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Genetic and Biochemical Characterization of Periplasmic-Leaky Mutants of *Escherichia coli* K-12

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Periplasmic-leaky mutants of *Escherichia coli* K-12 were isolated after nitrosoguanidine-induced mutagenesis. They released periplasmic enzymes into the extracellular medium. Excretion of alkaline phosphatase, which started immediately in the early exponential phase of growth, could reach up to 90% of the total enzyme production in the stationary phase. Leaky mutants were sensitive to ethylenediaminetetraacetic acid, cholic acid, and the antibiotics rifampin, chloramphenicol, mitomycin C, and ampicillin. Furthermore, they were resistant to colicin E1 and partially resistant to phage TuIa. Their genetic characterization showed that the *lky* mutations mapped between the *suc* and *gal* markers, near or in the *tolPAB* locus. A biochemical analysis of cell envelope components showed that periplasmic-leaky mutants contained reduced amounts of major outer membrane protein OmpF and increased amounts of a 16,000-dalton outer membrane protein.

The envelope of gram-negative bacteria consists of three compartments: the inner or cytoplasmic membrane, the outer membrane, and the periplasmic space between the two membranes (7, 25). Inner membrane proteins are involved in active transport, oxidative phosphorylation, and biosynthesis of envelope macromolecules (7). The protein composition of the outer membrane is much simpler than that of the cytoplasmic membrane (9). Outer membrane proteins are involved in conjugation, diffusion of small molecules, and adsorption of phages and colicins (18). The periplasmic space houses binding proteins involved in the active transport of various metabolites (4, 17, 28, 31, 37), degradative periplasmic enzymes (15), and a peptidoglycan layer covalently bound to the lipoprotein (6) and noncovalently associated with other outer membrane proteins (29, 36).

Cell envelope proteins, which account for approximately 25% of the total bacterial proteins, are synthesized in the cytoplasmic compartment and have to be transported in or across the cytoplasmic membrane (9). Many aspects of the export process for cell envelope proteins in bacteria agree with the predictions of the signal hypothesis elaborated for the secretion of proteins and peptide hormones in eucaryotic systems (5). A genetic analysis of protein export should help to identify cellular components involved in the export process and the final integration of cell envelope proteins.

There is little evidence for the secretion of any *Escherichia coli* periplasmic proteins into the culture medium. It seems likely that the outer membrane acts as a barrier to such release. We previously isolated periplasmic-leaky mutants which were unable to keep some periplasmic enzymes in their original compartment and which consequently released them into the extracellular medium (20). The periplasmic-leaky phenotype has been described in *E. coli* strains deficient in lipoprotein and lipopolysaccharide (14, 16, 42).

Here, we present genetic and biochemical properties of a new class of periplasmic-leaky mutants which contain reduced amounts of major outer membrane protein OmpF. (The nomenclature for major outer membrane proteins is as described by Bachmann [2] and Lugtenberg et al. [22].)

MATERIALS AND METHODS

Bacterial strains and phages. All bacterial strains used in this work were derivatives of *E. coli* K-12. Some of their relevant properties are listed in Table 1. All Hfr mutant strains derived from Hfr P4X. The characteristics of phages we used are listed in Table 2.

Media. Cells were grown in rich L medium (24) or in minimal Tris (T) medium (12). Solid media contained 1.5% agar. Minimal medium was supplemented with (per liter): glucose (G) 2 g; thiamine hydrochloride, 1 μg; appropriate amino acids, 40 μg; and adenine, 0.03 g, if required. Other substrates were used at a

TABLE 1. *Bacterial strains*

Strain	Sex	Relevant genotypes	Source
P4X	Hfr	*thi metB*	E. Wollman
Gal5	Hfr	*thi metB lacI*	β-Galactosidase constitutive derivative from P4X
188	Hfr	*thi metB lacI phoS,T*	Arsenate-resistant mutant of strain Gal5
207	Hfr	*thi metB lacI lky-207*	Periplasmic-leaky mutant of strain Gal5
207c	Hfr	*thi metB lacI phoS,T lky-207*	Arsenate-resistant mutant of strain 207
207R	Hfr	*thi metB lacI phoS,T*	Deoxycholic acid-resistant spontaneous revertant of strain 207c
236	Hfr	*thi metB lacI lky-236*	Periplasmic-leaky mutant of strain Gal5
236c	Hfr	*thi metB lacI phoS,T lky-236*	Arsenate-resistant mutant of strain 236
CGSC 4444	F^-	*thi leu suc bioA galT chlC rpsL*	B. J. Bachmann
PA601	F^-	*proA thr leuC argH his-1 thyA purE lacY galB malA xyl mtl tonA rpsL*	M. Schwartz

TABLE 2. *Phage characteristics*

Phage	Receptor membrane component	Source
U3, 6SR, Br2, Br10, C21, FP1, T3, T4, T7	Lipopolysaccharide	R. N. Picken
TuIa	Major protein OmpF	C. Verhoef
Mel	Major protein OmpC	C. Verhoef
K3	Major protein OmpA	C. Verhoef
T6	Tsx protein	Institut Pasteur Paris

final concentration of 2 g/liter, except for succinate (4 g/liter). TG medium supplemented with 0.25% proteose peptone was used to induce alkaline phosphatase synthesis (TGLP medium); 0.1 M phosphate was added to this medium to repress alkaline phosphatase synthesis (TGHP medium).

L-RNA and L-DNA plates contained 1.5% yeast RNA or 0.15% DNA. Selective medium for isolation of arsenate-resistant mutants has been described previously (41).

Bacterial growth. Growth, estimated by the increase of cell suspension turbidity, was measured in a Jean et Constant spectrophotometer: 100 U of absorbance at 600 nm corresponds to 3×10^8 cells per ml and 200 μg of bacteria (dry weight) per ml.

Mutagenesis and in situ plate assays for detecting alkaline phosphatase, RNase I, DNase I, or β-galactosidase excretion. Nitrosoguanidine-induced periplasmic-leaky mutants were isolated and characterized as previously described (20).

Enzyme assays. Standard published procedures were used for assaying alkaline phosphatase (34) (with 10^{-3} M *p*-nitrophenyl phosphate as the substrate) and β-galactosidase (24) (with 3×10^{-3} M *o*-nitrophenyl-β-D-galactopyranoside as the substrate). One unit of alkaline phosphatase or β-galactosidase activity was defined as the amount of enzyme which hydrolyzed 1 nmol of substrate per min. The differential rate of enzyme synthesis corresponds to the increase of enzyme activity for an increase of 1 mg of bacterial dry weight.

Cellular and extracellular extracts. Routinely, a 500-ml culture was centrifuged (6,000 × *g* for 20 min), and a sample of the supernatant (extracellular fraction) was dialyzed three times for 12 h against water. Cells were osmotically shocked as described by Heppel (15). Extracellular and periplasmic extracts were lyophilized. Intact or shocked cells were disrupted by sonication with a Sorensen oscillator (80 kc) for 15 min at a temperature below 10°C. Sonic extracts were centrifuged (13,000 × *g* for 30 min at 4°C), and supernatants were used to determine intracellular activities.

Preparation of membrane fractions. Cells harvested in the late exponential phase of growth were washed twice with 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.4, and sonicated as indicated above, in the same buffer. After the intact cells were discarded by low-speed centrifugation, the supernatant was ultracentrifuged (200,000 × *g* for 60 min at 4°C); the pellet contained envelope proteins.

Cytoplasmic membrane fractions were extracted twice with a solution containing 10 mM HEPES (pH 7.4), 2% Triton X-100, and 1 mM $MgCl_2$ as described by Schnaitman (32). Outer membrane fractions were extracted twice with the same buffer, except that 5 mM EDTA was used instead of $MgCl_2$. Under these conditions, about 40% of the total membrane proteins were solubilized. Membrane proteins were precipitated with cold ethanol as described previously (32).

Isolation of peptidoglycan-associated proteins. Peptidoglycan-associated proteins were isolated from total envelope proteins after solubilization of other membrane proteins in the presence of 2% sodium dodecyl sulfate (SDS) for 30 min at 60°C as described by Lugtenberg et al. (23). The lipoprotein-peptidoglycan complex was dissociated by lysozyme treatment (1 mg/ml in 100 mM Tris, pH 8, for 16 h at 37°C).

Electrophoretic analysis. SDS-polyacrylamide slab gel electrophoresis was performed by the method of Laemmli (19) with gels 12 or 18 cm long. Before electrophoresis, samples were boiled for 5 min under reducing conditions (in 0.1 M β-mercaptoethanol) in the presence of 1% SDS. After electrophoresis, gels were stained with 2% Coomassie blue in methanol-

acetic acid-water (50:10:40) and then diffusion destained for 12 h in successive baths of methanol-acetic acid-water (5:10:85) at 50°C. Gels were scanned with a Vernon Phi 5 densitometer (Vernon, Paris, France).

Labeling of cell envelope proteins. Labeling experiments were performed by adding 500 μCi of [^{3}H]-arginine (25 Ci/mmol) to 40 ml of culture. After electrophoresis, gels were fractionated into 1-mm fractions. Each fraction was suspended in a mixture containing Protosol (60 ml), Triton X-100 (500 ml), 2,5-diphenyloxazole (4 g), 1,4-bis[2(5-phenyloxazolyl)]-benzene (0.16 g), and toluene (1,000 ml) and stored overnight at 37°C before counting. The recovery of radioactive counts from the fractionated gels was routinely 80 to 100%.

Genetic techniques. Conjugation and transduction techniques using P1 *kc* were carried out as described by Miller (24). Mapping by the gradient of transmission was carried out as described by De Haan et al. (8).

Sensitivity to phages, colicin, and drugs. Sensitivity to phages and colicin E1 was estimated qualitatively, by cross-streaking phage or colicin stocks and bacteria on L medium + G plates, and quantitatively, by titering phage lysates on parental and mutant strains. Resistant mutants gave fewer plaque-forming units than did the sensitive parental strain.

Sensitivity to drugs was determined by testing the colony-forming ability of mutants on L medium containing inhibitors at concentrations which allowed growth of parental strains.

Other analytical methods. The protein content of samples was assayed by the procedure of Schacterle and Pollack (30), except that 5% SDS was added to Triton X-100–containing fractions to prevent interference with colorimetric determination (38). 2-Keto-3-deoxyoctulosonate and heptose were assayed as described by Osborn (27).

Chemicals. Yeast RNA (type VII), DNA (type III), Coomassie blue, alkaline phosphatase and β-galactosidase substrates, Triton X-100, acridine orange, mitomycin C, rifampin, Tris (Trizma base), and HEPES were purchased from Sigma Chemical Co., St. Louis, Mo. Other reagents were of the highest purity available. Radioactive [^{3}H]arginine was purchased from the Radiochemical Centre, Amersham, England, and Protosol was purchased from New England Nuclear Corp., Boston, Mass.

RESULTS

Isolation of periplasmic-leaky mutants. Nitrosoguanidine-induced periplasmic-leaky mutants were isolated from strain Gal5 and classified into several phenotypic classes on the basis of their responses to the plate assays we have developed for detecting the excretion of alkaline phosphatase, RNase I, and DNase I (20). Mutant strains 207 and 236, which leaked the three enzymes, were further analyzed. In this study, alkaline phosphatase was chosen as the periplasmic enzyme marker, and β-galactosidase was chosen as the cytoplasmic enzyme marker.

Strains 188, 207c, and 236c were selected as arsenate-resistant derivatives of strains Gal5, 207, and 236, respectively, and they synthesized alkaline phosphatase constitutively. Mutations responsible for constitutivity were cotransducible with the *ilvA* marker and mapped in the *phoS,T* locus (41, data not shown). The availability of constitutive leaky mutants allowed us to assay alkaline phosphatase activity after growth in L medium, which contains about 1 mM phosphate. In low-phosphate minimal medium, periplasmic-leaky mutants did not develop as well and occasionally lysed.

Growth of periplasmic-leaky mutants on different substrates requiring or not requiring binding proteins for their cytoplasmic accumulation (arabinose, ribose, galactose, succinate, glycerol, and glucose) was normal. However, strain 207 did not grow on maltose.

Time course of alkaline phosphatase excretion. When wild-type strain 188 was grown in L medium at 37°C, alkaline phosphatase excretion slowly increased with time: less than 2% of the enzyme activity was released in the late stationary phase of growth after 16 h of incubation (Fig. 1). On the other hand, alkaline phosphatase excretion by mutant strains 207c and 236c started immediately in the early exponential phase of growth and reached a plateau corresponding to a maximum release of 90% of the total enzyme content (Fig. 1).

Intracellular enzyme activity increased during growth of strain 188 but remained constant in mutant strains (Table 3). Total alkaline phosphatase activities of parental and mutant strains, however, were equivalent.

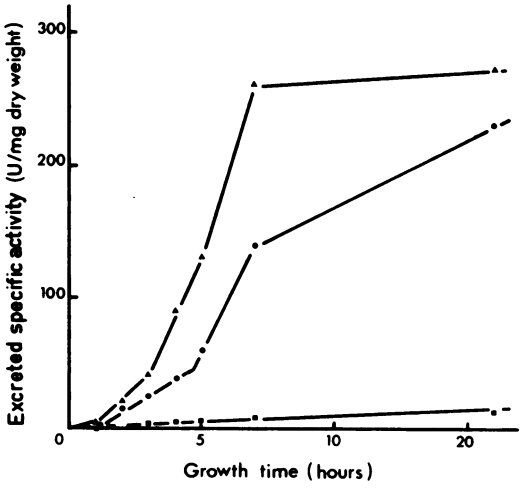

FIG. 1. *Kinetics of alkaline phosphatase excretion by parental and periplasmic-leaky mutant strains. Symbols: ■, strain 188; ▲, strain 207c; ●, strain 236c. Bacteria were grown at 37°C in L medium.*

In all experiments, β-galactosidase activity recovered in the extracellular fluids never exceeded 2% of the total enzyme activity.

Susceptibility of periplasmic-leaky mutants to osmotic shock. Alkaline phosphatase and β-galactosidase activities were assayed in the different extracts obtained after mutant and wild-type cells were exposed to osmotic shock (Table 4). The distribution of alkaline phosphatase activity within periplasmic-leaky mutants showed that the remaining intracellular enzyme could be released by osmotic shock. A significant fraction of this activity, however, was released during the first stage of the treatment, especially after cells had developed in low-phosphate medium (data not shown).

Drug-resistant revertants isolated from strain 207c (see below) behaved like the parental strain (Table 4). Periplasmic proteins which were not excreted by mutant 207c during growth could be released by osmotic shock (Fig. 2, lane IIb).

Sensitivity of periplasmic-leaky mutants to bacteriophages and colicin E1. Sensitivity of periplasmic-leaky mutants to colicin E1, lipopolysaccharide-requiring phages (U3, 6SR, Br2, C21, Br10, FP1, T3, T4, and T7), phages that require the major outer membrane proteins (TuIa, K3, Me1), and phage T6 was analyzed by cross-streaking. Parental and mutant strains were equally sensitive to all phages, except for phage TuIa, which gave 10 times fewer plaque-forming units after titration on mutant cells. Strain 207 was furthermore resistant to colicin E1.

Sensitivity to drugs. Periplasmic-leaky mutants were tested for sensitivity to a variety of drugs known to inhibit growth of cell envelope mutants (10, 33, 39). Mutants 207c and 236c were more sensitive than parental strain 188 to rifampin, mitomycin C, deoxycholic acid, and EDTA (Table 5), but they were resistant to SDS, Triton X-100, Brij 58, acridine orange, methylene blue, and crystal violet (data not shown). The addition of $MgCl_2$ did not restore the wild-type nonleaky phenotype.

Spontaneous revertants were isolated from leaky mutants as resistant clones to deoxycholic acid with a frequency of 4×10^{-8}. When tested

TABLE 3. *Kinetics of alkaline phosphatase excretion*

Growing time (h)	Alkaline phosphatase activity[a] of strain:								
	188 (wild type)			207c			236c		
	Extracellular	Intracellular	Total	Extracellular	Intracellular	Total	Extracellular	Intracellular	Total
1	0 (0)	6	6	2 (33)	4	6	2 (33)	4	6
2	1 (3)	31	32	22 (34)	43	65	16 (30)	40	56
3	2 (2)	83	85	42 (55)	35	77	25 (37)	40	65
4	2 (1)	180	182	139 (74)	49	188	38 (42)	52	90
5	3 (2)	190	193	139 (75)	46	185	60 (54)	51	111
7	5 (2)	269	274	259 (87)	40	299	141 (83)	30	171
16	7 (2)	314	321	265 (85)	45	340	230 (85)	40	270

[a] Cells were grown at 37°C in L medium. Enzyme activity, including total enzyme activity (intra- plus extracellular), is given in enzyme units and corresponds to the amount of enzyme contained in 1 ml of sonic extract prepared from a cell suspension containing 3×10^8 bacteria per ml. Values within parentheses indicate percentage of total enzyme activity.

TABLE 4. *Distribution of enzyme contents after osmotic shock*

Strain	Amt (%) of enzyme[a]							
	Alkaline phosphatase				β-Galactosidase			
	Extracellular fluid	Osmotic shock at stage[b]:		Sonicate[c]	Extracellular fluid	Osmotic shock at stage:		Sonicate
		I	II			I	II	
188 (*lky*$^+$)	1	0	99	0	0	0	0	100
207c (*lky*)	60	7.5	32	0.5	5	0	1.5	93.5
236c (*lky*)	48	3	48	1	2	0	0	98
207R (*lky*$^+$)	1	1	98	0	1	0	0	99

[a] Cells were grown at 37°C in L medium and harvested in the stationary phase of growth.
[b] Stage I, Tris-sucrose-EDTA treatment; stage II, water treatment (15).
[c] Intracellular residual activity after osmotic shock treatment.

for drug sensitivity and periplasmic-leaky phenotypes, deoxycholate revertants showed no leakage of periplasmic enzymes and no sensitivity to any drug. These results suggest that *lky* mutations, responsible for both sensitivity to inhibitors and release of periplasmic enzymes, are single mutations.

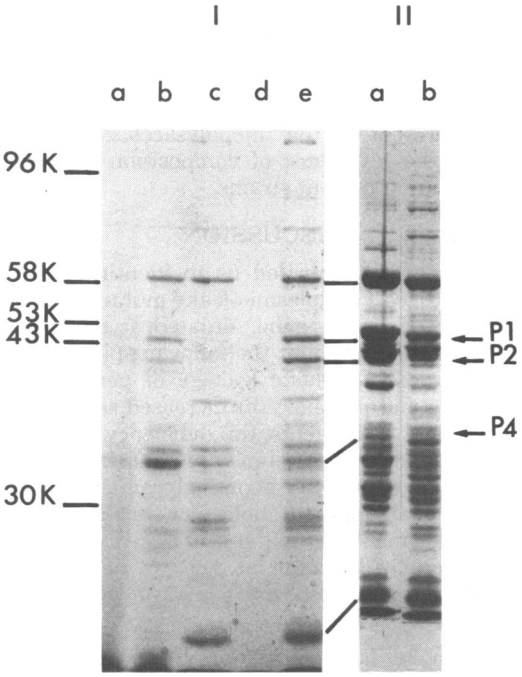

FIG. 2. *SDS-polyacrylamide gel (10% acrylamide gels) electrophoresis patterns of the extracellular proteins of parental and periplasmic-leaky mutant strains. Cells were grown in TGHP medium; strain 207 was also grown in TGLP medium (lane Ib). Standards: phosphorylase a (96K); catalase (58K); glutamic dehydrogenase (53K); E. coli alkaline phosphatase, monomer (43K); carbonic anhydrase (30K). I: Extracellular fluids from strains Gal5 (a), 207 (TGLP medium) (b), 207 (TGHP medium) (c), 188 (d), and 207c (e). II: Osmotic fluids, stage II (water treatment), from strains 188 (a) and 207c (b). Extracellular fluid samples applied on gels corresponded to* 5×10^9 *cells.*

Protein composition of extracellular fluids. The protein composition of extracellular fluids prepared from stationary-phase cultures was analyzed by electrophoresis on SDS-polyacrylamide slab gels (Fig. 2). Under these conditions, parental strains Gal5 and 188 did not excrete any protein (Fig. 2, lanes Ia and Id). On the other hand, comparison of the protein patterns corresponding to strains 207 and 207c, grown in low- (Figure 2, lane Ib) or high-phosphate medium (Fig. 2, lanes Ic and Ie), showed that several phosphate-regulated periplasmic proteins were released. Proteins P1 (alkaline phosphatase, monomer), P2, and P4 (phosphate-binding protein) were released by depressed cells of mutant 207, whereas only bands P1 and P2 were excreted by strain 207c; band P4 was absent in strain 207c, which was probably a *phoS* mutant (13). (The nomenclature of periplasmic proteins is as described by Morris et al. [26].)

Two other major periplasmic proteins were released: a 58,000-dalton (58K) protein, and a 17K protein, which was only present after growth in high-phosphate medium. Other unidentified minor bands, probably including RNase I, DNase I, and other periplasmic proteins, were excreted.

Genetic characterization of strains 207c and 236c. Preliminary results from interrupted and noninterrupted mating studies between Hfr strains 207c or 236c and the F^- strain PA 601 suggested that the two *lky* mutations mapped between *purE* and *gal* markers (20a).

Transduction studies with phage P1 *kc* were carried out, using strain 207c as a donor and strain CGSC 4444 as a recipient. gal^+ and suc^+ transductants were selected: 43 and 67%, respectively, of these transductants inherited the pleiotropic phenotype associated with the *lky-207* mutation (alkaline phosphatase and RNase excretion and sensitivity to deoxycholate).

To determine the position of the *lky-207* mutation relative to *suc* and *gal* markers, suc^+ lky^+ and gal^+ lky^+ transductants were analyzed. Since the great majority (85 to 95%) of such transductants segregated as *gal* for the first class and *suc* for the second class, we concluded that

TABLE 5. *Sensitivity of periplasmic-leaky mutants to growth inhibitors*[a]

Strain	Deoxycholate (%)			EDTA (M)			Rifampin (mg/ml)			Mitomycin C (μg/ml)		
	0.5	0.75	1	0.01	0.02	0.05	0.02	0.04	0.05	0.5	1	2
Gal5 (wild type)	+	+	+	+	+	−	+	+	+	+	+	+
207	+	+	−	+	−	−	+	−	−	+	−	−
236	+	−	−	+	−	−	+	+	−	+	+	−

[a] Sensitivity to drugs was determined by testing the colony-forming ability of wild-type and mutant strains at 37°C in L medium containing inhibitors at the concentrations indicated above. +, Growth; −, no growth.

the *lky-207* mutation is located between *suc* and *gal* markers at min 16.5 of the *E. coli* linkage map (2, 40). Unfortunately, we were unable to infect strain 207c with a bacteriophage P1 *kc* lysate prepared on strain CGSC 4444 or to transduce it for deoxycholic acid resistance.

The same kind of analysis, using a P1 *kc* lysate prepared on strain 236c, showed that the *lky-236* mutation mapped at min 16.6 of the *E. coli* linkage map.

Cell envelope composition. Strains Gal5 and 207 were grown at 30°C in TGLP medium in the presence of [^{3}H]arginine. Labeled cell envelope proteins were quantitatively estimated after SDS-polyacrylamide gel electrophoresis (Fig. 3).

The strains contained equal amounts of major outer membrane proteins e, OmpC, and OmpA but the periplasmic-leaky mutant reproducibly contained only half the amount of OmpF protein present in the parental strain. The OmpF protein was not excreted by periplasmic-leaky mutants (Fig. 2); we also checked to be sure that *ompF* and *omp*B mutants did not release alkaline phosphatase activity (data not shown).

The amount of a peptidoglycan-associated protein with an apparent molecular weight 16,000 was threefold higher in mutant strain 207 than in parental strain Gal5 (Fig. 3 and 4); the amount of the 16K protein which entered the gel was not increased by lysozyme treatment of peptidoglycan-protein complexes (Fig. 4, lanes 3 and 6).

The analysis of the lipopolysaccharide sugars, 2-keto-3-deoxyoctulosonate and heptose, specifically present in the lipopolysaccharide core, showed no difference of composition between parental and mutant strains.

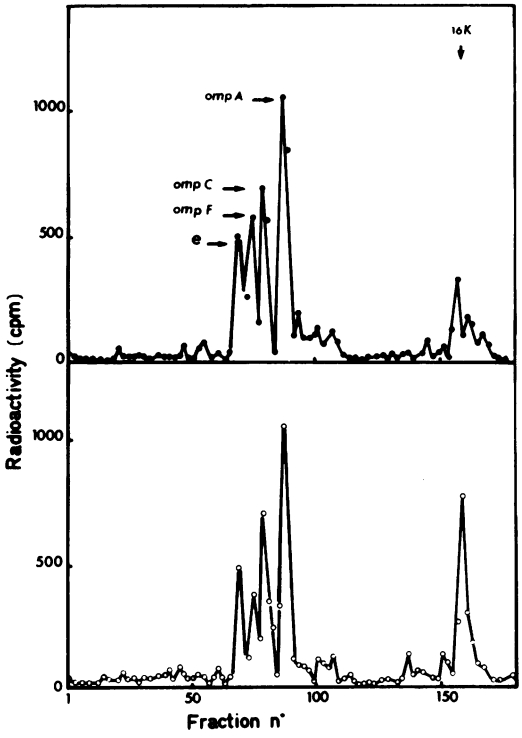

FIG. 3. *SDS-polyacrylamide gel (10% acrylamide gel) electrophoresis patterns of the total cell envelope proteins in wild-type and lky cells. Bacteria were grown in TGLP medium at 30°C; labeling and counting experiments were carried out as described in the text. Symbols: ●, strain Gal5; ○, strain 207. Total cell envelope samples applied on gel corresponded to 2 × 10^9 cells.*

DISCUSSION

This investigation led us to identify a new class of *E. coli* periplasmic-leaky mutants which are altered in one gene, situated at min 16.5. These mutants exhibit the following pleiotropic phenotype: (i) specific leakage of periplasmic enzymes and proteins, (ii) increased sensitivity to external toxic agents, (iii) deficiency in major outer membrane protein OmpF, and (iv) an increased amount of a peptidoglycan-associated protein of an apparent molecular weight of 16K.

Periplasmic-leaky mutants of *E. coli* were first described by Lopes et al. (21). Mutants with altered lipopolysaccharide (14, 33, 35) or murein-

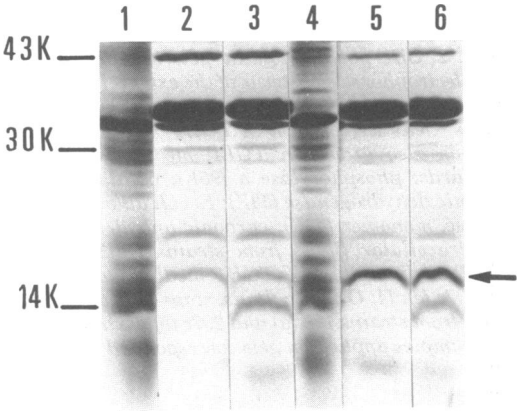

FIG. 4. *SDS-polyacrylamide gel (15% acrylamide gel) electrophoresis patterns of the SDS-soluble and -insoluble membrane proteins from parental and periplasmic-leaky mutant strains. SDS treatment and lysozyme digestion of peptidoglycan-protein complexes were carried out as described in the text. Strain Gal5: SDS-soluble (1) and -insoluble proteins (2); and insoluble proteins after lysozyme treatment (3). Strain 207: SDS-soluble (4) and -insoluble proteins (5); and insoluble proteins after lysozyme treatment (6).*

lipoprotein (11, 16, 39) and conditional mutants (10) have previously been isolated, and their periplasmic-leaky phenotype has been outlined.

Lugtenberg and his collaborators (14, 35) showed that heptose-deficient mutants of *E. coli* K-12, isolated as resistant strains to bacteriophages T3, T4, and T7, excreted RNase I, were supersensitive to various antibiotics and detergents, and contained decreased amounts of OmpF protein. These mutations mapped in genes located near min 7 and 72 of the *E. coli* genetic map. As the new periplasmic-leaky mutants that we have described above do not show any apparent alteration in 2-keto-3-deoxyoctulosonate or heptose content, are still sensitive to phages T3, T4, and T7 as well as to the other lipopolysaccharide-requiring phages, and map in a different region of the chromosome, their pleiotropic phenotype is likely to result from defects in other structural components of the cell envelope.

Mutants have been described in which lipoprotein was absent (*lpo* mutants) (16), had a modified structure (*mlpA* mutants) (42), or presented a defect in formation or stability of the murein-lipoprotein attachment (*lkyD* mutants) (11, 39). Such mutants leaked periplasmic enzymes during growth and showed an increased sensitivity to various drugs. Both *lpo* and *mlpA* mutations mapped in the structural gene for lipoprotein (*lpp*), located at min 36.4 on the *E. coli* linkage map (2). In *Salmonella typhimurium*, *lkyD* mutations mapped in the *proA-galE* region. *lkyD* mutants contained decreased amounts of bound lipoprotein and increased amounts of a 15K cell envelope protein and free lipoprotein; their major outer membrane protein content was not altered.

Recently, Anderson et al. (1) described a periplasmic-leaky mutant of *E. coli* K-12 carrying a pleiotropic *lky*::Mu *cts* mutation. This mutant was sensitive to detergents, antibiotics, and EDTA and tolerant to colicin E1, E2, and E3, but it remained normally sensitive to phage T4. It grew extremely poorly on glucose and sugars requiring osmotic shock-sensitive transport systems and did not grow on glycerol; it showed a twofold reduction in the neutral sugar content of the core lipopolysaccharide. The *lky*::Mu *cts* mutation was linked to the *gal* locus, near or in the *tolPAB* gene cluster. No cell envelope alteration was reported from this study.

Like the pleiotropic *tolPAB* colicin-tolerant mutants (3), the periplasmic-leaky mutants we have isolated map at min 16.5, are resistant to colicin E1, and are sensitive to deoxycholate and EDTA. None of the previously described *tolPAB* mutants, however, showed any major deficiency in the protein composition of outer membrane preparations (3). A complementation analysis will be necessary to determine whether the new *lky* mutations belong to the *tolPAB* group.

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