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Defective Transport and Other Phenotypes of a Periplasmic "Leaky" Mutant of *Escherichia coli* K-12

JAMES J. ANDERSON,* JAMES M. WILSON, AND DALE L. OXENDER
Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48109

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A mutant of *Escherichia coli* K-12 deficient in high-affinity leucine transport and related binding proteins was obtained by selecting for azaleucine resistance after bacteriophage Mu mutagenesis. We determined that the cause was a generalized loss of periplasmic binding proteins and a sharp decrease in the activity of transport systems requiring them. Other transport systems, resistant to osmotic shock and present in membrane vesicles, were affected to a lesser degree or not at all. The mutation, designated *lky*::Mu*cts*, was shown to be a pleiotropic envelope mutation, rendering the mutant sensitive to ionic and nonionic detergents, antibiotics, and ethylenediaminetetraacetic acid; the strain had also acquired tolerance to colicins E1, E2, and E3, while remaining normally sensitive to a variety of bacteriophages. An analysis of the lipopolysaccharide of parent and mutant strains revealed a twofold reduction in the neutral sugar content of the core oligosaccharide of the *lky* strain, but no change in sensitivities to phages which utilize lipopolysaccharide or outer membrane proteins for absorption. The *lky*::Mu*cts* locus was mapped by transduction and found to be located near, or in, the *tolPAB* gene cluster linked to *gal*. Secondary mutations suppressing the detergent sensitivity of *lky* arose at a frequency of 10^{-7}, yielding a variety of new phenotypes. The *lky*::Mu*cts* mutation did not give rise to obvious alterations in the gross morphology of the cell or in cell division.

The envelope of *Escherichia coli* and other gram-negative bacteria contains a compartment termed the periplasmic space (23), which is delimited by the outer and inner membranes. When the outer membrane has been compromised by treatments such as cold osmotic shock or by preparation of spheroplasts (25), a large number of proteins are selectively released. Within this group of periplasmic proteins are degradative enzymes and certain proteins capable of binding a variety of ligands, organic and inorganic (27). Binding proteins have been associated with active transport processes, since their loss by osmotic shock or mutation results in selective loss of kinetically defined components of active transport (27). We have been investigating the role of the leucine-binding proteins in leucine transport, one aspect of which is the interactions that occur between the binding proteins and other macromolecules in the periplasmic milieu.

One approach we are using is to examine the properties of mutants in which the binding proteins are unable to function due to disturbances in the envelope; such mutants include strains which mimic the results of osmotic shock in that periplasmic proteins are spontaneously released from the cell surface, i.e., "leaky" mutants (18). Previously described examples are the lipopolysaccharide (LPS)-defective "deep rough" mutants of *Salmonella typhimurium* (17) and the lipoprotein-defective strains of *E. coli* (13, 31) and *S. typhimurium* (7). During the routine screening of phage Mu*cts*-induced mutants of *E. coli* K-12 defective in leucine transport (1), we discovered a class of "leaky" mutants which secreted periplasmic components, including the leucine-binding proteins, in large amounts. In this paper we describe the transport characteristics of a representative strain carrying the mutation *lky*::Mu*cts*, the pleiotropic nature of this mutation upon membrane properties, and its map location.

MATERIALS AND METHODS

Bacteria and phage. The bacterial strains used (Table 1) were derivatives of *E. coli* K-12. Bacteriophages P1*vir*, P2*vir*, and 434 were gifts of D. Friedman; phage C21 was a gift from B. A. D. Stocker, and phages T2 and T4 were provided by R. Greenberg.

Materials. Reagents were of the highest purity available; nutritional supplements were products of Difco Laboratories (Detroit, Mich.) and Sigma Chemical Co. (St. Louis, Mo.).

Media and growth conditions. Minimal medium based on morpholinepropanesulfonic acid (MOPS) and Luria broth with thymine (LBT) have been de-

scribed previously (1). Carbon sources were generally provided at 0.2% concentration except as noted. Cells were grown aerobically at 32°C by shaking, growth was monitored by absorbance at 420 nm, and absorbance was converted to dry weight as previously described (1). Cells used for transport assay were harvested in mid-log phase.

Isolation of azaleucine-resistant mutants. Strain AE84 was infected with the mutagenic phage Mu*cts* and permitted to grow overnight at 32°C as previously described (1). Culture samples were harvested, washed three times by centrifugation with minimal medium, and plated on glucose-minimal plates containing 100 μg of DL-azaleucine per ml. This strain is sensitive to this level of azaleucine due to the derepression of the LIV-I leucine transport system (1). Resistant colonies were purified on LBT plates and assayed for leucine transport and shockable LIV-binding protein as previously described (1). Binding protein-negative strains were patched on LBT plates containing a 3-ml agar overlay of anti-LIV-binding protein antisera (1); after overnight growth, the colonies were washed away with tap water. Approximately 20% of these strains showed precipitin zones in the agar underneath the colonies. Several strains showed particularly intense secretion correlated with reduced leucine transport; these were also found to secrete RNase by a similar plate assay (18) which reveals RNase activity by rendering RNA in an agar overlay nonprecipitable by acid. These mutant strains were determined to be Mu*cts* lysogens by their temperature sensitivity at 42°C, resistance to Mu, and release of Mu*cts* phage upon heat induction. One mutant (strain AE84064) was selected for intensive study.

Transport assays. Transport assays for ^{3}H- or ^{14}C-labeled amino acids and sugars were carried out on mid-log cells grown in minimal medium at 32°C. After extensive washing in MOPS-salts solution, 10-s uptakes were determined by filtration as previously described (1).

Osmotic shock of ^{35}S-labeled cultures. Cells were grown overnight in 2-ml volumes of MOPS minimal medium containing 0.02% glucose, which limits growth at an absorbance of 1 (420 nm). In the morning glucose was added back to 0.4%, and after recovery (0.5 h) the cultures were centrifuged and washed in an Eppendorf microfuge. Fresh medium containing 50 μCi of $^{35}SO_4^{2-}$ was added, and the cultures were allowed to double in mass. The labeled cells were centrifuged and washed with 1.5 ml of 33 mM Tris-hydrochloride (pH 7.6), resuspended in 33 mM Tris-hydrochloride (pH 7.6) containing 0.5 M sucrose and 1 mM EDTA, and centrifuged again. The minute pellet was then rapidly resuspended in 0.2 ml of ice-cold 0.5 mM $MgCl_2$ to effect osmotic shock and release of periplasmic proteins. This material was lyophilized and suspended in solubilization buffer (see below) for sodium dodecyl sulfate (SDS)-acrylamide gel electrophoresis. Release of binding proteins during growth was quantitated by adding 0.2 ml (packed volume) of Sepharose 4B conjugated with anti-LIV binding protein immunoglobulin G (21) to the 2-ml cultures at the time of ^{35}S addition. After one mass doubling, the beads were recovered by a brief spin in the Eppendorf microfuge. After at least five washes in deionized water to remove cells, the beads were lyophilized and resuspended in solubilization buffer; after heating at 100°C to dissociate the antibody-antigen complex, the bead mixture was loaded into sample wells for SDS-polyacrylamide gel electrophoresis according to Laemmli (14). After being electrophoresed and dried, the gel was autoradiographed. The position of the LIV-binding protein was determined by protein standards, and the appropriate sections of labeled gel were cut out and counted by scintillation methods.

TABLE 1. *E. coli K-12 strains*

Strain	Relevant genotype	Source
AE84[a]	*livR argG6 his-1 trp-31 mtl-2 xyl-7 malA1 gal-6 pdxC3 lacY1* or *-Z4 rpsL104 tonA2 tsx-1 supE44*	Anderson and Oxender (1)
AE84064	*livR gal-6 lky*::Mu*cts*	Mu*cts* lysogen of AE84
AB1623	*gltA5 gal*	CGSC[b]
JP5053	*nagA1*	CGSC[b]

[a] This strain was originally derived from JC1552 (CGSC).
[b] *E. coli* Genetics Stock Center.

Inhibition assays. The inhibitory effects of various agents were assayed by the filter disk method. Cells (10^7) were plated in a 3-ml overlay of 0.65% agar on LBT plates. A 50-μl sample of the test substance was added to a 1-cm antibiotic assay disk (Difco), and the disk was placed in the center of the seeded plate. After overnight growth at 30°C, the zone of growth inhibition from the edge of the disk to the turbid zone was measured.

Phage and colicin sensitivity tests. Sensitivity to phages was determined by seeding 10^7 cells in soft agar on an LBT plate and spotting with a phage dilution containing approximately 10^7 plaque-forming units. After overnight growth, sensitivity was scored as clearing of the bacterial lawn beneath the spot. Colicin sensitivity was scored by the method of Fredericq (6). Specific colicin-producing strains were streaked on an LBT plate, allowed to grow for 8 h at 37°C, and then killed by chloroform vapor. A 3-ml overlay of soft agar was added, and strains to be tested were streaked 90° across the prior streak; sensitivity was determined by the presence of clear zones over the colicin-secreting streak.

Envelope composition analysis. Core LPS was isolated from strains grown to stationary phase in 1 liter of LBT broth by the method of Galanos et al. (8). After two washes by centrifugation from distilled water (100,000 × *g*, 4 h), the LPS was lyophilized and stored desiccated. Neutral sugars were released from 1-mg samples of LPS by a 64-h hydrolysis in 0.1 N HCl at 100°C, according to the procedure of Hancock and Reeves (9). The preparation of alditol derivatives of the sugars and their separation, identification, and quantitation by gas-liquid chromatography were as previously described (9). Glucosamine was determined from the hydrolysate by means of a Beckman amino acid analyzer. KDO (2-keto-3-deoxyoctonate) was determined colorimetrically by the method of Osborne (26), and phosphorus was determined from the perchloric acid digest of LPS by the method of Lowry et al. (19).

RESULTS

Secretion of periplasmic proteins. Colonies of strain AE84064 *lky*::Mu*cts* were found to release LIV-binding protein and RNase into the medium (see Materials and Methods). We wished to determine whether this reflected a general inability to retain periplasmic proteins and if the phenomenon was characteristic of healthy cells or simply reflected envelope fragility at senescence. Washed, mid-log cultures of parent strain AE84 *lky*$^+$ and AE84064 *lky*::Mu*cts* were allowed to grow one generation in ^{35}S-containing minimal medium in order to label newly synthesized proteins. LIV-binding protein secreted during this time was complexed with antibody-Sepharose (see Materials and Methods) and separated from the cells. The washed cells were then osmotically shocked to release residual periplasmic proteins. Figure 1 shows the autoradiogram of antibody-complexed labeled protein and crude shock fluid proteins from equivalent cell weights of parent and mutant strains. It is evident that much less periplasmic protein was released from the *lky* strain, and although the patterns of residual protein were similar, differences existed which suggested that not all proteins were released to the same degree. The continuous loss of LIV-binding protein from the periplasm of strain AE84064 *lky*::Mu*cts* is reflected in the appearance in the medium of antibody-trapped radioactivity. The parent strain AE84 secreted undetectable levels of the binding protein under these conditions, and we estimate that the rate of release in the *lky* mutant was at least 20-fold higher than in the *lky*$^+$ parent. The *lky* mutation, therefore, caused continuous secretion of newly synthesized periplasmic protein during growth.

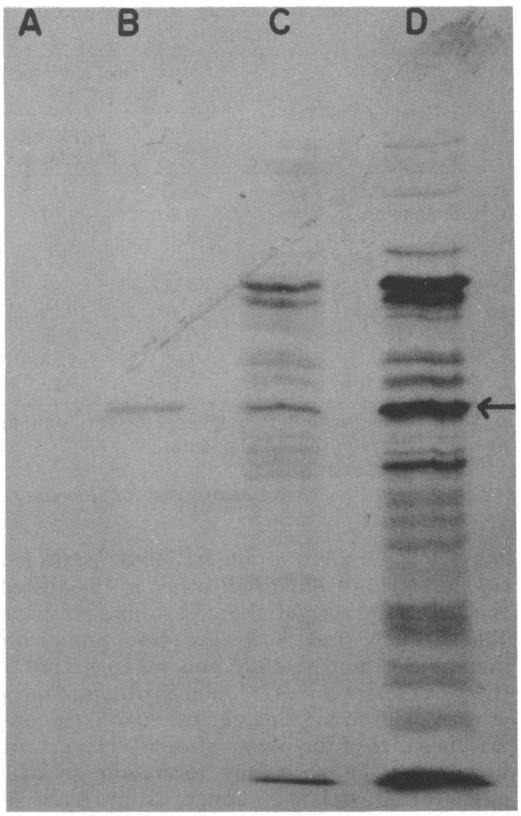

FIG. 1. *Autoradiogram of $^{35}SO_4^{2-}$-labeled proteins electrophoresed on an SDS–11% acrylamide gel according to Laemmli (16). Lanes A and B contain material eluted from anti-LIV-binding protein antibody conjugated to Sepharose which had been present during growth of, respectively, strains AE84 lky$^+$ and AE84064 lky::Mucts. Lanes C and D represent equal volumes of osmotic shock fluid from, respectively, strains AE84064 lky::Mucts and AE84 lky$^+$. The arrow shows the position of a stained standard LIV-binding protein.*

Transport activity. Table 2 compares the transport capacity of strain AE84 *lky*$^+$ with that of AE84064 *lky*::Mu*cts* for a variety of transport solutes. The particular ligands, and their concentrations, were chosen to distinguish osmotic shock-sensitive and -insensitive systems. The transport of galactose by the methylgalactoside system, isoleucine by the threonine-inhibitable LIV-I system, arginine by the lysine-arginine-ornithine system, and glutamine by the high-affinity glutamine transport system were lowered three- to fivefold in the *lky* mutant compared with its parent (Table 2); all these systems have been previously shown to be sensitive to osmotic shock and associated with periplasmic binding proteins (27). In contrast to these results, the threonine-insensitive LIV-II isoleucine transport system, the proline transport system, and the alanine transport system were only slightly reduced in the *lky* mutant (a portion of alanine uptake is through the LIV-1 system [29]). These latter uptake systems are osmotic shock resistant, are found in membrane vesicles free of periplasmic proteins, and are presumably composed of integral inner membrane-bound components. Methionine transport, which has been reported to be sensitive to osmotic shock (13), does not occur in membrane vesicles, and no periplasmic component has been identified; however, it is lowered by twofold in the *lky* strain, supporting evidence for a periplasmic component.

Growth. Strain AE84064 *lky*::Mu*cts* was compared with the parent, AE84 *lky*$^+$, for growth on a variety of carbon sources at 32°C. In a rich broth (LBT), the doubling times differed slightly: strain AE84 *lky*$^+$ (36 min) grew

TABLE 2. *Transport activity in parent and lky strains*[a]

Compound (μM)	Sp act[b] (nmol min^{-1} mg [dry wt]$^{-1}$)	
	AE84 *lky*$^+$	AE84064 *lky*::Mu*cts*
D-Galactose (1)[c]	0.41	0.11
L-Isoleucine (1)	3.80	1.01
L-Isoleucine (1) + L-threonine (200)	0.46	0.44
L-Arginine (5)	4.50	1.81
L-Glutamine (1)	1.40	0.32
L-Proline (10)	0.42	0.38
L-Alanine (40)	1.92	1.23
L-Methionine (5)	0.50	0.22

[a] Cells were grown in 0.2% glucose–MOPS minimal medium plus required supplements.
[b] Average of duplicates.
[c] Growth in 1% glycerol substituted for glucose.

slightly faster than strain AE84064 *lky*::Mu*cts* (38 min). However, on all other media tested, the *lky* mutation put the cell to disadvantage. The doubling time of the *lky* strain on glucose minimal medium was 120 min, whereas that of the parent strain was 80 min; furthermore, the growth yield in limiting glucose (0.02% glucose) was half that of the parent strain. When growth was measured by colony formation on agar plates over extended periods, strain AE84064 *lky*::Mu*cts* grew qualitatively as well as the parent, AE84 *lky*$^+$, on the sugars D-glucose, D-mannose, *N*-acetyl-D-glucosamine, and D-arabinose. When growth was tested on D-ribose (30 mM) or glycerol (110 mM), strain AE84064 *lky*::Mu*cts*, unlike the parent strain, failed to show growth after 2 days of incubation but did form colonies by 4 days (observation communicated to us by S. Schwartz and R. Macnab). This pattern of utilization suggests that the sugars transported by the phosphotransferase (30) system (D-glucose, D-mannose, *N*-acetylglucosamine) enter the cell in sufficient amount to support growth, but D-ribose, which is transported by an osmotic shock-sensitive, binding protein-mediated uptake system, cannot. The fact that L-arabinose supports growth despite also possessing a binding protein-mediated uptake system is explained by the existence of a low-affinity, membrane-bound transport system for this sugar (12). The lack of utilization of glycerol is puzzling. Glycerol is believed to enter the cell at low concentrations by facilitated diffusion (15), but reportedly independent of carrier above 5 mM concentration. The plate concentration of glycerol which failed to support growth of strain AE84064 *lky*::Mu*cts* was much higher than this (110 mM). Either there is an as yet unknown periplasmic component of glycerol entry which has been lost in the *lky* strain, or the *lky* mutation is altering some subsequent event in glycerol dissimilation.

Sensitivity to toxic agents. Pleiotropic envelope-defective mutants of *E. coli* and *S. typhimurium* which secrete periplasmic proteins (7, 17, 32) have been reported to show altered sensitivities to agents such as EDTA, antibiotics, and detergents. We compared strain AE84064 *lky*::Mu*cts* with its parent, AE84 *lky*$^+$, for growth inhibition by the agents listed in Table 3. These agents either attack the envelope directly or have their penetration dependent on the barrier function of the outer membrane. The *lky* strain had acquired varying degrees of sensitivity to antibiotics and to EDTA and had become dramatically more sensitive than its parent to detergents (Table 3). The latter sensitivity was also seen by growth inhibition on MacConkey plates, which contain bile salts. We determined that as little as 0.2% bile salts completely inhibited growth of strain AE84064 *lky*::Mu*cts*, whereas 1.5% bile salts had no effect on the parent, AE84 *lky*$^+$. This property proved useful in mapping the *lky* mutation (see below). We conclude that the ability of the outer membrane to erect a permeability barrier against a variety of antibiotics and to prevent detergent attack had been compromised in the *lky* strain.

Bacteriophage and colicin sensitivities. The outer membrane of *E. coli* functions also as the site of attachment or absorption of bacteriophage (16). We tested the plating ability of phages with known receptor requirements on strain AE84064 *lky*::Mu*cts* and its parent, AE84 *lky*$^+$, to determine whether the outer membrane retained this functional characteristic. We found that the *lky* and *lky*$^+$ strains were equally sensitive to the LPS-requiring phages (16) T4, P1,

TABLE 3. *Growth inhibition by various agents of parent and lky strains growing on agar*[a]

Strain	Growth inhibition by[b]:						
	AMP	KAN	CAM	SDS	EDTA	Triton	PEA
AE84 *lky*$^+$	9	6	8	0	2	0	8
AE84064 *lky*::Mu*cts*	12	8	10	10	3	6[c]	8

[a] Cells (10^7) were plated in soft agar on an LBT agar plate and overlaid with a 1-cm paper disk impregnated with the inhibitor. Growth proceeded at 30°C for 24 h. The zone of inhibition was measured from the edge of the disk to the area of growth.
[b] Expressed as zone of inhibition (in millimeters) around a filter-disk. Abbreviations: AMP, ampicillin (80 μg); KAN, kanamycin (300 μg); CAM, chloramphenicol (120 μg); SDS, sodium dodecyl sulfate (1,000 μg); EDTA, ethylenediaminetetraacetic acid (6 μmol); Triton, Triton X-100 (25 mg); PEA, phenethyl alcohol (50 mg).
[c] This zone was partly turbid.

and P2. Furthermore, phage C21, which is capable of attacking only cells with defective core oligosaccharide (16), could not plate on either the *lky*$^+$ or *lky* strain. The phages T2 and 434 have been shown to require the presence of, respectively, outer membrane proteins Ia and Ib (10) for absorption; both phages plate equally well on the *lky* and *lky*$^+$ strains, suggesting that the outer membrane has the minimal structural requirements for these functions.

Colicins, which affect sensitive cells by a variety of mechanisms, also must interact with the outer membrane, and mutations affecting this structure which result in tolerance or resistance to colicins have been reported (3–5, 24). We tested both *lky*$^+$ and *lky* strains for colicin resistance by the soft agar overlay method (Materials and Methods), using a variety of strains producing defined colicins. The results were that strain AE84064 *lky*::Muc*ts* had acquired resistance to colicins E1, E2, and E3 (both the parent and the *lky* mutant were resistant to colicin Ia) and remained sensitive to colicin B. Quantitatively, whereas a dilution of crude colicin E1 (3×10^3) was able to clear a lawn of strain AE84 *lky*$^+$, the undiluted colicin failed to clear a lawn of strain AE84064 *lky*::Muc*ts*. The pattern of drug and detergent sensitivity and broad range of colicin resistance suggests similarities to the *tolA*-type mutation (3–5, 24).

Mapping. The map position of *lky*::Muc*ts* was initially established by gradient of transmission studies (data not shown), using a variety of Hfr strains and using sensitivity to bile salts as a screen for the *lky* phenotype. These data suggested linkage with *gal*, a position similar to that of the *tolPAB* region (3). When the F-prime F100 was introduced into a *recA* derivative of strain AE84064 *lky*::Muc*ts*, selecting for Gal$^+$, the detergent sensitivity, RNase leakage, and colicin E1 resistance phenotypes of the *lky* mutation were lost (Fig. 2), verifying the position of *lky* near *gal* and establishing that the *lky* mutation is recessive to the wild-type allele. Transduction mapping using markers in the *gal* region as unselected characters was carried out (Table 4). The data are consistent with the gene order *gltA lky gal* (Fig. 2), and the linkage of *lky* to *gal* is similar to that reported for *tolA* (3). The lack of reciprocity when *lky* was used as the unselected marker (last cross, Table 4) and *gal*$^+$ was selected was probably due to phenotypic loss of galactose utilization in a *lky* background (as, for example, the previously described loss of ribose utilization). Ten *lky*$^+$ transductants from each cross were purified and tested for the presence of the Muc*ts* insertion by heat sensitivity (42°C) and resistance to Mu phage; all were found to be free of Mu by these criteria. This result suggests that one and only one Muc*ts* phage is associated with the *lky* mutation. We have noted that when *lky*$^+$ transductants are selected by resistance to 1% bile acids on LBT plates, a considerable phenotypic lag is encountered in the expression of resistance; we have found it necessary to permit growth overnight in broth to fully express detergent resistance. Apparently the envelope defect in the *lky* mutant is very slowly repaired after introduction of a *lky*$^+$ allele.

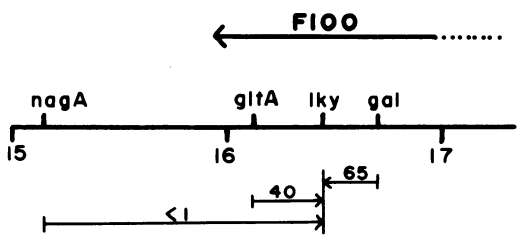

FIG. 2. *Segment of the E. coli genetic map showing the position of lky relative to other markers in this region. The upper arrow shows the known extent of the F-prime F100; the numbers over the lower arrows designate cotransduction frequencies, with the arrowhead pointing to the selected marker.*

Envelope composition. Mutants of *E. coli* and *S. typhimurium* which "leak" periplasmic proteins and become detergent sensitive have been described, and their phenotypes have been attributed to neutral sugar deficiencies in the core LPS (17, 28) or to loss of outer membrane protein, specifically the lipoprotein (9). We wished to determine if the *lky* mutation fell into one or the other of these categories. First, we extracted the core LPS. The yields of core LPS from strains AE84 *lky*$^+$ and AE84064 *lky*::Muc*ts* were, respectively, 0.6 and 0.7% of cellular dry weight. As a molar ratio, relative to the level of KDO, strain AE84064 *lky*::Muc*ts* had levels of the distal neutral sugars glucose, galactose, and heptose one-half those of strain AE84 *lky*$^+$ (Table 5). This lack, on a weight basis, accounts for the lowered total percentage of nonlipid constituents of the LPS (last column) of strain AE84064

TABLE 4. *Mapping of lky by cotransduction with markers in the gal region*

Donor	Recipient	Selected character	% Cotransduction of donor marker
AB1623 *gltA*	AE84064 *lky*	*lky*$^+$[a]	40 (40/100)
JP5053 *nag*	AE84064 *lky*	*lky*$^+$	<1 (0/200)
JP5053 *gal*$^+$ *lky*$^+$	AE84064 *lky gal*	*lky*$^+$	65 (130/200)
JP5053 *gal*$^+$ *lky*$^+$	AE84064 *lky gal*	*gal*$^+$	100 (200/200)

[a] *lky*$^+$ was selected by plating on 1% bile salts–LBT plates.

TABLE 5. *Composition[a] and molar ratios of LPS extracted from wild-type and lky strains of E. coli K-12*

Strain	% Composition, dry wt						
	Galactose	Glucose	Heptose	KDO	Glucosamine	Phosphorus	Total
AE84 *lky*$^+$	4.5 (1.26)[b]	11.4 (3.19)	14.5 (3.48)	9.4 (2)	3.7 (1.05)	6.8 (11.1)	50.3
AE84064 *lky*::Mu*cts*	2.9 (0.60)	7.1 (1.45)	7.9 (1.38)	12.9 (2)	3.2 (0.66)	4.5 (5.3)	38.5

[a] Average of duplicates; see Materials and Methods.
[b] Figures in parentheses denote the molar ratio with KDO normalized to 2.0.

lky::Mu*cts* compared with the parent, AE84 *lky*$^+$. However, the ratios of the neutral sugars to each other in the residual LPS of strain AE84064 *lky*::Mu*cts* (1:2.4:2.3, Gal:Glu:Hep) were not dramatically different from those of the parent (1:2.5:2.8), AE84 *lky*$^+$. One explanation for these data is that two populations of LPS molecules are present in the *lky* mutant, one with a complete core and another with the oligosaccharide moiety terminated at KDO. Whether this altered pattern is the primary outcome or an indirect effect of the *lky* mutation cannot be determined.

The loss of the major outer membrane protein, the murein lipoprotein, has been reported to result in detergent and antibiotic sensitivity, as well as the periplasmic leaky phenotype (7, 11). To test whether the constellation of envelope leakiness and detergent sensitivity, per se, also conferred colicin resistance, we compared a strain, JE5505 carrying a deletion for the lipoprotein (*lpo*; 11), with its parent, strain JE5506, *lpo*$^+$ for resistance to colicn E1, by the dilution endpoint method. We found no difference in susceptibility to the colicin. Since preliminary data from SDS-polyacrylamide gel electrophoresis analysis showed no differences in murein lipoprotein between strains AE84 *lky*$^+$ and AE84064 *lky*::Mu*cts* (data not shown), we suggest that the combined phenotypes of the latter strain are not due to alterations involving the lipoprotein, and that periplasmic "leakiness" per se does not result in colicin resistance.

Phase microscopy of strains AE84 *lky*$^+$ and AE84064 *lky*::Mu*cts* showed no marked differences in cell morphology. Specifically, no increase in blebbing of vesicles from the *lky* strain was noted, even when the strain was grown in low-magnesium medium, in contrast to that reported for the periplasmic leaky strains lacking murein lipoprotein (7).

Reversion. Revertant colonies resistant to 1% bile salts in LBT medium arose from freshly cloned cultures of strain AE84064 *lky*::Mu*cts* at a frequency of 10^{-7}. These colonies were not results of reversal of the original mutation; they were still temperature sensitive due to the presence of the original Mu*cts* insertion. Although the revertants were resistant to detergent, they also showed only partial return to the colicin sensitivity of the parent, strain AE84. More strikingly, the revertants grew poorly on glucose or any other sugar in minimal medium and showed a very mucoid colony morphology. These data suggest that the *lky*:Mu*cts* mutation had been suppressed by a compensating alteration in another component of the envelope which protected the cell from detergent, but which compromised the permeability of the envelope in other ways that restricted the ability of the cell to grow on some carbon sources.

DISCUSSION

The mutation *lky*::Mu*cts* is a pleiotropic envelope mutation which apparently affects the permeability barrier of the outer membrane in a unique manner. At the macromolecular level, this is expressed by the loss of the periplasmic proteins, as shown by continuous secretion of the LIV-binding protein during balanced growth; however, the entry of the colicin molecule (E1, E2, or E3) is apparently prevented, resulting in the phenotype of colicin tolerance. The loss (or partial loss, since some periplasmic proteins remain in the osmotic shock compartment) of the periplasmic binding proteins in strain AE84064 *lky*::Mu*cts* resulted in reduced solute transport by osmotic shock-sensitive transport systems. At first the *lky* mutant resembled outer membrane cryptic mutants (20, 22), except that transport by inner membrane-associated transport systems such as proline and alanine was not significantly affected. A further consequence of the periplasmic leaky phenotype is that the cells grow extremely poorly on sugars requiring osmotic shock-sensitive transport systems, such as ribose. The lack of growth on glycerol might reflect an as yet unknown periplasmic component of transport or utilization of this molecule (15). Indirect effects of the *lky*::Mu*cts* mutation upon metabolism cannot be eliminated, since both growth rate and growth yield are reduced even on glucose.

Accompanying the leaky phenotype is a marked sensitivity to detergents such as bile salts, SDS, and Triton X-100 and an increase in sensitivity to antibiotics and EDTA. Such sensitivities are common in certain outer mem-

brane-defective mutants, particularly those strains of *S. typhimurium* and *E. coli* shown to be deficient in the murein lipoprotein (7, 11, 32) or the neutral sugars of the LPS core (17, 28). Strain AE84064 *lky*::Mu*cts* does not appear to be a mutant of the latter type, since the LPS core sugars are present, although in reduced amounts. Furthermore, the mutant can absorb the LPS-requiring phages P1, P2, and T4 and be resistant to phage C21, which has been shown to attack cells with depleted core sugars (16). The murein lipoprotein also is apparently not involved, since strains carrying deletions of this protein are not colicin resistant.

The mutation *lky*:Mu*cts* appears to be related to the family of colicin tolerance loci linked to *gal* (3–5, 24). Our mapping data suggest a position similar to that of *tolPAB*. The aggregate pattern of detergent sensitivity and resistance to colicins of the A group of strain AE84064 *lky*:: Mu*cts* resembles the pattern of mutants in the colicin tolerance class tolXVIII of Davies and Reeves (4). This communication represents the first indication of the periplasmic leaky phenotype for colicin-tolerant mutants and the consequences of this defect upon the transport properties. Since it is clear that there are at least three complementation groups within the *tolPAB* group which yield similar phenotypes (3), complementation analysis will be necessary to determine whether *lky*::Mu*cts* corresponds to one of the previously identified gene loci. Since the effect of a Mu insertion is highly polar, it is likely that the product of the *lky* gene is totally inactive, and likewise any downstream gene products as well.

Operationally, strain AE84064 *lky*::Mu*cts* resembles an osmotically shocked cell. Cold osmotic shock of wild-type *E. coli* causes sensitivity to agents such as EDTA, to which it is ordinarily resistant (2), causes a loss of the binding protein-dependent transport systems (29), and results in a lag in growth on minimal medium until the envelope has been repaired (2). However, unlike osmotically shocked cells which are rendered sensitive to colicins they are normally resistant to (31), the *lky* mutation renders the cell resistant to these colicins. We suggest that the plasma membrane of strain AE84064 *lky*::Mu*cts* is not freely accessible to colicin molecules because a connection between the outer membrane colicin receptors and the cytoplasmic membrane has been severed. This assumes that colicins must penetrate the cytoplasmic membrane in order to inhibit; this is very likely true for colicin E3, which attacks the ribosome (31). If such connections are also necessary for the mechanical stability of the outer membrane, it may explain the loss of periplasmic enzymes as well as the acquisition of detergent sensitivity.

Strain AE84064 *lky*::Mu*cts*, in addition to providing information about the architectural determinants of the cell envelope, should prove useful in assessing periplasmic components of various processes, especially transport systems. Since periplasmic proteins are exported proteins in strains carrying the *lky* mutation, we can take advantage of this fact to study the secretion and processing of such proteins. For example, we are using strains carrying the *lky* mutation in a search for mutants defective in the latter functions.

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