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Shielding of *Escherichia coli* Outer Membrane Proteins as Receptors for Bacteriophages and Colicins by O-Antigenic Chains of Lipopolysaccharide

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The accessibility of several outer membrane proteins for bacteriophages and colicins in isogenic smooth and rough *Escherichia coli* strains was investigated. The results show that O antigen carrying lipopolysaccharide is able to prevent access of all phages and colicins tested to their outer membrane protein receptors.

Outer membrane proteins of *Escherichia coli* are known to function as receptors for many bacteriophages and colicins (4). This receptor function has generally been demonstrated in rough strains such as *E. coli* K-12 which lack the O-antigen part of lipopolysaccharide (LPS). It is conceivable that the long O-antigenic chains present on most wild-type *E. coli* strains (5) can sterically hinder access of phages and colicins to their outer membrane protein receptors. This possibility has often been suggested (for example, see references 7, 8, and 12), but never systematically investigated. In the present study, the effect of O antigen on the ability of a number of different phages and colicins to reach their outer membrane protein receptor sites has been investigated.

Twelve outer membrane protein-specific phages were tested for their ability to plate on four smooth *E. coli* strains, i.e, strains U20, F2, F3, and F8, and their isogenic O-antigen-deficient derivatives, strains U20-3, F2-1, F3-2, and F8-5. Strains in the latter group have been isolated as spontaneous mutants resistant to phages which recognize the O antigens of strains in the former group as their receptors (11). All 12 phages failed to form plaques on the smooth strains U20 and F2, but 10 and 6 phages, respectively, were able to infect derivative strains U20-3 and F2-1 with strongly reduced amounts of O antigen (Table 1). Apparently, in strains U20 and F2, the O-antigenic part of LPS creates a barrier to these outer membrane protein-specific phages. Resistance of the rough strains to the other phages may be caused by lack of receptors or by digestion of the phage DNA by restriction endonucleases. A more complex picture was obtained with strains F3 and F8 (Table 1), which contain less O antigen than strains U20 and F2 (Fig. 1). Exclusive plating on the rough strain F3-2 or F8-5 or both was observed only for phages K20 and T6. All other phages that infected the rough strains F3-2 or F8-5 were also capable of plating on the smooth parental strain. Apparently, the amount of O antigen produced by strains F3 and F8 is insufficient to create an effective barrier to all of the outer membrane protein-specific phages.

We also investigated the ability of eight different colicins to reach their receptor sites on several outer membrane proteins in the presence or absence of O antigen (Table 2). In contrast to their rough derivatives, strains U20 and F2 were resistant to all colicins tested. Similarly, five colicins were

TABLE 1. Bacteriophage sensitivity or resistance of smooth *E. coli* strains and their rough derivatives[a]

Phage	Receptor[b]	Bacteriophage plating characteristics of strain:							
		U20	U20-3	F2	F2-1	F3	F3-2	F8	F8-5
TuIa	OmpF	R	R	R	R	R	R	0.2<<	0.1<<
K20	OmpF	R	0.1=t	R	R	R	R	R	0.01<<
TuIb	OmpC	R	1=	R	0.5=t	0.05<<	R	0.5<	0.8<
Me1	OmpC	R	0.2<<	R	R	R	R	R	R
PA-2	OmpC	R	0.2<t	R	R	R	R	R	R
HK253hrk	OmpC	R	R	R	R	0.2<<	1=	R	R
SS1	LamB, OmpC	R	1=t	R	1=t	R	R	0.2<<	0.2<<
TC45	PhoE	R	0.2<<t	R	0.2<t	R	R	0.3<	R
TC45hrN3	PhoE	R	0.5<<t	R	0.5<	R	R	0.7<	R
TuII*	OmpA	R	0.2<t	R	R	R	R	R	R
K3	OmpA	R	0.2=	R	0.2<t	R	R	R	R
T6	Tsx	R	0.5<<	R	0.1<<	R	0.3<<	R	0.3<

[a] Smooth *E. coli* U20, F2, F3, and F8 and their rough derivatives U20-3, F2-1, F3-2, and F8-5 were grown overnight at 37°C in L broth (10). Bacteria were mixed with approximately 300 PFU (as measured on a sensitive *E. coli* K-12 strain) of the indicated bacteriophages and applied as a top layer of soft L-broth agar on L-broth agar plates. The plates were scored for plaque formation after incubation overnight at 37°C. The numbers indicate efficiency of plating relative to *E. coli* K-12. Symbols: R, no plaques; =, similar plaque size; <, smaller plaques; <<, pinpoint plaques; t, turbid plaques.

[b] See reference 4, except for the OmpC-specific phage HK253hrk (C. Verhoef, personal communication).

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TABLE 2. Colicin sensitivity or resistance of smooth *E. coli* strains and their rough derivatives[a]

Colicin (producing strain[b])	Receptor[c]	Colicin inhibition in strain:							
		U20	U20-3	F2	F2-1	F3	F3-2	F8	F8-5
A (PC1403)	OmpF, BtuB	−	++	−	++	++	++	−	++
B (PC1396)	FepA	−	++	−	++	++	++	++	++
E1 (PC2247)	BtuB	±	++	±	++	++	++	++	++
E2 (PC2338)	BtuB	±	++	−	++	−	−	−	++
E3 (PC2339)	BtuB	−	++	−	++	−	−	−	++
K (PC2340)	Tsx	−	+	−	+	+	+	−	+
S4 (PC2406)	?	−	++	−	++	−	−	−	++
V (PC1395)	Cir?	−	++	−	++	++	++	++	++

[a] Sensitivity of smooth and rough strains to colicins was tested in a double-layer test as described by Havekes et al. (3). Colonies of colicin-producing strains on L-broth agar plates were irradiated with UV light (40 ergs/mm^2 for 10 s) and incubated for 2 h at 37°C. After the cells were killed with chloroform vapor, the plates were layered with 4 ml of L-broth soft agar containing about 10^8 cells of the smooth or rough *E. coli* strains, and the presence or absence of a halo around the colicin-producing colonies was scored after overnight incubation at 37°C. Symbols: −, completely resistant; ±, inhibition zone of approximately 1 mm; +, inhibition zone of 2 to 5 mm; ++, inhibition zone larger than 5 mm.

[b] PC, Phabagen Collection, Department of Molecular Cell Biology, Section Microbiology, State University of Utrecht, The Netherlands.

[c] See reference 6. ?, Receptor unknown.

able to kill cells of the rough strain F8-5 but not of its smooth parental strain. The latter strain, however, was sensitive to colicins B, E1, and V. The resistance-sensitivity pattern of strain F3, which contains the lowest amount of O antigen of the four smooth strains (Fig. 1), was identical to that of its rough derivative, strain F3-2.

In conclusion, our results show that O-antigen-carrying LPS is able to prevent access of all phages and colicins tested to their outer membrane protein receptors, provided that a sufficient amount of O antigen is present. The same conclusion has been reached previously for antibodies directed against PhoE outer membrane protein (11). A shielding effect of long-chain LPS has also been demonstrated with the neutrophil bactericidal permeability-increasing protein (13) and with the bactericidal activity of normal serum (9).

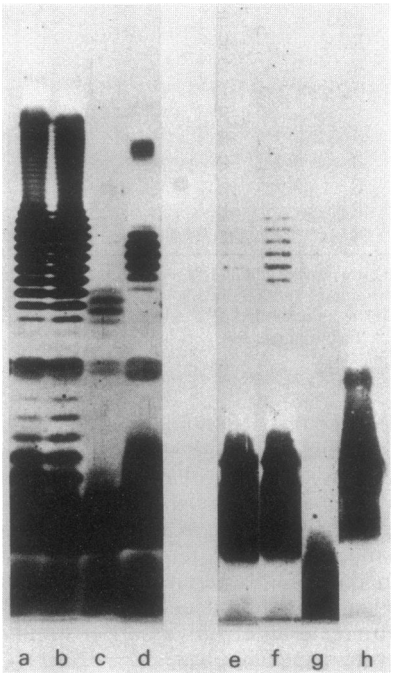

FIG. 1. Silver-stained sodium dodecyl sulfate-polyacrylamide gel of proteinase K-treated cell envelopes (11), showing the LPS patterns of smooth strains U20 (lane a), F2 (lane b), F3 (lane c), and F8 (lane d) and of their rough derivatives, strains U20-3 (lane e), F2-1 (lane f), F3-2 (lane g), and F8-5 (lane h).

The implications of these results are twofold. First, they provide support for the notion that shielding of deeper, more conserved layers of the bacterial cell surface from noxious agents is an important function of the O-antigenic part of LPS. Second, they are relevant for structure-function studies of outer membrane proteins. Sequencing studies of *ompA* (2) and *phoE* (P. van der Ley, A. Bekkers, J. Van Meersbergen, and J. Tommassen, unpublished data) genes of several different species of the family *Enterobacteriaceae* revealed the presence of conserved and variable regions. It has been suggested that the OmpA (2) and LamB (1) proteins present their most variable regions at the cell surface and that the observed interspecies differences in these regions are the result of selective pressure exerted by phages and colicins. Our demonstration of outer membrane protein shielding by O-antigenic chains makes the last part of this interpretation seem less plausible, since most wild isolates of *Enterobacteriaceae* synthesize smooth, O-antigen-carrying LPS (5). When phages or colicins or both exert a significant selective pressure, synthesis of a covering layer of O antigen is clearly a much more efficient way to obtain resistance to multiple agents than amino acid substitutions in many different outer membrane proteins. It is therefore much more likely that the conserved and variable regions in outer membrane proteins reflect instead the different degrees of constraint on the amino acid sequence in membrane-spanning versus exposed parts.

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