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Cell Surface Properties of Organic Solvent-Tolerant Mutants of *Escherichia coli* K-12

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In this study, we examined cell surface properties of mutants of *Escherichia coli* for which organic solvent tolerance levels were elevated. The cell surface of each mutant was less hydrophobic than that of the parent, probably due to an increase in lipopolysaccharide content. OmpF synthesis was repressed in the mutants. Organic solvent bound readily to viable *E. coli* cells in response to the polarity of the solvent. The mutants were bound less abundantly with the organic solvent than was the parent.

The toxicity of an organic solvent correlates negatively with the parameter log P_{OW} (3, 16). Log P_{OW} is defined as the common logarithm of the partition coefficient (P_{OW}) of a particular solvent between *n*-octanol and water phases (10, 11). Any microorganism grows in the presence of organic solvents whose log P_{OW} values are equal to or higher than a certain value. However, it had not been clear why the toxicity of an organic solvent was determined in response to the parameter.

Organic solvent molecules intercalate into biological membranes (6, 13, 19, 31, 32, 35) and disturb the structure of the microbial membrane (4, 7). Putative mechanisms of solvent tolerance have been proposed for *Escherichia coli*, *Pseudomonas putida*, and *Pseudomonas aeruginosa* (i.e., increased chain length of fatty acids or proportion of saturated fatty acids [14], increase in *trans*-unsaturated fatty acid levels [13, 32], lack of the OprF porin (22), modification of lipopolysaccharides [LPSs] [29], and activation of an efflux system for hydrophobic compounds [5, 18, 26]). However, the microbial solvent tolerance mechanism is not fully understood.

We have isolated a number of solvent-tolerant mutants of *E. coli* K-12 (3). These mutants provide a means to understand the log P_{OW}-dependent toxic effect and the microbial tolerance mechanisms. The organic solvent-tolerant mutants are also tolerant of low levels of multiple hydrophobic antibiotics whose structures and functions are unrelated to one another (5). Organic solvent tolerance levels are improved in several mutants which became tolerant of low levels of hydrophobic antibiotics. The mutants are more susceptible to a relatively hydrophilic antibiotic, kanamycin, than is the parent. These facts suggest that envelope structures of the organic solvent-tolerant mutants are altered to reduce penetration of hydrophobic compounds. In this study, cell surface properties of the organic solvent-tolerant mutants were examined to elucidate the tolerance mechanism acquired by these mutants.

This report mainly shows that the cell surfaces of organic solvent-tolerant mutants derived from *E. coli* K-12 have low hydrophobicity and that organic solvents bind to *E. coli* cells in response to the polarity of the solvents and the hydrophobicity of the cells.

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MATERIALS AND METHODS

Microbial organisms. The *E. coli* strains used were the streptomycin-resistant K-12 JA300 (F^- *thr leuB6 trpC1117 thi rpsL20 hsdS*) (20) and its organic solvent-tolerant mutants, OST3408, OST3410, OST3301, OST3101, and OST3121. OST3408 and OST3410 are cyclohexane-tolerant mutants that were derived independently from JA300. OST3301, OST3101, and OST3121 were derived sequentially from OST3408 (3, 5). Organic solvent tolerance levels of these organisms are shown in Table 1.

Culture conditions. The bacteria were grown aerobically at 37°C in LBG medium, consisting of 1% Bacto Tryptone (Difco Laboratories, Detroit, Mich.), 0.5% Bacto Yeast Extract (Difco), 1% NaCl, and 0.1% glucose (pH 7.0) (24). This medium supplemented with 10 mM $MgSO_4$ (LBGMg medium) was also used (3). In some experiments, the organisms were grown in LBGMg medium overlaid with a particular organic solvent (10 to 20% of the volume of the medium).

Measurement of the organic solvent tolerance level. Organic solvent tolerance levels of the bacteria were determined by measuring colony formation on LBGMg agar overlaid with a particular organic solvent (4).

Measurement of the relative cell surface hydrophobicities of the organisms. (i) Microbial adhesion to hydrocarbon (MATH) method (30). Cells grown in LBG medium were harvested by centrifugation (3,000 × *g*, 10 min, 4°C) at stationary phase of growth. The cells were suspended in cold 0.8% saline to an optical density at 660 nm (OD_{660}) of 0.6. The absorbance of this suspension is referred to as A_1. Aliquots of the suspension (3 ml) were transferred to two glass tubes. *n*-Octane (0.6 ml) was added to one tube (sample) but not to the other (control). Both suspensions were agitated vigorously for 2 min and then allowed to stand for 15 min to allow separation into *n*-octane and saline layers. The OD_{660} of the saline phase of the sample (A_{2S}) and of the control (A_{2C}) was measured. A MATH value was calculated as the percentage of the decrease in turbidity of the saline phase, using the following equation:

$$[(A_{2C} - A_{2S})/A_1] \times 100$$

(ii) Adhesion to polystyrene bead (APSB) method. For the APSB method (25), the cell suspension was prepared as described above. After the OD_{660} (A_1) of the suspension was measured, 0.2 mg of hydrophobic polystyrene SM-2 Biobeads (100/200 mesh; Bio-Rad Laboratories, Richmond, Calif.) was added to 4 ml of the suspension. This mixture was stirred gently for 30 s at room temperature and filtered through glass wool. An aliquot of the suspension lacking polystyrene beads was used as a control. The OD_{660} values for the filtrate were referred to as A_{2S} (for the sample) and A_{2C} (for the control). The frequency of cell adherence to the beads, or APSB value, was calculated by the following equation:

$$[(A_{2C} - A_{2S})/A_1] \times 100$$

Determination of binding of organic solvent to cells. The organisms were grown in LBGMg medium overnight. The cells were harvested by centrifugation (3,500 × *g*, 10 min, 15°C) and then resuspended in one-fourth of the supernatant. Half of this suspension was diluted twofold with the supernatant. Ten milliliters of each suspension was shaken at 37°C for 1 h with 2 ml of a particular organic solvent. The cells harvested by centrifugation were washed twice with cold 0.8% saline and resuspended in 2.1 ml of the saline. An aliquot of the suspension (2 ml) was transferred to a glass tube. The cells were extracted with chloroform in an ice-water bath. The extract was assayed for the organic solvent with a high-performance liquid chromatography apparatus equipped with a reverse-phase column. The elution pattern was monitored by measuring the A_{280} or the differential refractive index. The organic solvent level was determined using an authentic organic solvent as a standard.

TABLE 1. Organic solvent tolerance levels and cell surface hydrophobicity of organic solvent-tolerant mutants of *E. coli*

Strain	Relative hydrophobicity (%)[a]		Organic solvent tolerance level[b]	
	MATH	APSB	Index solvent	Index value
JA300	72.5 ± 1.5	32.5 ± 0.8	*n*-Hexane	3.9
OST3408	50.1 ± 5.0	21.1 ± 2.2	Cyclohexane	3.4
OST3301	26.9 ± 3.4	19.6 ± 0.4	*n*-Pentane	3.3
OST3101	12.9 ± 1.1	NT[c]	*p*-Xylene	3.1
OST3121	10.0 ± 2.2	14.2 ± 0.4	*p*-Xylene	3.1
OST3410	5.9 ± 0.9	7.1 ± 0.4	Cyclohexane	3.4

[a] The values shown are the means of the values determined in two experiments.

[b] The index solvent is the most toxic solvent among the solvents to which the strain is tolerant, and the index value is the log P_{OW} of the index solvent.

[c] NT, not tested.

Preparation of envelope, outer membrane protein, and peptidoglycan fractions. Cells grown in LBGMg medium were harvested (OD_{660}, 0.6) by centrifugation (5,000 × *g*, 10 min, 4°C). The cells were resuspended in 10 mM NaH_2PO_4–NaOH buffer (pH 7.0) and broken by sonication (20 W, 2 min) in an ice-water bath. Unbroken cells were removed by centrifugation. The envelope was pelleted by centrifugation (100,000 × *g*, 60 min, 4°C) and washed once with the phosphate buffer.

The envelope (3 mg of protein/ml) was incubated at room temperature for 30 min in 0.5% sodium dodecyl sarcosinate (sarcosyl)–10 mM NaH_2PO_4–NaOH (pH 7.4) (8). The suspension was centrifuged at 100,000 × *g* for 45 min at 5°C. The pellet was washed with the sarcosyl-phosphate buffer and used as the outer membrane protein fraction. A portion of this fraction was further extracted with sodium dodecyl sulfate (SDS) at 100°C for 5 min and then used as the lipoprotein-bearing peptidoglycan fraction.

Extraction of LPS from cells. Cells grown in 100 ml of LBG medium were harvested and washed with chloroform-methanol (1:2, vol/vol). The cells were extracted with warm phenol-water (34). LPS was pelleted from the extract by centrifugation (10,000 × *g*, 45 min, 4°C).

SDS-polyacrylamide gel electrophoresis of protein. Samples were electrophoresed on an SDS-urea-polyacrylamide gel (1). Protein was stained with Coomassie brilliant blue R-250.

Quantitative analyses. (i) Protein. Protein was determined by the method of Lowry et al. (23), using bovine serum albumin as a standard.

(ii) LPS. 2-Keto-3-deoxyoctonate (KDO) was determined with thiobarbituric acid reagent after weak hydrolysis and periodate oxidation (33). LPS was estimated on the basis of the KDO content, with LPS prepared from *E. coli* K-12 (2) as a reference.

(iii) Peptidoglycan. After the sample was hydrolyzed in 6 M HCl at 105°C for 14 h, diaminopimelic acid was determined with an amino acid analyzer. The peptidoglycan content was estimated from this determination on the assumption that 1 μmol of diaminopimelic acid corresponds to 0.92 mg of peptidoglycan (2).

(iv) Peptidoglycan-binding lipoprotein. The amino acid composition of the lipoprotein-bearing peptidoglycan was determined as described above. Lipoprotein was determined from molar ratios of the protein-constituting amino acids (17) to diaminopimelic acid.

Materials. The organic solvents used were of the highest quality available. The log P_{OW} values of the solvents listed in Table 1 were calculated by the addition rule (21).

RESULTS

Low-level adherence of organic solvent-tolerant mutants to *n*-octane. A vigorous agitation of the *n*-octane–saline mixture containing strain JA300 yielded many stable *n*-octane droplets in the saline phase. These droplets, which adhered to numerous JA300 cells, were maintained stably in the interface region between the saline and *n*-octane layers (Fig. 1A and 2). As a result, a considerable number of the cells were transferred from the saline to the interface. The *n*-octane droplets that formed in the saline containing the organic solvent-tolerant mutants adhered to a relatively small number of cells (Fig. 1B and C). In particular, the droplets scarcely adhered to OST3121 cells. These *n*-octane droplets readily fused with one another. A homogeneous *n*-octane phase was formed immediately after agitation was halted. The interface region was narrower for the mutants than for the parent (Fig. 2). These results indicate that the cell surfaces of the mutants have low affinity for the *n*-octane droplets.

Cell surface hydrophobicity of organic solvent-tolerant mutants. The relative cell hydrophobicity was measured by two methods (Table 1). MATH values show adhesion of the cells to *n*-octane droplets stabilized by the adherent cells (Fig. 2). APSB values indicate directly the frequency of adherence of the cells to polystyrene beads. The hydrophobicity values determined by the two methods correlated with each other. By each method, the values for the organic solvent-tolerant mutants were lower than that for the parent, indicating that the

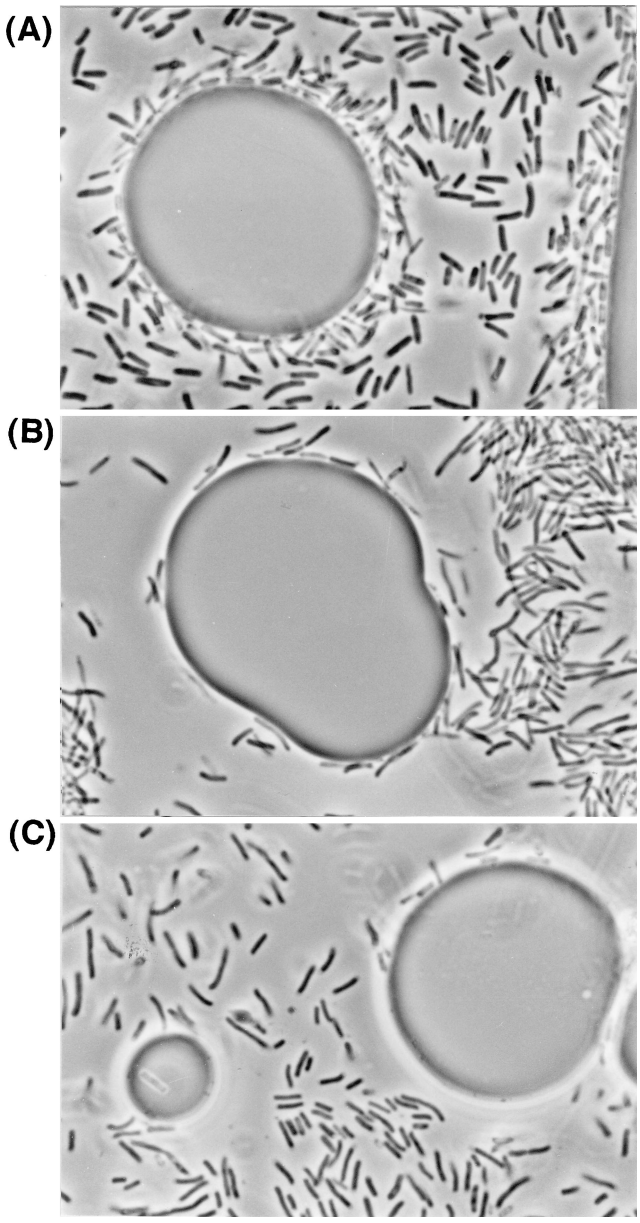

FIG. 1. *n*-Octane droplets with adherent *E. coli* cells. *E. coli* cell suspensions overlaid with *n*-octane were agitated with a vortex mixer. *n*-Octane droplets that formed in the saline were observed microscopically. (A) JA300; (B) OST3408; (C) OST3121.

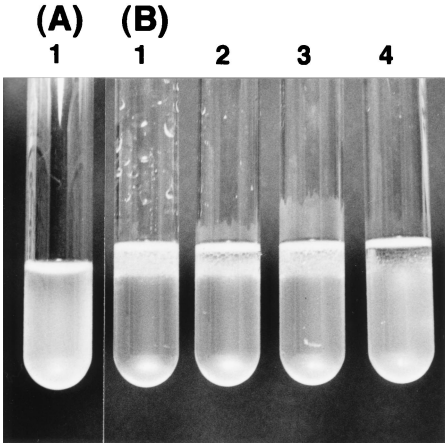

FIG. 2. Decrease in turbidity of the aqueous suspension of *E. coli* caused by adhesion of cells to *n*-octane droplets maintained in the interface region. The cell suspensions were vigorously agitated in the absence (A) or presence (B) of *n*-octane. Tubes: 1, JA300; 2, OST3408; 3, OST3301; 4, OST3121.

cell surfaces of the mutants were less hydrophobic than that of the parent. It was found that the hydrophobicity of the mutants decreased in response to the organic solvent tolerance level, except for OST3410, which showed extremely low hydrophobicity.

Binding of organic solvent to cells. When a large volume of a hydrophobic organic solvent is added to an aqueous medium, a small portion of the solvent is dissolved in the medium and adheres to the cell surfaces. The molecules adhering to the cells seem to penetrate across the outer membrane into the periplasm and cytoplasm. The total amounts of organic solvent molecules in these several steps were measured en bloc and are shown as binding amounts in Table 2.

Cyclohexane, *p*-xylene, and toluene, which are toxic to JA300, bound to JA300 cells abundantly (0.10 to 0.16 μmol/mg of cellular protein). Binding of the low-toxicity solvent (cyclooctane) or nontoxic solvent (*n*-nonane) was low or undetectable. Thus, highly toxic organic solvents bound more abundantly to JA300 cells than did solvents of low toxicity with low polarity. Organic solvents with decreasing log P_{OW} values also bound abundantly to each organic solvent-tolerant mutant. Each organic solvent bound less abundantly to the mutants than to the parent. The amount of each organic solvent that bound to the mutants was small in response to the cell surface hydrophobicity and was negatively correlated with organic solvent tolerance levels, with the exception of the binding of cyclooctane and *p*-xylene to OST3410. Organic solvents for which log P_{OW} values were equal to or higher than the index value bound to *E. coli* in amounts less than 0.04 to 0.05 μmol/mg of cellular protein.

TABLE 3. Fragility of organisms grown in the presence of organic solvent[a]

Strain	Index value[b]	Decrease in turbidity of cell suspension (%)[c]				
		n-Nonane (5.5)	Isooctane (4.8)	Cyclooctane (4.5)	*n*-Hexane (3.9)	Cyclohexane (3.4)
JA300	3.9	<2	16.2	20.1	NT[d]	NT
OST3408	3.4	<2	10.5	19.6	22.3	NT
OST3121	3.1	<2	<2	<2	13.7	18.4
OST3410	3.4	<2	18.7	30.5	NT	NT

[a] Each organism was grown in LBGMg medium containing 10% (vol/vol) organic solvent at 37°C for 15 h. The cell suspension was agitated vigorously with a vortex mixer for 2 min. The turbidity of the suspension was measured at 660 nm before and after agitation. Cell fragility is represented by the percent decrease in turbidity during agitation versus the original turbidity.
[b] The index value is the log P_{OW} of the index solvent.
[c] The log P_{OW} values are shown in parentheses after the solvent names.
[d] NT, not tested.

Fragility of *E. coli* cells grown in the presence of organic solvents. *E. coli* cells grown in the presence of organic solvents which were less toxic than the relevant index solvent became mechanically fragile in stationary phase of growth (Table 3). This fragility was observed typically in cells of strains JA300, OST3408, and OST3410 grown with prolonged incubation in the presence of isooctane or cyclooctane. JA300 cells grown in the presence of one of the more polar organic solvents were more fragile. The JA300 cells grown in the presence of *n*-nonane, which scarcely bound to *E. coli* cells (Table 2), were as stable as those grown without the organic solvent. The fragility was probably caused, directly or indirectly, by intercalation of organic solvent molecules into the membranes.

OST3121 cells grown in the presence of isooctane or cyclooctane were mechanically stable. OST3121 cells grown in the presence of *n*-hexane or cyclohexane were as fragile as JA300 or OST3408 cells grown with isooctane or cyclooctane. The affinity of binding of cyclohexane to OST3121 was similar to that of cyclooctane to JA300 or OST3408 (Table 2). These results supported the theory that the binding of organic solvent molecules made the cells fragile. Cyclooctane was shown to be less adherent to OST3408 and OST3410 than to JA300 cells, at least after 1 h of incubation with cyclooctane (Table 2). However, OST3408 and OST3410 grown in the presence of cyclooctane were not more stable than JA300. The OST3410 cells were significantly fragile.

TABLE 2. Binding of organic solvents to *E. coli* cells

Strain	Amount of organic solvent binding to cells[a]				
	n-Nonane (5.5)	Cyclooctane (4.5)	Cyclohexane (3.4)	*p*-Xylene (3.1)	Toluene (2.7)
JA300	<0.001	0.046	0.10	0.13	0.16
OST3408	<0.001	0.032	0.036	0.075	0.088
OST3121	<0.001	0.009	0.024	0.036	0.071
OST3410	<0.001	0.012	0.021	0.049	0.053

[a] Each organism was grown overnight in LBGMg medium. Then the cells were incubated with organic solvent for 1 h. The log P_{OW} values of the organic solvents are shown in parentheses. Units are micromoles of solvent per milligram of cellular protein.

TABLE 4. LPS content in envelope preparations from organic solvent-tolerant mutants of *E. coli*

Strain	Ratio of LPS content to amount of[a]:		
	Whole envelope protein (mg/mg)[b]	Sarcosyl-insoluble protein (mg/mg)[b]	Peptidoglycan (mg/mg)[c]
JA300	0.27	0.49	3.8
OST3408	0.31	0.58	4.4
OST3121	0.39	0.86	5.2
OST3410	0.38	0.89	4.8

[a] Envelope was prepared from cells grown in the absence of organic solvent.
[b] Envelope protein was determined before and after extraction with sarcosyl.
[c] Peptidoglycan content was calculated from the diaminopimelic acid content of the envelope preparation.

TABLE 5. Amino acid composition of peptidoglycan bearing lipoprotein

Strain	Molar ratio of amino acid (mol/mol)[a]									LP/PG (mg/mg)[b]
	Asx	Thr	Val	Met	Ile	Leu	Tyr	Lys	Arg	
JA300	0.88 (14)	0.14 (2.2)	0.26 (4.1)	0.13 (2.0)	0.07 (1.1)	0.25 (4.0)	0.06 (1.0)	0.30 (4.7)	0.27 (4.3)	0.51
OST3408	0.84 (14)	0.14 (2.3)	0.27 (4.5)	0.11 (1.9)	0.07 (1.2)	0.26 (4.3)	0.06 (1.0)	0.29 (4.9)	0.26 (4.3)	0.50
OST3121	1.36 (14)	0.23 (2.3)	0.39 (4.0)	0.17 (1.7)	0.11 (1.1)	0.40 (4.1)	0.10 (1.0)	0.47 (4.9)	0.44 (4.5)	0.78
OST3410	0.60 (14)	0.09 (2.1)	0.21 (4.8)	0.12 (2.7)	0.06 (1.5)	0.19 (4.4)	0.04 (1.0)	0.22 (5.2)	0.18 (4.3)	0.39
Theoretical amino acid composition of lipoprotein	(14)	(2)	(4)	(2)	(1)	(4)	(1)	(5)	(4)	

[a] The amino acid composition of the HCl hydrolysate of peptidoglycan-bearing lipoprotein was measured. Molar ratios of protein-constituting amino acids to diaminopimelic acid are shown. The molar ratios of the amino acids to aspartic acid are shown in parentheses, with the aspartic acid content being taken as 14.
[b] The ratios of lipoprotein to peptidoglycan (LP/PG) were calculated with the mean values of the ratios of amino acids to diaminopimelic acid.

Increase of LPS in organic solvent-tolerant mutants. The amounts of LPS in the envelopes of the mutants were compared with those of other components, such as whole envelope protein, outer membrane protein, and peptidoglycan (Table 4). The ratios of LPS content to the amounts of these components were higher in the organic solvent-tolerant mutants than in the parent. In particular, it is expected that the ratio of LPS content to the content of sarcosyl-insoluble protein correlates with the surface area of the outer leaflet occupied by LPS. It was shown that this ratio increased with decreasing cellular hydrophobicity (Table 1), indicating that the cell surfaces of the tolerant mutants were made less hydrophobic by an increase in LPS content.

LPS molecules present in the envelope preparations were analyzed on SDS-polyacrylamide gels (results not shown). All the organisms produced a rough type of LPS. No detectable changes in the electrophoretic mobilities of LPS bands were found among the organisms. The molar ratios of the constituent sugars of LPS—KDO, neutral sugars, and amino sugars—were from 1:2.6:1.2 to 1:2.9:1.3 in all the LPS samples prepared from the cells (results not shown). These results suggested that the LPSs of the mutants were not significantly altered in chemical composition, although small changes in the molecules could not be ruled out.

Repression of OmpF synthesis in organic solvent-tolerant mutants. The parent produced OmpC and OmpF porins even when grown in LBGMg containing 1% NaCl. In this medium, the level of OmpF porin protein in the organic solvent-tolerant mutants decreased extremely compared with that of the parent (Fig. 3). All the mutants produced as much OmpC as the parent when grown in medium with 1% NaCl. Repression of OmpF is consistent with the fact that the ratio of LPS to sarcosyl-insoluble protein was high in the mutants (Table 4). The parent and the organic solvent-tolerant mutants synthesized OmpF when grown in the absence of NaCl (results not shown), indicating that OmpF synthesis was derepressed in the parent regardless of the NaCl concentration and was repressed in the mutants at 1% NaCl.

Amounts of peptidoglycan-binding lipoprotein in organic solvent-tolerant mutants. The lipoprotein-bearing peptidoglycan contained no protein capable of being electrophoresed on an SDS-polyacrylamide gel (results not shown). Several amino acids and amino sugars were released from the preparation by the HCl hydrolysis (Table 5). It was hard to determine the amounts of serine, glutamic acid, and alanine derived from protein, because serine coeluted with muramic acid under the conditions used for the amino acid analysis and because the last two amino acids were also constituents of peptidoglycan. Table 5 shows the molar proportions of amino acids that were obviously constituents of proteins.

In all samples, the molar ratios of amino acids were almost identical to the theoretical ratios of peptidoglycan-binding lipoprotein (17). These results indicated that the amino acids were derived from the lipoprotein. Therefore, it was possible to calculate the amounts of peptidoglycan-binding lipoprotein from the amino acid compositions shown in Table 5. The amount of peptidoglycan-binding lipoprotein was high in OST3121 and low in OST3410. These different amounts might affect the fragility of the cells grown in the presence of organic solvents.

DISCUSSION

There is increasing interest in organic solvent-tolerant microorganisms and microbial tolerance mechanisms. Several putative tolerance mechanisms have been proposed. It has been reported that organic solvents with higher log P_{OW} values intercalated more readily into phospholipid liposomes (31). However, it is known that the more polar organic solvents are more toxic to microorganisms (3, 15, 16). In this study, we found that organic solvents with lower log P_{OWs} bound more abundantly to viable cells of *E. coli* (Table 2). This finding

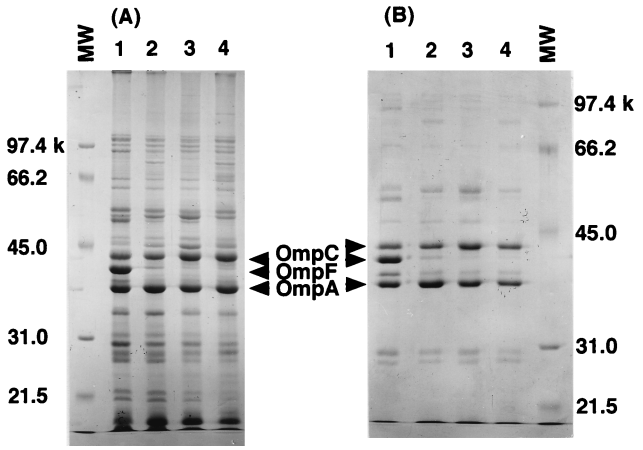

FIG. 3. SDS-urea-polyacrylamide gel electrophoresis of envelope protein of organic solvent-tolerant mutants. Whole-envelope preparation containing 50 μg of protein (A) or the sarcosyl-insoluble fraction obtained from the whole envelope (30 μg of protein) (B) was electrophoresed on 0.1% SDS–4 M urea–10% (wt/vol) polyacrylamide gels. Protein was stained with Coomassie brilliant blue R-250. Lanes: MW, molecular size markers (in kilodaltons); 1, JA300; 2, OST3408; 3, OST3410; 4, OST3121.

explains the basis of the empirical log P_{OW}-toxicity rule and suggests that some structure or function of the outermost cell surface prevents binding of hydrophobic organic solvents. Each organic solvent bound less abundantly to the organic solvent-tolerant mutants than to the parent. This low-level binding of organic solvent is likely one of the mechanisms of tolerance to organic solvents. The low level of binding might be due to the low cell surface hydrophobicity (Fig. 1 and 2; Table 1), although efflux activity levels of the mutants must be examined to determine this.

It is rational to consider that the low hydrophobicity of the organic solvent-tolerant mutants resulted from the quantitative increase in LPS without any chemical alterations. LPS molecules make a monolayer on the cell surface to provide *E. coli* with a permeation barrier to hydrophobic compounds (28). Saturated fatty acid residues of LPS are anchored into the outer leaflet of the outer membrane. Therefore, an abundance of LPS results in an increased proportion of saturated fatty acids in the outer leaflet. An increase in the proportion of saturated fatty acids has been proposed as an ethanol tolerance mechanism of *E. coli* (14), although the fatty acid composition of the phospholipid component of each mutant was similar to that of the parent (results not shown). The oligosaccharide chain of LPS protrudes into the environment. As a result, the surfaces of cells that are enriched in LPS become less hydrophobic. It has been reported that the saccharide chain of LPS is elongated in *P. putida* exposed to *o*-xylene (29). The intensity of the lateral interaction between LPS molecules is important to the improvement of organic solvent tolerance levels of *E. coli* (4).

OmpF synthesis is usually repressed and OmpC synthesis is derepressed at high osmolarities (9, 12). It was shown that JA300 was a mutant in which OmpF synthesis was not under the osmoregulation of NaCl. OmpF synthesis in the organic solvent-tolerant mutants was controlled by this osmoregulation, although genetic alterations in the mutants are still not clear. At least, OST3408 is a *marR* mutant (5a). It has been reported that the porin protein OprF is lost in a toluene-tolerant mutant of *P. aeruginosa* (22). Hydrophobic β-lactam antibiotics pass through OmpF channels rather than OmpC channels (27). These findings imply that organic solvent molecules could pass through the OmpF porin. Loss of OmpF might contribute to improving the level of organic solvent tolerance in *E. coli* directly by decreasing the number of channels for organic solvents and indirectly by increasing the surface area available for LPS arrays.

The cells grown in the presence of organic solvent became mechanically fragile (Table 3). It has been reported that *n*-octane intercalates into the outer membrane of *E. coli* (7). The outer and inner membranes are displaced from each other in *E. coli* cells grown in the presence of organic solvents (4). The periplasm is expanded by this displacement. These structural perturbations may cause such cell fragility. The fragility correlates positively with the cell surface hydrophobicity and negatively with the amount of peptidoglycan-binding lipoprotein (Table 5). The lipoprotein seems to contribute to the mechanical stabilization of surface structures of cells bound with organic solvents.

In this study, it was shown that the organic solvent-tolerant mutants differed from the parent in the amounts of several cell surface components (LPS, OmpF, and peptidoglycan-binding lipoprotein). These quantitative alterations probably contribute synergistically to the stepwise elevation of organic solvent tolerance levels of the mutants.

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