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Increase in Sensitivity to Antibiotics and Lysozyme on Deletion of Lipopolysaccharides in *Escherichia coli* Strains

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Lipopolysaccharides on the cell surface of *Escherichia coli* prevent penetration of lysozyme or certain low-molecular-weight drugs.

Previously we found that the polysaccharide portion of lipopolysaccharides (LPS) in *Escherichia coli* K-12, strain JE1011, are required for resistance to several antibiotics (e.g., novobiocin, spiramycin, actinomycin D) and for adsorption of several phages (T4, T7, P1, and Mu1) (2). In this work we examined other strains of *E. coli* and found that LPS-deficient mutants were hypersensitive to lysozyme.

Mutants were obtained from *E. coli* strain W4573 (Lederberg) by lysogenization with phage Mu1 to cause insertion mutations (3) and then selection of strains resistant to phage T2 (S. Tamaki and M. Matsuhashi, unpublished experiments). Spontaneous mutants of *E. coli* strain B-1 (IAM 1268) were selected for resistance to phage T4. LPS was isolated and analyzed as described previously (2). Sensitivities to antibiotics and phages were assayed essentially as described previously (2).

As shown in Table 1, among the T2-resistant mutants of strain W4573, those strains which had little or no phosphates and glucose (galactose not measured) in the polysaccharide fraction of their LPS (except mutant TM9) were hypersensitive to novobiocin and spiramycin and were partially resistant to phage T4. Those strains which had normal amounts of phosphates (mutant TM34 and many other strains not listed in the table) were resistant to the antibiotics and sensitive to phage T4. Similar results were obtained with T4-resistant mutants of strain B-1 (T42, T44, T41 and T43).

Sekiguchi and Iida (1) reported that some *E. coli* mutants with increased sensitivity to actinomycin D are also hypersensitive to externally added lysozyme. However, they did not suggest which part of the cell surface was responsible for resistance to these compounds. Our results (Table 2) indicate that the absence

TABLE 1. *Lipopolysaccharide composition, antibiotic sensitivity, and phage adsorption of E. coli strains*

Strain	LPS[a]			Antibiotics[b]		Sensitivity to phage T4[c]
	Heptose	Phosphate	Glucose	Novobiocin	Spiramycin	
W4573 parent	0.55	0.46	0.22	30	20.7	S
TM25	0.36	0.05	0.00	0.6	1.2	PR
TM6	0.55	0.14	0.01	0.5	1.4	PR
TM9	0.62	0.27	0.33	2.0	13.8	PR
TM34	0.74	0.58	0.34	30	28	S
B-1 parent	0.64	0.55	0.33	0.52	3.5	S
T42	0.51	0.09	0.00	0.10	0.32	R
T44	0.69	0.09	0.00	0.07	0.35	R
T41	0.49	0.28	0.07	0.21	0.80	R
T43	0.64	0.36	0.03	0.26	0.55	R

[a] Micromoles in acetic acid supernatant fraction per milligram of LPS.
[b] Concentrations (μg/ml) required for 50% inhibition of growth of bacteria.
[c] S, Sensitive; PR, partially resistant; R, resistant.

TABLE 2. *Lysozyme sensitivity of parent and mutant strains*

Strain	Lysozyme sensitivity	
	Exponential phase[a]	Stationary phase[b]
JE1011 parent	>10	—[c]
NS1	0.5	—
NS3	0.5	—
NS7	0.25	—
W4573 parent	>10	>60
TM25	0.5	0.3
TM6	0.5	>60
TM9	1.0	>60
TM34	>10	>60
B-1 parent	>10	>60
T42	0.5	0.2
T44	0.25	0.5
T41	0.9	>60
T43	0.2	—

[a] Cells were cultured in nutrient broth at 37 C. They were harvested in the exponential phase and suspended in 0.01 M Tris hydrochloride, pH 8.0 (10^8 cells per ml). Suspensions were mixed with various amounts of lysozyme (Eizai Co., Tokyo, Japan) and incubated at 37 C. Turbidity at 660 nm was measured in an ADS photoelectric colorimeter (Fuji Kogyo Co., Tokyo, Japan) or a Hitachi spectrophotometer. Results are expressed as micrograms of lysozyme per milliliter required to cause 10% (JE1011) or 20% (W4573 and B-1) decrease in turbidity on incubation in 6 min.

[b] Overnight cultured cells were tested as above. Results are expressed as micrograms of lysozyme per milliliter required to cause 3% decrease in turbidity on incubation in 6 min.

[c] Results uncertain because cells aggregated.

of phosphate or glucose residues in the polysaccharide portion of LPS is closely correlated with sensitivity of the cells to lysozyme. These results suggest that a part of LPS on the cell surface forms a structure which makes the cells resistant to the penetration of macromolecular lysozyme as well as low-molecular-weight antibiotics.

Table 2 lists the mutants of strain JE1011 (NS1, NS3, and NS7) selected by their hypersensitivity to novobiocin (2). They were also resistant to phage T4 and lacked phosphate and several sugar residues in the polysaccharide region of their LPS (2). These mutant strains were also more sensitive to added lysozyme than their parent, strain JE1011 (Table 2). Similarly, some T2-resistant mutants of strain W4573 and T4-resistant mutants of strain B-1 which were hypersensitive to novobiocin and spiramycin were also hypersensitive to lysozyme (assay with exponential-phase cells). Strains practically lacking phosphate and glucose residues of LPS (mutants TM25, T42, and T44) were, moreover, sensitive to lysozyme in assay with stationary-phase cells (Table 2).

LITERATURE CITED

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