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Outer Membrane Proteins of *Escherichia coli*

VII. Evidence That Bacteriophage-Directed Protein 2 Functions as a Pore

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Protein 1, a major protein of the outer membrane of *Escherichia coli*, has been shown to be the pore allowing the passage of small hydrophilic solutes across the outer membrane. In *E. coli* K-12 protein 1 consists of two subspecies, 1a and 1b, whereas in *E. coli* B it consists of a single species which has an electrophoretic mobility similar to that of 1a. K-12 strains mutant at the *ompB* locus lack both proteins 1a and 1b and exhibit multiple transport defects, resistance to toxic metal ions, and tolerance to a number of colicins. Mutation at the *tolF* locus results in the loss of 1a, in less severe transport defects, and more limited colicin tolerance. Mutation at the *par* locus causes the loss of protein 1b, but no transport defects or colicin tolerance. Lysogeny of *E. coli* by phage PA-2 results in the production of a new major protein, protein 2. Lysogeny of K-12 *ompB* mutants resulted in dramatic reversal of the transport defects and restoration of the sensitivity to colicins E2 and E3 but not to other colicins. This was shown to be due to the production of protein 2, since lysogeny by phage mutants lacking the ability to elicit protein 2 production did not show this effect. Thus, protein 2 can function as an effective pore. *ompB* mutations in *E. coli* B also resulted in loss of protein 1 and similar multiple transport defects, but these were only partially reversed by phage lysogeny and the resulting production of protein 2. When the *ompB* region from *E. coli* B was moved by transduction into an *E. coli* K-12 background, only small amounts of proteins 1a and 1b were found in the outer membrane. These results indicate that genes governing the synthesis of outer membrane proteins may not function interchangeably between K-12 and B strains, indicating differences in regulation or biosynthesis of these proteins between these strains.

The outer membrane of enteric bacteria contains pores or transmembrane channels which allow diffusion of small, hydrophylic solutes such as sugars, amino acids, and inorganic ions across the outer membrane (15). It has recently been shown that a major outer membrane protein of *Escherichia coli* termed "protein 1," and related proteins present in *Salmonella typhimurium*, form these pores or channels, and these proteins have been termed "porins." In *E. coli* K-12, protein 1 consists of two subcomponents, proteins 1a and 1b (see reference 1 for other nomenclature applied to these proteins), which can be differentiated by polyacrylamide gel electrophoresis (1). Protein 1 of *E. coli* B is a single component which comigrates with protein 1a of *E. coli* K-12 on gel electrophoresis (1).

Evidence that protein 1 is the porin comes both from the in vitro studies of Nakae (14, 15), who showed that fractions enriched in this protein or analogous proteins from *S. typhimurium* could confer the proper permeability to phospholipid vesicles, and from a variety of studies on mutants lacking protein 1. By selection for poor utilization of glucose, von Meyenburg obtained mutants of *E. coli* B defective in the transport of sugars, amino acids, and inorganic ions (23). These mutants have been shown to lack protein 1 (P. Bavoil, H. Nikaido, and K. von Meyenburg, submitted for publication). Lutkenhaus obtained similar pleiotropic transport mutants in *E. coli* B by selecting for copper resistance (12), and these too lacked protein 1. Mutants of *S. typhimurium* lacking proteins analogous to the subcomponents of protein 1 of *E. coli* K-12 exhibit reduced diffusion of the antibiotic cephaloridine across the outer membrane (16).

E. coli K-12 mutants lacking protein 1 have also been selected on the basis of colicin resistance (4), and Sarma and Reeves (19) have shown that mutations giving rise to this phenotype map near *aroB* at a locus they termed *ompB*. The mutations in *E. coli* B that give rise to the

pleiotropic transport defect map at the same locus (Bavoil et al., submitted for publication).

We have shown that lysogeny by the lambdoid bacteriophage PA-2 results in the production of a new major outer membrane protein, protein 2 (1). This protein, which is a distinctly different polypeptide from protein 1 (6), almost entirely replaces protein 1 in lysogens. This is a form of surface exclusion, since protein 1b is the receptor for phage PA-2.

Protein 2 is very similar to protein 1, both in its chemical properties and in its interaction with the peptidoglycan layer (6). In this work, we demonstrate that protein 2 is also a functional analog of protein 1 in *E. coli* K-12, in that its presence can repair many of the permeability defects resulting from the loss of protein 1 caused by mutations at the *ompB* locus.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. The bacterial strains used in this study, excluding colicinogenic strains, are listed in Table 1. Phage Mel (22) was obtained from B. Lugtenberg, and phages TuIa and TuIb (3) were obtained from U. Henning. The λ-PA-2 hybrid phages hy2, hy7, and hy8 have been described previously (1).

Culture media. The complex media used in this study are modifications of Luria broth (13). Medium TY contained 1% tryptone and 0.5% yeast extract, adjusted to pH 7.2 with KOH. Medium TYS was the same medium plus 0.5% NaCl, and medium TYHS was the same medium plus 2.5% NaCl. The minimal medium was that of Davis and Mingioli (5), supplemented when necessary with amino acids (0.01%), thiamine and pyridoxine (0.001%), and *p*-hydroxybenzoic acid and *p*-aminobenzoic acid (0.0007%). Carbon sources (generally 0.5%) and isopropyl-β-D-thiogalactopyranoside were added after autoclaving. For experiments on sulfate uptake, the Davis and Mingioli formulation was modified by replacing the $(NH_4)_2SO_4$ and $MgSO_4$ with equimolar amounts of NH_4Cl and $MgCl_2$, and 0.01% methionine was added as a source of S. Citrate was omitted from the minimal medium used for demonstrating copper sensitivity. Difco agar (1.5%, or 0.75% for soft agar) was added for solid medium.

Preparation of colicins and assay of colicin sensitivity. Colicins A, E2, E3, K, and L were prepared by a modification of a previous method (17). Cultures were grown on TYS broth, and colicin production was induced by the addition of 0.4 μg of mitomycin C per ml. The induced cells were broken in a French pressure cell, and colicin activity was partially purified from the cytoplasmic fraction by stepwise precipitation with $(NH_4)_2SO_4$. Colicin sensitivity was determined by the cross-streak method (4), using TYS agar, or by dilution spot tests in which 1:2 dilutions of colicin were spotted onto lawns of 3×10^6 indicator cells in 3 ml of soft agar. Plates were read after 16 h at 37°C, and the titer is expressed as the reciprocal of the last dilution giving a clear zone of inhibition. Colicinogenic strains were CA31 (colA.CA31), T20 (colB.K260), UB1082 (colD.CA23), K53 (colE1.K53), CA42 (colE2.CA42), K-12.CA38 (colE3.CA38), CA46 (colG.CA46), CA58 (colH.CA58), CA53 (colIa.CA53), K-12.P9 (colIb.P9), JF246 (colL.JF246), K235 (colK.K235), M32.T19 (colM.K260), P1 (colS1.P1), P15 (colS4.P15), CA7 (colV.CA7), and K-12.185 (colX.K235).

TABLE 1. *Bacterial strains*

Strain no.	Genotype/characteristics	Source
E coli K-12		
W1485F⁻	*thi*	CGSC[a]
CS136	W1485F⁻ · hy7	This study
CS197	*ompB151* of W1485F⁻	This study
CS228	CS197 · hy7	This study
CS208	*par-10* of W1485F⁻	This study
CS231	CS208 · hy7	This study
JF568	*aroA357 ilvC277 metB65 his-53 purE41 proC24 cys-1 xyl-14 lacY29 str-77 tsx-63*	J. Foulds
CS252	JF568 · hy7	This study
JF703	*aro⁺ tolF4* of JF568	J. Foulds
CS253	JF703 · hy7	This study
AB2847	*aroB351 malA354 tsx supE42*	CGSC
Lin221	*phoA8 glpT6 relA1 tonA22*	CGSC
E. coli B		
834	*met gal bfe*, r⁻, m⁻[b]	R. Benzinger
CS227	834 · hy7	This study
CS198	As 834 but *ompB152*	This study
CS229	CS198 · hy7	This study
CS199	As 834 but *ompB153*	This study
CS230	CS199 · hy7	This study

[a] CGSC, Coli Genetic Stock Center. *ompB* and *par* allele numbers were allocated by the CGSC.
[b] This strain and its derivatives lack *E. coli* B restriction and modification.

Antibiotic and metal ion sensitivity. Cells grown to a density of 3×10^8/ml in TYS broth were diluted 1:100 in fresh TYS, and 0.01 ml was spotted onto TYS plates containing different antibiotic concentrations. After incubation for 24 to 48 h at 37°C, plates were scored for growth, with the minimal inhibitory concentration being defined as the lowest antibiotic concentration at which no growth occurred. The relative resistance of the mutant strains was determined by comparison of the minimal inhibitory concentration with that of the parent strains. Antibiotic resistance was also examined by using sensitivity disks (Difco or Colab Multidiscs) containing polymyxin B, chloramphenicol, methicillin, streptomycin, penicillin, lincomycin, tetracycline, and oxytetracycline, which were placed on TYS plates, overlaid with 3×10^6 cells in soft agar, and incubated for 16 h at 37°C. Mutant and parent strains were compared by measuring the width of the inhibition zones. Resistance to silver and copper was determined by spotting dilutions of cells as above on plates containing various concentrations of $AgNO_3$ or $CuSO_4$, except that cells were grown in TY broth and plated on TY agar in the case of silver resistance and in minimal medium in the case of copper resistance.

Bacteriophage sensitivity. Phage resistance was determined by cross-streaking on TYS plates, or by measuring the efficiency of plating by mixing dilutions of phage with 3×10^8 cells, incubating for 20 min at 37°C, and then plating in soft agar to obtain plaque counts.

Uptake of labeled substrates. Cells were grown to a density of 10^9/ml, harvested, washed once, resuspended at the same density in minimal medium containing 0.1 mg of chloramphenicol per ml, and incubated for 15 min at the assay temperature. The labeled substrate was then added, 0.1-ml samples were taken at various times and filtered through a membrane filter, and the filter was rinsed, dried, and counted (18). Comparisons of rates of uptake between parent and mutant strains are based on the initial rates measured over the time period where uptake was linear with time. Amino acid uptake was assayed using cells grown in TYS or glucose minimal medium and resuspended in glucose minimal medium. Sulfate uptake was measured in cells grown in either glucose or glycerol minimal medium and resuspended in sulfur-free medium with the same carbon source. Mannitol uptake was determined using cells grown in TYS broth containing 0.5% mannitol plus 0.5% glycerol and resuspended in glycerol minimal medium. For measurement of maltose uptake, cells were grown in either TYS broth or minimal medium containing 0.5% maltose plus 0.5% glycerol, and they were assayed in minimal salts medium containing glycerol. The assay of β-D-thiomethylgalactoside uptake was the same as that for mannitol uptake except that 0.5 mM isopropyl-β-D-thiogalactopyranoside was used in place of mannitol as inducer. In experiments on maltose and mannitol uptake, several samples were taken over the first minute to overcome problems due to metabolism of these substrates by the cells. The concentrations of the transport substrates are given in Results. The radiochemicals used were [^{14}C]proline, [^{14}C]glutamine, [^{14}C]mannitol, [^{35}S]sodium sulfate, [^{14}C]maltose, and β-D-[^{14}C]thiomethylgalactoside. The uptake mixture contained 5 to 0.04 μCi/ml, depending on the transport substrate and experimental conditions.

Transduction. Transduction experiments with phage P1*kc* were performed as described by Miller (13). In each experiment, a minimum of 150 recombinants were examined for transfer of unselected markers. Unselected markers (*ompB, par*) were scored for colicin or bacteriophage resistance, and representatives of each class of transductants were also examined for the presence of outer membrane proteins 1a and 1b by gel electrophoresis.

Polyacrylamide gel electrophoresis. Outer membranes were isolated from midlog-phase cells grown on various media as indicated in Results. Cell harvesting and fractionation were as previously described (18), and the outer membrane protein profiles were examined using a modification (1) of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis system of Laemmli (11).

Storage of mutants. Since *ompB* mutants are at a disadvantage in all growth media, revertants tend to accumulate, and precautions were necessary to insure against reversion. All of the *ompB* mutants used in this study showed a strong tendency to revert and could not be stored on slants. To overcome this problem, mutant cultures were frozen in broth containing 20% glycerol at −70°C, and were thawed and cloned before each experiment. Cultures were checked for several known features of the OmpB phenotype, such as colicin and phage resistance, before and after each experiment. In some cases, broth cultures that were used in experiments were plated to obtain single colonies, which were checked as above to determine if revertants were present at significant levels.

RESULTS

Isolation and preliminary characterization of mutants with reduced amounts of protein 1. Three classes of mutants of *E. coli* K-12 which lack subcomponents of protein 1 have been described. Phage-resistant mutants with mutations in the *par* (or *meo*; 22) locus lack protein 1b (1). Strains with mutations in the *tolF* locus have been selected for chloramphenicol resistance, and these produce protein 1b but lack protein 1a (2, 7). Mutants selected for colicin resistance that have the TolIV or TolXIV phenotype lack both proteins 1a and 1b (4) and result from mutation in *ompB* (19). Mutants of *E. coli* B that lack protein 1 have been selected for copper resistance (12) or on the basis of delayed color change on indicator plates containing a low level of glucose (23; Bavoil et al., submitted for publication).

We have used copper and chloramphenicol as selective agents, since resistance to these agents is likely to be the result of reduced uptake. We plated 10^{10} cells of strain W1485F$^-$ or 834 onto citrate-free minimal medium containing 400 μM $CuSO_4$ (12) or onto TYS agar containing 2 μg (for strain W1485F$^-$) or 0.6 μg (for strain 834) of

chloramphenicol per ml. A wide range of chloramphenicol- and copper-resistant mutants was obtained, and these were differentiated by their colicin and phage resistance patterns. Many of the mutants were resistant to various combinations of colicins A, K, L, S4, and X and appeared similar to mutants with alterations in lipopolysaccharide composition, which have been described (9). Mutants with the TolXIV phenotype (presumed *ompB* mutations) were found among both chlormaphenicol- and copper-resistant mutants of *E. coli* B (strain 834), but only among the chloramphenicol-resistant mutants of *E. coli* K-12 strain W1485F⁻. We were unable to isolate mutants of W1485F⁻ with the TolF phenotype by selection for either copper or chloramphenicol resistance, although both agents have been reported to be effective for such selection (6, 12). Only TolXIV and mucoid mutants were obtained when phage TuIa resistance (3) was used for selection with strain W1485F⁻. Thus, we were unable to select additional mutants that lacked protein 1a but produced protein 1b. Additional *par* mutants were obtained by selection for phage hy2 resistance.

Each of the mutants was examined for several characteristics of the *par* and *ompB* mutations. Representative results are shown in Table 2 and agree with previous descriptions of these mutations (1, 3, 22). Interestingly, all of the *E. coli* K-12 *ompB* mutants obtained by selection for chloramphenicol resistance (15 independent isolates tested) exhibited partial sensitivity to phage hy2. Strain CS197 (Table 2) was selected for further study, because its relatively high level of resistance to phage hy2 indicates that little protein 1b remains. All 15 of these mutants and the *tolF* strain JF703 were also partially sensitive to phage TuIa (efficiency of plating reduced by 10^2 to 10^4 as compared to parent strains), which is reported (3) to use protein 1a as receptor. *E. coli* B strain 834 was fully resistant to phage TuIa, even though this strain produces a protein that resembles protein 1a of *E. coli* K-12. The resistance of strain 834 to phages hy2, TuIb, and Mel does not appear to be due to a mutation at a locus corresponding to the *par* locus of *E. coli* K-12. Using strain 834 as the donor, we transduced the K-12 strain Lin221 to *glp*⁺. Of 200 transductants tested, none exhibited resistance to these phage (see Table 2 and reference 1 for comparison).

Effect of hy7 lysogeny (protein 2 production) on phage and colicin resistance. Lysogenization of strain W1485F⁻ by hy7 resulted in a reduction in the efficiency of plating of phages TuIa, TuIb, hy2, and Mel by a factor of 10 to 20. This is presumably due to a reduction in the amount of proteins 1a and 1b resulting from the production of protein 2 (1). Similarly, hy7 lysogens of both W1485F⁻ and 834 were less sensitive to colicins A and L than nonlysogens (Table 3). Killing by these colicins appears to require protein 1a, as demonstrated by the high level of colicin A and L resistance of *tolF* and *ompB* mutants (Table 3).

van Alphen and Lugtenberg (21) have shown that the relative amounts of proteins 1a and 1b can be changed by increasing the osmolarity of the culture medium. The reduction in protein 1a resulting from increased osmolarity was accompanied by a reduction in sensitivity to colicins A and L (Table 4). This effect was observed in both *E. coli* K-12 (W1485F⁻), where the reduc-

TABLE 2. *Characteristics of mutants altered in protein 1 production*

Strain[a]	Colicin resistance[b]								Phage resistance[b]				Outer membrane[c] proteins		Cotransduction frequency (%)		
	A	E1	E2	E3	K	L	S4	X	hy2	Mel	TuIa	TuIb	1a	1b	*aroB*	*malA*	*glpT*
E. coli K-12																	
W1485F⁻ (wild type)	S[d]	S	S	S	S	S	S	S	S	S	S	S	+	+			
CS197 (*ompB*)	R	S	R	R	R	R	R	P	R	R	S	R	−	−	53	59	0
CS208 (*par*)	S	S	S	S	S	S	S	S	R	R	S	R	+	−	0	0	12
E. coli B																	
834 (wild type)	S	P	R	R	S	S	S	S	R	R	R	R	+	−			
CS198 (*ompB*)	R	P	R	R	R	R	R	P	R	R	R	R	−	−	56	38	0
CS199 (*ompB*)	R	P	R	R	R	R	R	P	R	R	R	R	−	−	51	34	0

[a] Strains CS197 and CS198 were obtained by selection for chloramphenicol resistance. Strain CS208 was selected for hy2 resistance. Strain CS199 was selected for copper resistance. Transduction analysis was performed using strain AB2847 with selection for *aro*⁺ or *mal*⁺, and with strain Lin221 selecting for *glp*⁺.

[b] Colicin and bacteriophage sensitivity were determined by the cross-streak method.

[c] Proteins were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of outer membranes.

[d] S, Sensitive; R, resistant (tolerant); P, partially resistant. All strains were sensitive to colicins B, D, G, Ia, Ib, M, S1, and V.

TABLE 3. *Colicin resistance of omp, par, and tolF mutants and their corresponding hy7 lysogens*

Strain	Relevant characteristics	Control strain	Relative resistance[a]				
			A	E2	E3	K	L
E. coli K-12							
CS136	Wild type, hy7	W1485 F⁻	16	0.5	0.125	0.25	8
CS197	*ompB*		>2.0 × 10³[b]	>1.6 × 10⁴	>1.3 × 10⁵	128	>1.6 × 10⁴
CS228	*ompB* hy7		>2.0 × 10³	8	8	128	>1.6 × 10⁴
CS208	*par*		1	0.25	1	2	1
CS231	*par* hy7		8	0.125	0.25	1	16
CS252	Wild type, hy7	JF568	32	0.5	0.125	0.5	16
JF703	*tolF*		>4.1 × 10³	>1.6 × 10⁴	64	>1.6 × 10⁴	>1.6 × 10⁴
CS253	*tolF* hy7		>4.1 × 10³	>1.6 × 10⁴	64	>1.6 × 10⁴	>1.6 × 10⁴
E. coli B							
CS228	Wild type, hy7	834	4	—[d]	—[d]	1	128
CS199[c]	*ompB*		>2.1 × 10³	—	—	>1.6 × 10⁴	>8.2 × 10³
CS230[c]	*ompB* hy7		>2.1 × 10³	—	—	>1.6 × 10⁴	>8.2 × 10³

[a] The relative resistance is the colicin titer in arbitrary units as measured with the control strain divided by the colicin titer in arbitrary units as measured with the mutant strain or lysogen. The cultures were grown and tested using TYS medium.

[b] The symbol > denotes full resistance to the highest concentrations of colicin available.

[c] Strains CS198 (*ompB*) and CS229 (*ompB* hy7) gave results identical to those with strains CS199 andCS230, respectively.

[d] The parent strain (834) is resistant to these colicins.

TABLE 4. *Protein 1 content and colicin sensitivity in TY media containing varying levels of NaCl*

Determination	*E. coli* K-12 (W1485 F⁻)			*E. coli* B (834)		
	TY (no NaCl)	TYS (0.5% NaCl)	TYHS (2.5% NaCl)	TY (no NaCl)	TYS (0.5% NaCl)	TYHS (2.5% NaCl)
Protein[a]						
1a	++	+	±	++++	+++	+
1b	++	+++	++++	−	−	−
Colicin titer (arbitrary units/ml)						
A	1.6 × 10³	200	32	1.6 × 10³	200	32
E2	2.6 × 10³	3.2 × 10³	1.6 × 10³	—[b]	—	—
E3	5.1 × 10⁴	2.6 × 10⁴	2.6 × 10⁴	—	—	—
K	100	400	1.6 × 10³	400	800	1.6 × 10³
L	2.6 × 10⁴	3.2 × 10³	64	1.3 × 10⁴	1.6 × 10³	16

[a] Proteins were determined by visual examination of stained gels. In *E. coli* K-12, the total amount of protein 1 (1a + 1b) appeared to be the same under all growth conditions.

[b] Strain 834 is resistant to these colicins.

tion in 1a was accompanied by an increase in protein 1b, and *E. coli* B (834), which produces no protein analogous to protein 1b and in which increasing osmolarity resulted in a decrease in the level of protein 1 in the outer membrane. This further illustrates the similarity between the single protein 1 band of *E. coli* B and protein 1a of *E. coli* K-12.

As noted previously (1) and in Table 3, lysogenization of *E. coli* K-12 *ompB* mutants by hy7 restored sensitivity to colicins E2 and E3. However, this effect appears to be specific for these colicins, since sensitivity to colicins A, K, and L was not restored by lysogenization. The resistance of JF 703 to colicins was also not affected by lysogenization with hy7.

Effect of hy7 lysogeny on antibiotic and metal ion resistance. *ompB* and *tolF* mutants were more resistant to tetracycline and chloramphenicol than the parent strains, as indicated by both a decreased zone diameter around antibiotic disks and an increased minimal inhibitory concentration (Table 5). *E. coli* B was more sensitive to these antibiotics than the K-12 strains. *par* mutants and hy7 lysogens of the parent strains exhibited sensitivity identical to that of the parent strains. *E. coli* K-12 *ompB* and *tolF* strains lysogenic for hy7 were slightly

more sensitive to chloramphenicol and tetracycline than were the nonlysogenic *ompB* and *tolF* mutants. Lysogenization by hy7 had no effect on the chloramphenicol sensitivity of the *ompB* mutant of *E. coli* B. Absence of proteins 1a, 1b, or both, or lysogenization by hy7 had no effect on sensitivity to any of the other antibiotics tested (see Materials and Methods).

E. coli K-12 *ompB*, *tolF*, and *par* mutants were as sensitive, or almost as sensitive, to copper as the parent strains (Table 5). This was not the case for silver sensitivity; the *ompB* mutant CS197 was substantially more resistant to silver than its parent strain. The *ompB* mutants of *E. coli* B were resistant to both copper and silver. Lysogenization by hy7 almost completely abolished the increased silver resistance of CS197, but had much less effect on the copper and silver resistance of the *E. coli* B *ompB* mutants.

Effect of hy7 lysogeny on nutrient transport. Table 6 illustrates that *ompB* mutants of both *E. coli* B and *E. coli* K-12 are defective in their ability to accumulate proline, glutamine, sulfate, mannitol, and β-D-thiomethylgalactoside. Maltose presumably passes through the outer membrane via the *lamB* gene product (20) instead of through the protein 1 "pore," since we observed normal maltose transport in *ompB* mutants grown on maltose to induce the *lamB* gene product. We tested proline uptake at various substrate concentrations (Table 7) and found that the rate of uptake in the *ompB* mutants approached that of the *omp*$^+$ strains at high substrate concentrations, indicating, as previously shown by von Meyenberg (23), that the effect of the *ompB* mutation is to increase the apparent K_m for transport. The rates of uptake of transport substrates by the *par* and *tolF* mutants, and by hy7 lysogens of these and the parent strains, were almost identical to those of the parent strains (not shown).

In the case of the *E. coli* K-12 *ompB* mutants, lysogenization by hy7 resulted in complete restoration of wild-type transport rates. However, this was not true of the *E. coli* B *ompB* mutants (CS198 and CS199), even though lysogenization with hy7 did result in production of protein 2 as judged by gel electrophoresis of the outer membrane proteins. Lysogenization of CS198 and CS199 increased their ability to accumulate proline and glutamine, particularly at higher concentrations of these substrates (Tables 6 and 7), but the effect was far less dramatic than in the K-12 strains. The limited ability of hy7 lysogeny to restore transport of nutrients to *E. coli* B *ompB* mutants was also demonstrated by growth of the strains in minimal medium containing limiting amounts of glucose (0.1%). Strain CS199 (*ompB*) grew poorly in this medium, as evidenced by slow growth in the logarithmic phase of growth and low final yields. Growth of a hy7 lysogen of this strain (CS230) was somewhat improved, but was still not comparable to that of the parent *omp*$^+$ strain 834.

The restoration of normal transport properties to *ompB* mutants of *E. coli* K-12 by hy7 lysogeny is a result of the production of protein 2, and not an indirect consequence of phage lysogeny. By

TABLE 5. *Chloramphenicol, tetracycline, copper, and silver resistance of ompB, tolF, and par mutants and their corresponding hy7 lysogens*

Strain	Relevant characteristics	Relative MIC[a]			
		CAP	TET	Cu^{2+}	Ag^+
W1485F$^-$		1.00 (0.66)	1.00 (2.60)	1.00 (132)	1.00 (16.4)
CS136	hy7	0.91	1.00	1.00	1.00
CS197	*ompB*	2.9	1.5	1.00	10.0
CS228	*ompB* hy7	2.4	1.4	1.00	1.39
CS208	*par*	1.00	1.00	1.00	1.00
CS231	*par* hy7	1.00	1.00	1.00	1.00
JF568		1.00 (0.48)	1.00 (2.28)	1.00 (66.2)	1.00 (16.4)
CS252	hy7	1.00	1.00	1.00	1.00
JF703	*tolF*	2.4	1.54	1.5	1.38
CS253	*tolF* hy7	2.2	1.54	1.5	1.20
834		1.00 (0.098)	1.00 (1.64)	1.00 (132)	1.00 (19.6)
CS227	hy7	1.00	1.16	1.00	1.00
CS199[b]	*ompB*	2.5	1.8	7.5	14.8
CS230[b]	*ompB* hy7	2.5	1.8	5.0	11.6

[a] MIC, Minimal inhibitory concentration; CAP, chloramphenicol; TET, tetracycline. Main columns indicate MIC relative to parent strain. Numbers in parentheses indicate the MIC of the parent strains in μg of CAP or TET per ml or μM Cu^{2+} or Ag^+.

[b] Strains CS198 (*ompB*) and CS229 (*ompB* hy7) gave results identical to those with strains CS199 and CS230, respectively.

TABLE 6. *Uptake of nutrients by ompB mutants and by their hy7 lysogens*

Transport substrate (μM)	Growth medium[a] and temp (°C)	Relative initial uptake rate[b]					
		E. coli K-12			*E. coli* B		
		W1485F⁻	CS197	CS228	834	CS199[c]	CS230[c]
Proline (2)	TYS, 25	1.00 (215)	0.23	1.12	1.00 (225)	0.12	0.23
	37	1.00 (410)	0.11	0.83	1.00 (410)	0.18	0.24
	MS, 25	1.00 (450)	0.87	0.98	1.00 (380)	0.08	0.25
	37	1.00 (720)	0.86	0.92	1.00 (610)	0.07	0.21
Glutamine (2.6)	TYS, 25	1.00 (70)	0.20	1.22	1.00 (46)	0.30	0.74
Mannitol (5)	TYS, 25	1.00 (7,800)	0.10	0.97	1.00 (7,800)	0.22	0.19
TMG (5)[d]	TYS, 25	1.00 (120)	0.38	0.82	1.00 (170)	0.37	0.53
Maltose (3.5)	TYS, 25	1.00 (2,900)	0.93	0.97	1.00 (3,200)	0.88	0.91
	MS, 25	NT[e]	NT	NT	1.00 (2,400)	0.71	0.67
Sulfate (5)	MS, 37 (glucose)	1.00 (64)	0.70	0.65	1.00 (48)	0.06	0.15
	MS, 37 (glycerol)	1.00 (70)	0.34	0.89	NT	NT	NT

[a] See Materials and Methods for details of supplementation of these media. MS, Minimal salts.
[b] Initial uptake rate relative to parent under same conditions. Numbers in brackets indicate actual rates in pmol/10^9 cells per min.
[c] Strains CS198 and CS229 gave results similar to those shown for strains CS199 and CS230, respectively.
[d] TMG, β-D-Thiomethylglactoside.
[e] NT, Not tested.

TABLE 7. *Effect of proline concentration on proline uptake by ompB mutants and their corresponding hy7 lysogens*

Proline concn (μM)	Initial uptake rate relative to parent strain[a]			
	E. coli K-12		*E. coli* B	
	CS197 (*ompB*)	CS228 (*ompB* hy7)	CS199 (*ompB*)	CS230 (*ompB* hy7)
0.4	0.14	0.85	0.09	0.09
0.9	0.17	1.07	0.09	0.12
2.0	0.23	1.14	0.08	0.13
9.5	0.33	0.99	0.13	0.27
30	0.51	0.96	0.27	0.62
95	0.78	0.93	0.47	0.93
500	1.11	1.04	0.87	0.93

[a] Parent strains were W1485F⁻ (for CS197 and CS228) and 834 (for CS199 and CS230). Cells were grown in TYS broth, and uptake was measured at 25°C. Similar results were obtained in assays performed at 37°C.

selecting for a silver-resistant mutant of strain CS228, we were able to obtain a mutant of phage hy7 which, when used to lysogenize wild-type K-12 strains, resulted in no detectable production of protein 2 as determined by gel electrophoresis of the outer membrane proteins. When *E. coli* K-12 *ompB* strains were lysogenized with this mutant phage, they exhibited no restoration of transport ability or colicin sensitivity (data not shown). Wild-type K-12 strains lysogenic for this mutant phage produced the same amount of protein 1 as nonlysogenic strains.

We could not demonstrate defects in transport of proline or glutamine by strain CS197 (K-12 *ompB*) if the cells were grown in glucose minimal medium. Similarly, sulfate uptake by this strain was normal in cultures grown on glucose minimal medium (Table 6). In the case of the various amino acids and sugars, we were able to overcome this problem by growth of the cultures on TYS broth. This was not possible with sulfate, since TYS broth contains sufficient sulfate (ca. 6 μM) to suppress sulfate transport. A partial defect in sulfate transport could be demonstrated with cultures grown on glycerol minimal medium (Table 6).

The reason that *ompB* mutants of *E. coli* K-12 are not transport defective when grown on minimal medium appears to be due to a new peptidoglycan-associated protein which appears when these mutants are grown on glucose minimal medium. This protein, which shows approximately the same electrophoretic mobility on the Laemmli slab gel system (1) as protein 2, is similar, if not identical, to "protein Ic" described recently by Henning et al. (10) and to a similar protein observed by Foulds and Chai (8) as a consequence of a secondary mutation in a *tolF par* mutant strain. We have found this protein to be produced in both *ompB* and *tolF par* mutants of *E. coli* K-12. Our preliminary data are in agreement with the reports cited above that the production of this protein is a consequence of a secondary mutation at a locus other than *ompB*, *par*, or *tolF*. Such secondary mutants appear to accumulate in cultures of *E. coli* K-12 grown on minimal medium. In K-12 cultures grown on complex medium, these second-

ary mutants appear less frequently, and we have not observed this protein in outer membrane from *E. coli* B grown under either of these growth conditions. We are presently investigating this protein and the conditions that lead to its formation in more detail.

Expression of the *E. coli* B *ompB* region in *E. coli* K-12. Since protein 2 could replace protein 1 in *E. coli* K-12 but only partially replaced protein 1 in *E. coli* B, we carried out a reciprocal experiment to determine if protein 1 (or at least the *ompB* region, which may code for protein 1) from *E. coli* B could function in *E. coli* K-12. Using *E. coli* B strain 834 as donor, *E. coli* K-12 strain AB2847 was transduced to *aroB*$^+$. From these transductants, several strains were selected which were also *malA*$^+$, indicating that the entire *aroB-malA* region (including *ompB*) had been transferred from *E. coli* B. When the outer membrane proteins from these strains were examined by gel electrophoresis, it was found that both proteins 1a and 1b were produced, but these proteins were produced in much lower amounts (about 20%, by visual estimate) than in strain AB2847 (data not shown). The transductants carrying the *aroB-malA* region from *E. coli* B were also significantly less sensitive to colicins A, K, and L than was a strain of AB2847 transduced simultaneously to *aro*$^+$ *mal*$^+$ by P1*kc* grown on W1485F$^-$.

DISCUSSION

The results of this study lead to several conclusions regarding the roles of various outer membrane proteins of *E. coli* in determining either the permeability of the outer membrane or the sensitivity of the cells to colicins.

Protein 1b, which is present in K-12 strains but not in B strains, is not in itself required for the either normal permeation of the outer membrane or the mediation of killing by colicins. Loss of protein 1b as a consequence of *par* mutations has no effect on either of these properties.

Protein 1a plays an essential role in determining the sensitivity of cells to colicins A and L (and probably also colicins S4, K, and X), since loss of this protein in either *ompB* or *tolF* mutants results in resistance to these colicins and this resistance is not altered by lysogeny by PA-2 phage derivatives and the resultant production of protein 2. Protein 1a also plays a role in the permeation of the outer membrane by chloramphenicol and tetracycline.

Loss of both proteins 1a and 1b by K-12 strains as a result of mutation at the *ompB* locus results in a pleiotropic permeability defect, evidenced by inability to transport a number of nutrients and by resistance to silver ions, and possibly also in a structural alteration evidenced by resistance to colicins E2 and E3. Both the permeability defect and the structural defect are reversed when protein 2 is produced as a consequence of phage lysogeny, indicating that protein 2 can function both as a porin and as a structural analog of protein 1.

Protein 2 is synthesized by PA-2 lysogens of *E. coli* B, but it is only partially able to repair the permeability defect caused by the loss of the single species of protein 1 present in this strain. Two hypotheses may be advanced to explain the inability of protein 2 to function in *E. coli* B. First, it is possible that some of the steps in biosynthesis or post-translational modification of outer membrane proteins are different in B strains than in K-12 strains, and that the outer membrane proteins produced by the two strains are altered to compensate for these differences. Second, it is possible that the outer membrane proteins of the two strains are altered to compensate for the differences in chemical composition of the lipopolysaccharide of these strains and that protein from one strain cannot interact properly with lipopolysaccharide from another strain. A consequence of either hypothesis is that proteins from *E. coli* B would function improperly in *E. coli* K-12, and this is consistent with our observation that only small amounts of proteins 1a and 1b were inserted into the outer membrane of an *E. coli* K-12 strain carrying the *ompB* region of *E. coli* B.

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