gel system, protein 2 migrates more slowly than either the 1a or 1b forms of protein 1. Strain PB102 (ParII) lysogenic for hy7 was identical to the nonlysogenic PB102 and is not shown. The strain of E. coli B used in this experiment was strain BE.*

The relative amounts of proteins 1a and 1b produced by wild-type *E. coli* K-12 depend upon the growth medium (Fig. 6). When cultures are grown on Trypticase soy broth, there is much more 1b than 1a, and exactly the reverse is observed when the cultures are grown on nutrient broth. Cultures grown on minimal medium with lactate or glucose as the carbon source produce significant levels of both 1a and 1b, with, generally, a slight excess of 1b over 1a. These differences correspond almost exactly to the differences in total protein 1 observed by scanning Bragg-Hou gels of ParI and ParII mutants. The amount of protein 1 produced by these mutants appears proportional to the amount of protein 1a observed in Par$^+$ strains grown under the same conditions. Thus, ParI and ParII mutants lack the ability to produce protein 1b, and they are unable to compensate for this loss by increased production of 1a.

These results suggest a physiological control over the relative levels of 1a and 1b. The control cannot be catabolite repression, since similar amounts of 1a and 1b are made on minimal medium with either lactate or glucose as carbon source. Similarly, this control cannot be due to culture pH, since glucose-grown cultures become more acid, whereas lactate-grown cultures become more alkaline. The reduction of protein 1b in cultures grown in nutrient broth suggests that the production of 1b may require a large pool of some glycolytic intermediate, since this medium has no fermentable carbon source. Figure 7 shows the effect of various supplements on protein 1a and 1b production by cultures grown on nutrient broth. Lactate and serine, both of which feed readily into the 3-carbon "pool", and to a lesser extent glycerol, all enhanced the production of protein 1b, whereas succinate had no effect. This indicates that either a sugar or a 3-carbon intermediate such as phosphoenolpyruvate might regulate or be essential for the formation of 1b.

Since protein 1b might represent a form of protein 1 modified by attachment of a sugar or sugars, we examined the effect of various mutations in sugar metabolism on the production of protein 1b. Protein 1b was formed by strains DF2000, M226, and D31m3 grown on nutrient broth plus lactate. Since these strains are all blocked under these growth conditions in the formation of uridine 5'-diphosphate-glucose, it is evident that protein 1b does not require attachment of glucose or galactose to the protein or carbohydrates derived from these sugars.

These experiments do not rule out modification by attachment of sugars, such as glucosamine or mannose, derived from fructose-6-phosphate.

Growth conditions have other pronounced effects on the outer membrane protein composition (Fig. 6). Cultures grown on minimal medium, regardless of the carbon source, produce large amounts of proteins 1 and 3a. These proteins together account for about 70% of the total outer membrane protein. On complex media, the proteins migrating more rapidly than protein 3a are enhanced with respect to the "major" proteins. However, proteins 1 and 3a are greatly reduced on complex media, so that the sum of proteins 1a, 1b, and 3a represents only about 12 to 20% of the total outer membrane protein. It should be noted that the amount loaded on the gel shown in Fig. 6 was not constant for each of the samples, but was adjusted so that protein 3a was roughly constant in stain intensity.

CNBr peptides of proteins 1a and 1b. Schmitges and Henning (19) reported differences in migration between cyanogen bromide (CNBr) peptides of protein 1 from *E. coli* B, which makes only protein 1a, and *E. coli* K-12, which makes both 1a and 1b. We have isolated protein 1b by QAE-Sephadex fractionation of protein 1 from a lactate-minimal medium-grown culture that produced both 1a and 1b, and compared this with protein 1a produced by an isogenic ParI mutant. The Par^+ strain used for the isolation of 1b was labeled with [^{3}H]leucine. Protein 1b was retarded slightly on QAE-Sephadex with respect to protein 1a, so, after two cycles of such chromatography, we obtained a preparation consisting primarily of 1b (Fig. 8). This was mixed with a sample of protein 1a from a [^{14}C]leucine-labeled culture of a ParI mutant, and the protein was cleaved with CNBr and analyzed on a phosphate-buffered 15% polyacrylamide gel (8). Both proteins yielded the same CNBr peptides and partial cleavage products in roughly the same amounts, but there were significant differences in the migration of peptides D1, D2, and D3 (Fig. 9). Since these peptides are not contiguous (8), this rules out modification occurring only at the N- or C-terminal ends of the proteins.

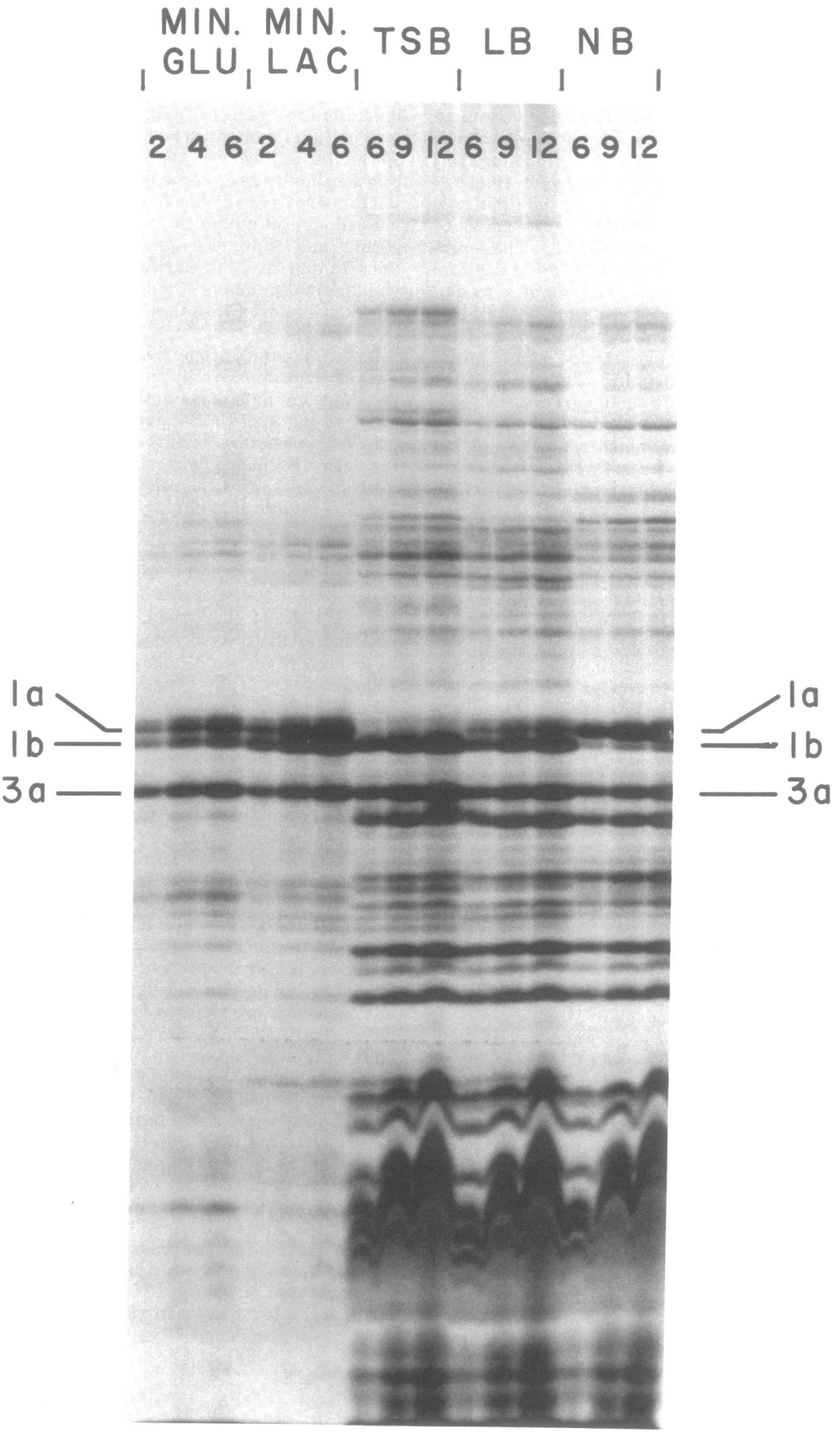
Production of protein 2 by *E. coli* B. Since *E. coli* B resembles ParI and ParII mutants in inability to produce protein 1b, it was of interest to determine if this strain can produce protein 2 when lysogenic for PA-2. To infect *E. coli* B, we used a mutant strain (B837) lacking the B restriction system. When this strain was lysogenized by hy7, it produced a large amount of protein 2, and in fact protein 1 could no longer be detected (Fig. 10). Thus, *E. coli* B resembles ParI mutants of *E. coli* K-12.

E. coli B is thought to be a double mutant in *lon* and *non*. However, there appears to be no relationship between these genes and ParI and ParII phenotypes in *E. coli* K-12. We have examined several authentic *lon* mutants in K-12 strains, and all produce normal amounts of proteins 1a and 1b. Both ParI and ParII mutants yield normal numbers of mucoid colonies when streaked against T7, so they do not appear mutated in *non*.

Isolation and properties of TolIV and TolXIV mutants. We did not observe colicin-resistant mutants among the mutants (both spontaneous and EMS-induced) selected only for PA-2 resistance. This is apparently due to the lower frequency (or recovery) of colicin-tolerant mutants with respect to the Par mutants, since mutants having the phenotype reported for TolIV and TolXIV (6) were isolated at a low frequency when EMS-mutagenized cultures of W1485F^- were challenged with both phage (either PA-2*c* or hy2) and colicin E3. For the purposes of this study, we define TolIV mutants as those sensitive to phage BF23 and colicin E1 but resistant (tolerant) to colicins E2, E3, and K, and TolXIV mutants are the same as TolIV except sensitive to colicin K. Sarma and Reeves (manuscript in press) recently found that these phenotypes are the result of mutations at the same locus, designated as *ompB*, which is located between *malA* and *aroB* at approximately minute 73.8 on the *E. coli* K-12 linkage map.

A number of TolIV and TolXIV mutants were examined by the thin-slab gel system. All were found to produce either no detectable protein 1 or very slight traces of protein 1a. Some of these mutants also produced small amounts of a protein with a higher apparent molecular weight than that of protein 1, which may represent some form of precursor polypeptide (Fig. 13). When TolIV or TolXIV mutants were lysogenized with hy7, the resulting lysogens produced normal amounts of protein 2.

Since the amount of protein 2 produced was normal, and comparable to the amount of protein 1 produced by the Tol^+ parent, we were interested in determining whether the production of protein 2 by the hy7 lysogens would repair the outer membrane defect leading to colicin tolerance. The results of such experiments indicate that replacement of the missing protein by protein 2 does result in almost complete restoration of sensitivity to colicins E2 and E3 (Table 5). Lysogeny for phage λ had no effect on colicin sensitivity.


MIN. GLU
MIN. LAC
TSB
LB
NB
2 4 6 2 4 6 6 9 12 6 9 12 6 9 12
1a
1b
3a
1a
1b
3a

Properties of *tolF* mutants. Mutations at a locus designated as *tolF* (11), now known to be identical with the *cmlB* locus (10), result in a tolerance to colicins E2, E3, K, and L similar to that observed in TolIV mutants missing protein 1. Chai and Foulds (submitted for publication) have found that *tolF* mutants produce protein 1b but lack protein 1a. We have confirmed their observation. When we compared the outer membrane protein profiles of JF568 *tolF4* grown on either LB or nutrient broth with those of an isogenic JF568 *tol*$^+$ strain, we observed that the levels of protein 1b were the same in both strains, but the *tolF* was specifically lacking protein 1a.

Strain JF568 *tolF4* exhibits normal sensitivity to phage PA-2 (Table 3). To examine this mutation in more detail, we obtained a number of spontaneous mutants in this strain resistant to PA-2*c* and hy2. These mutants exhibited the same level of tolerance to colicins E2, E3, and L

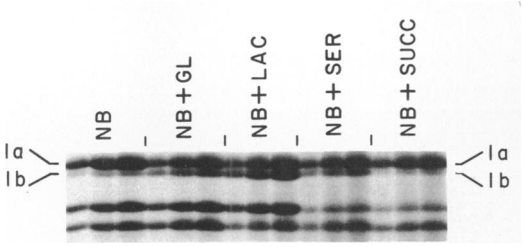


FIG. 7. *Slab gels as in Fig. 6 showing the major outer membrane proteins of W1485F$^-$ grown on nutrient broth (NB) supplemented with 0.25% glycerol (GL), 0.25% potassium lactate (LAC), 0.1% L-serine (SER), or 0.25% sodium succinate (SUCC).*

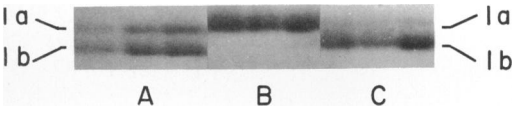


FIG. 8. *Slab gels as in Fig. 6 showing (A) protein 1 purified as previously described (8) from a culture of W1485F$^-$, (B) protein 1a purified as described in the text from a ParI mutant (PA101), and (C) protein 1b from a culture of W1485F$^-$ separated from protein 1a by two cycles of chromatography on QAE-Sephadex. All cultures were grown on lactate-minimal medium.*

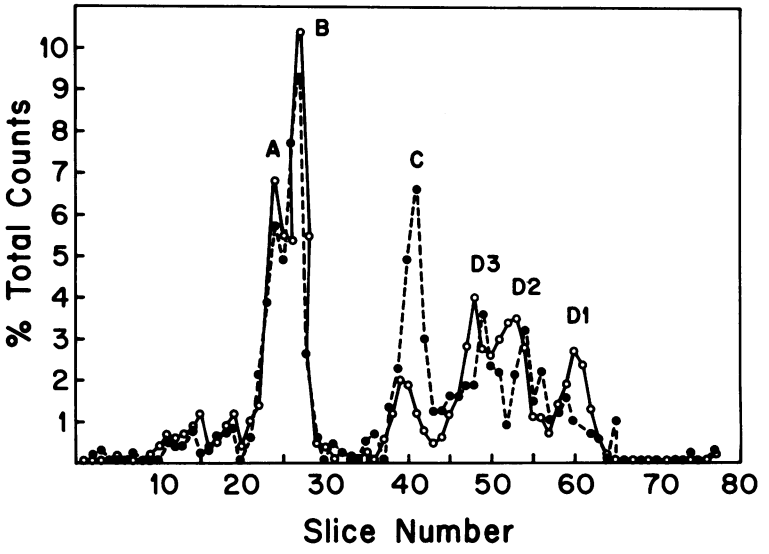


FIG. 9. *Comparison of CNBr peptides of purified protein 1a from a ParI mutant (PB101) and of protein 1b isolated from W1485F$^-$. Protein 1a was from a culture labeled with [^{14}C]leucine (dashed line), and protein 1b was from a culture labeled with [^{3}H]leucine (solid line). The two preparations of protein were mixed prior to CNBr cleavage. The two preparations are the same as shown in Fig. 8. The peptides are labeled as in Schmitges and Henning (19).*

FIG. 6. *High-resolution slab gels (Laemmli buffer system) of the outer membrane proteins of W1485F$^-$ grown on various media: minimal-glucose, minimal-lactate, Trypticase soy broth (TSB), LB without glucose, and nutrient broth (NB). The numbers above the various lanes indicate the amount of protein (in micrograms) applied to each slot. These amounts were chosen so that the intensity of protein band 3a was similar in each of the samples.*

Tol IV
Tol IV (hy7)
Tol XIV
Tol XIV (hy7)
B
B(hy7)
2
1a
1b
3a
2
1a
1b
3a

TABLE 5. *Effect of lysogeny by PA-2 (hy7) on colicin sensitivity of TolIV and TolXIV mutants*

Strain	Colicin sensitivity[a]	
	Colicin E2	Colicin E3
W1485F⁻	1:3,125	1:78,125
W1485F⁻·hy7	1:3,125	1:78,125
W1485F⁻·λ	1:3,125	1:78,125
PB108 (TolIV)	Undiluted	1:5
PB108 (TolIV)·λ	Undiluted	1:5
PB108 (TolIV)·hy7	1:625	1:15,625
PB109 (TolXIV)	Undiluted	1:5
PB109 (TolXIV)·λ	Undiluted	1:5
PB109 (TolXIV)·hy7	1:625	1:15,625

[a] Sensitivity is the highest dilution of a colicin stock solution that gave a clear zone when spotted on a soft agar lawn of the test strain. Serial fivefold dilutions were tested.

as did the parent *tolF* strain, indicating that they were not revertants at the *tolF* locus. One such mutant was characterized in detail, and it was shown to be of the ParI phenotype since it produced normal levels of protein 2 when lysogenized with hy7. When the *tolF par* mutant was transduced to *nalA* with a P1*kc* lysate obtained from a *nalA par*$^+$ strain, the majority of the transductants were Par$^+$, indicating that the mutation was at the *par* locus.

A surprising result was obtained when the outer membrane of this *tolF par* double mutant was analyzed on gels. Whereas the parent *tolF* produced normal levels of protein 1b and no detectable protein 1a, the *tolF par* double mutant produced no protein 1b and almost normal levels of protein 1a. To further rule out reversion at the *tolF* locus, we examined both the colicin sensitivity and the outer membrane protein profiles of several of *tolF nalA par*$^+$ transductants. All of these had the same colicin sensitivity as JF568 *tolF4*, and all produced protein 1b but no protein 1a.

Since JF568 *tolF4* produced normal levels of protein 2 when lysogenic for hy7, we examined the colicin sensitivity of these lysogens as compared with the nonlysogenic *tolF* strain. The results were essentially identical to those shown for the TolIV and TolXIV strains in Table 5. Lysogeny of JF568 *tolF4* with hy7 restored the colicin E2 and E3 sensitivity to almost that of the isogenic *tolF*$^+$ strain. A similar restoration of sensitivity was observed when the *tolF par* double mutant was lysogenized with hy7.

DISCUSSION

Four lines of evidence now indicate that protein 1 is an essential component of the receptor for phage PA-2: (i) it was previously shown (21) that cells lysogenic for PA-2 and in which protein 2 has largely replaced protein 1 exhibit reduced ability to bind phage. (ii) Mutants (TolIV and TolXIV) selected on the basis of colicin resistance and lacking protein 1 are resistant to the phage. (iii) Isolated envelope fractions enriched for protein 1 by differential SDS extraction, and solubilized, partially purified preparations of protein 1 from such fractions, inactivate the phage. The addition of LPS, chloroform, or detergents is not necessary to observe this phage inactivation. (iv) The majority of mutants selected for resistance to virulent or clear-plaque derivatives of PA-2 exhibit obvious alterations in protein 1.

Several observations indicate that the 1b component of protein 1 is the phage receptor. First, *E. coli* B, which is resistant to phage PA-2, lacks protein 1b but produces large amounts of protein 1a (19). Second, the predominant classes of mutants selected for resistance to PA-2 are missing protein 1b but have normal levels of protein 1a. Third, *tolF* mutants missing protein 1a but producing protein 1b exhibit normal phage sensitivity even though they are altered in colicin sensitivity.

Though LPS need not be added to purified protein 1 to observe phage inactivation, it is possibly required as a component of the receptor since the protein preparations we have used are undoubtedly contaminated with small amounts of LPS. The phage-resistant mutants described by Schmitges and Henning (19) that are missing protein 1b are also resistant to T4, suggesting a LPS defect in these mutants. Unlike these, ParI mutants are sensitive to phage T4 and to a number of other phages that use LPS receptors and, hence, must have normal LPS.

It is not clear whether proteins 1a and 1b represent different forms of the same polypeptide or are the products of different structural genes. However, both genetic and biochemical evidence favor the hypothesis that proteins 1a and 1b are derived from the same structural gene product and differ in some form or forms of post-translational modification. With respect to the two Par phenotypes associated with mutations at the *par* locus, we have

FIG. 10. *Slab gels as in Fig. 6 showing the outer membrane proteins of TolIV and TolXIV mutants of W1485F⁻ and E. coli B strain as well as the corresponding strains lysogenic for hy7. The cultures were grown on LB.*

examined more than 20 such mutants and have not observed any that produced any detectable protein 1b or any band that might represent an electrophoretic variant of 1b. Using a very sensitive phage recombination test, we have been unable to detect any mutants with the ParI phenotype that were at all leaky with respect to PA-2 resistance. The mutants with the ParII phenotype are defective both in the production of protein b and in the production of protein 2 and PA-2 lysogens. Since protein 1 and protein 2 are clearly the products of different structural genes (8), these mutants are affected in the production of more than one gene product. All of these observations suggest that the ParI and ParII mutants are not altered in the structural gene for the polypeptide of protein 1b. Likewise, the observation that *tolF* mutants lacking protein 1a will produce this protein when a second mutation in the *par* locus is introduced indicates that the *tolF* locus does not represent the structural gene for the polypeptide of protein 1a.

Several lines of evidence suggest that the *ompB* locus near *malA* may represent the structural gene for a polypeptide precursor of proteins 1a and 1b. First, mutations mapping at this locus (TolIV and TolXIV phenotypes) are defective in both 1a and 1b. Second, these mutants produce normal amounts of protein 2 when lysogenic for PA-2 derivatives. Since protein 2 is clearly a different polypeptide (8) than protein 1, mutations in the structural gene for protein 1 should have no effect on the production of protein 2. Finally, we recently observed that a revertant of a TolIV mutant produced a polypeptide that migrated as a single band on both Laemmli slab gels and Bragg-Hou gels and exhibited an altered mobility with respect to wild-type protein 1 on both of these gel systems (data not shown).

The nutritional studies indicating that the formation of protein 1b depends upon a high level of some intermediate in the pathway from fructose-6-phosphate to pyruvate suggest that protein 1b may differ from protein 1a through modification of the polypeptide chain by addition of some small, acidic molecule derived from one of these glycolytic intermediates. Protein 1b is clearly more acidic than 1a, as shown by its retention on QAE-Sephadex and by the electrofocusing studies of Schmitges and Henning (19). The studies of the CNBr peptides of proteins 1a and 1b also favor the idea that these proteins differ by modification of amino acid residues or by addition of a small molecule at several points along the polypeptide chain. Both forms of the protein yielded the same number of cleavage products (including partial cleavage fragments A and C; 8), and these exhibited similar, although not identical, electrophoretic mobility. It is difficult to imagine two distinct polypeptides that yielded such similar CNBr peptides, since this would imply that the methionine residues in both proteins would have to be the same in number and would occur at almost exactly the same positions along the polypeptide chain.

Protein 1b has no obvious function, since the ParI mutants lacking this protein grow normally under the conditions we have tested and exhibit wild-type phage and colicin sensitivity. Protein 1a seems to play a role in organization of the outer membrane, since the *tolF* mutants specifically lacking this protein and the *ompB* mutants lacking both forms of protein 1 exhibit tolerance to several group A colicins. That lysogenization of both *tolF* and *ompB* mutants with derivatives of phage PA-2 restores colicin sensitivity indicates that protein 2, which is produced by these lysogens, is a functional as well as a structural analog of protein 1.

This restoration of colicin sensitivity by protein 2 also aids in the elucidation of one of the colicin tolerance mechanisms. We have previously shown that the vitamin B_{12} transport protein, which is the receptor for colicin E3, can exist in different functional states with respect to its participation in colicin killing (3, 4). Although both old and new receptor molecules can function in B_{12} transport, only newly synthesized receptor molecules can mediate killing by colicin E3. These results indicate that the newly synthesized receptor must exist, for a period of time, fixed in juxtaposition or in a particular orientation with respect to some site on the cytoplasmic membrane for the colicin to penetrate and kill the cell. Eventually, this juxtaposition or orientation is lost as the receptor moves away from its original site of insertion in the outer membrane as a consequence of growth or lateral diffusion, and this portion of the cell surface becomes "tolerant" in the sense that colicin can still be bound but can no longer penetrate and kill.

It is likely that the vitamin B_{12} receptor, which is a very minor component of the outer membrane, is stabilized or constrained with respect to diffusion by the major components (LPS, proteins) of the outer membrane. Defects in these major components, for example, inability to produce protein 1, would allow more rapid diffusion of the newly synthesized receptor away from its site of insertion and render the cell tolerant to the colicin. It would be predicted in this case, if the missing protein could be replaced by another protein (protein 2) of similar function, that sensitivity to the colicin

would be restored. The data presented here and in our previous studies of colicin E3 sensitivity (3, 4) are entirely consistent with such a model for colicin tolerance.

While this work was being prepared for publication, we became aware of work by Verhoef et al. (C. Verhoef, P. J. de Graff, and E. J. J. Lugtenberg, Mol. Gen. Genet., in press) on a virulent *E. coli* phage, termed Mel, that they have isolated. Mutants of *E. coli* K-12 resistant to this phage are also missing protein 1b (which they term protein c), and the mutations to phage resistance map at a locus exhibiting 35% cotransduction with *nalA*. It appears likely that this locus, termed *meo* by Verhoef et al., is identical with the *par* locus described in this paper.

APPENDIX

Nomenclature of outer membrane proteins from *Escherichia coli* K-12. As a result of exchange of data, strains, and phage stocks between various laboratories, it is now possible to reconcile the nomenclature systems for the "major" outer membrane proteins as follows:

Lugtenberg et al. (14)	Schmitges and Henning (19)	Schnaitman (20)
–	–	2
a	–	3b
b	Ia	1a
c	Ib	1b
d	II*	3a

The proteins are listed above in the order in which the bands appear (in terms of increasing mobility) on SDS-gels in the Laemmli buffer system (1, 14). It should be noted that the relative order of migration of proteins 2 and 3b is quite different in this gel system from that observed with the Bragg-Hou buffer system (20). Protein 2 is present only in strains lysogenic for phage PA-2 or its derivatives and thus was not detected by Lugtenberg or Henning. Protein 3b is not produced at low temperature and thus was not detected by Henning in cultures grown at 30°C.

Protein 1a from *E. coli* K-12 appears to be very similar or identical to the "matrix protein" isolated by Rosenbusch from *E. coli* B (8, 19). Proteins 3a and 3b are "heat-modifiable" proteins according to the terminology of Bragg and Hou (5), and proteins 1a, 1b, and 2 are "peptidoglycan-associated" proteins (8).

We are grateful to U. Henning, P. Manning, and B. Lugtenberg for supplying unpublished data that aided in the reconciliation of these nomenclature systems.

ACKNOWLEDGMENTS

This research was supported by grant BMS-04973 from the National Science Foundation and Public Health Service grant GM18006 from the National Institute of General Medical Sciences. D.L.D. was supported by fellowship Public Health Service grant AI05049 from the National Institute of Allergy and Infectious Diseases.

We thank John Foulds and Ben Lugtenberg for making their manuscripts available prior to publication and David Lee for assistance with the studies on *tolF* colicin sensitivity.

LITERATURE CITED

1. **Ames, G. F.-L.** 1974. Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. Membrane, soluble and periplasmic fractions. J. Biol. Chem. 249:634-644.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. 40:116-167.
3. **Bassford, P. J., Jr., R. J. Kadner, and C. A. Schnaitman.** 1977. Biosynthesis of the outer membrane receptor for vitamin B_{12}, E colicins, and phage BF23: a study of the kinetics of phenotypic expression following the introduction of *bfe*$^+$ and *bfe* alleles. J. Bacteriol. 129:265-275.
4. **Bassford, P. J., Jr., C. A. Schnaitman, and R. J. Kadner.** 1977. Functional stability of the *bfe* and *tonB* gene products in *Escherichia coli*. J. Bacteriol. 130:750-758.
5. **Bragg, P. D., and C. Hou.** 1972. Organization of proteins in the native and reformed outer membrane of *Escherichia coli*. Biochim. Biophys. Acta 274:478-488.
6. **Davies, J. K., and P. Reeves.** 1975. Genetics of resistance to colicins in *Escherichia coli* K-12: cross-resistance among colicins of group A. J. Bacteriol. 123:102-117.
7. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. 60:17-28.
8. **Diedrich, D. L., A. O. Summers, and C. A. Schnaitman.** 1977. Outer membrane proteins of *Escherichia coli*. V. Evidence that protein 1 and phage-directed protein 2 are different polypeptides. J. Bacteriol. 131:598-607.
9. **Fairbanks, G., T. L. Steck, and D. F. H. Wallach.** 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606-2617.
10. **Foulds, J.** 1976. Chromosomal locations of the *tolF* locus. J. Bacteriol. 128:604-608.
11. **Foulds, J., and C. Barrett.** 1973. Characterization of *Escherichia coli* mutants tolerant to bacteriocin JF246: two new classes of tolerant mutants. J. Bacteriol. 116:885-892.
12. **Hane, M. W., and T. H. Wood.** 1969. *Escherichia coli* K-12 mutants resistant to nalidixic acid: genetic mapping and dominance studies. J. Bacteriol. 99:238-241.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 222:680-685.
14. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the major outer membrane protein of *Escherichia coli* K-12 into 4 bands. FEBS Lett. 58:254-258.
15. **Manning, P. A., and P. Reeves.** 1976. Outer membrane of *Escherichia coli* K-12: differentiation of proteins 3A and 3B on acrylamide gels and further characterization of *con* (*tolG*) mutants. J. Bacteriol. 127:1070-1079.
16. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
17. **Sabet, S. F., and C. Schnaitman.** 1971. Localization and solubilization of colicin receptors. J. Bacteriol. 108:422-430.
18. **Sabet, S. F., and C. Schnaitman.** 1973. Purification and properties of the colicin E3 receptor of *Escherichia coli*. J. Biol. Chem. 248:1797-1806.

19. **Schmitges, C. J., and U. Henning.** 1976. The major proteins of the *Escherichia coli* outer cell-envelope membrane. Heterogeneity of protein I. Eur. J. Biochem. **63**:47-52.
20. **Schnaitman, C. A.** 1974. Outer membrane proteins of *Escherichia coli*. IV. Differences in outer membrane proteins due to strain and cultural differences. J. Bacteriol. **118**:454-464.
21. **Schnaitman, C. A., D. Smith, and M. Forn de Salsas.** 1975. Temperate bacteriophage which causes the production of a new major outer membrane protein by *Escherichia coli*. J. Virol. **15**:1121-1130.
22. **Skurray, R. A., R. E. W. Hancock, and P. Reeves.** 1974. Con mutants: a class of mutants in *Escherichia coli* K-12 lacking a major cell wall protein, and defective in conjugation and adsorption of a bacteriophage. J. Bacteriol. **119**:726-735.
23. **White, D. A., W. J. Lennarz, and C. A. Schnaitman.** 1972. Distribution of lipids in the wall and cytoplasmic membrane subfractions of the cell envelope of *Escherichia coli*. J. Bacteriol. **109**:686-690.
24. **Wood, W. B.** 1966. Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16**:118-133.
25. **Zierdt, C. M., F. A. Fox, and G. F. Norris.** 1960. A multiple syringe bacteriophage applicator. Am. J. Clin. Pathol. **33**:233-237.