ON THE STRUCTURE OF THE ESCHERICHIA COLI C CELL WALL LIPOPOLYSACCHARIDE CORE AND ON ITS ØX174 RECEPTOR REGION

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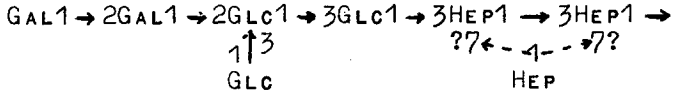
SUMMARY

MUTANTS OF ESCHERICHIA COLI C WERE SELECTED FOR RESISTANCE TOWARDS A SET OF CELL WALL LPS CORE SPECIFIC BACTERIOPHAGES, INCLUDING ØX174. INCREASINGLY DEFICIENT LPS'S FROM WT AND MUTANT E.COLI C WERE TESTED FOR INACTIVATION OF ØX174, AND THE CORE OLIGOSACCHARIDES WERE SUBJECTED TO STRUCTURAL ANALYSIS BY METHYLATION/G.L.C./M.S. LOSS OF THE TERMINAL GALACTOSE IN THE FOLLOWING BASIC STRUCTURE OF THE E.COLI C WT CORE WAS FOUND TO LEAD TO ADSORPTION RESISTANCE TOWARDS ØX174:



IN PURSUIT OF AN UNDERSTANDING OF MOLECULAR PRINCIPLES IN BACTERIOPHAGE-HOST SURFACE INTERACTIONS (E.G. 1-3), WE HAVE TAKEN UP THE STRUCTURAL ANALYSIS OF THE CELL WALL LIPOPOLYSACCHARIDE OF ESCHERICHIA COLI C, THE HOST OF BACTERIOPHAGE ØX174. E.COLI C LPS IS KNOWN TO CAUSE ECLIPSE OF ØX174 (4,5), AND TO COMPRISE STRUCTURES WITH SPECIFIC AFFINITY FOR ONE OF THE BINDING SITES OF ØX174 SPIKE H PROTEIN ("PILOT PROTEIN") (5-7). IN THE FOLLOWING WE REPORT ON THE PRIMARY STRUCTURE OF THE E.COLI C LPS CORE, AND ON A REGION WITHIN IT WHICH IS NECESSARY FOR ØX174 ATTACHMENT.

MATERIALS AND METHODS

MEDIA. UNLESS STATED OTHERWISE, MERCK STANDARD I MEDIUM WAS USED.

Bacteria and bacteriophages. Escherichia coli C was obtained from Dr. R.L. Sinsheimer (California Institute of Technology, Pasadena), and bacteriophage øX174 from Dr. M.E. Bayer (Institute for Cancer Research, Philadelphia). The typing phages GSR, T3, T4, T7, BR10, BR30, Fp1, and Fp3 (see 8) were kindly supplied by Dr. G. Schmidt at this institute.

Isolation and typing of E.coli C LPS mutants. An excess of each of the phages listed above was added to young broth cultures of E.coli C - not mutagenized, or treated with acridine orange -, and resistant clones were isolated. These were then plated and typed with the same set of viruses, applying drops of suspensions with about 3×10^8 PFU*/ml (compare 8).

Adsorption of øX174 to E.coli C and to its mutants. The kinetics of attachment to whole bacteria were determined in starvation buffer, as detailed by Newbold and Sinsheimer (9).

Inactivation of øX174 by LPS's from E.coli C and from its mutants. The cell wall LPS's of E.coli C and of its mutants were isolated by the method of Galanos et al. (10); the 50% inactivation doses (ID_{50}; see 11) were estimated by titrating the residual infectivity after incubation (1 h at 37°C) of increasing amounts of homogenized LPS's with 1000 PFU per ml starvation buffer, or MERCK medium (compare 4).

Analyses of complete cell wall LPS's. After hydrolysis (0.1 N HCl, 48 h at 100°C), the aldoses were determined by g.l.c. of the alditol acetates (12), using a VARIAN aerograph (model 1520B), and glass columns filled with ECNSS-M on Gaschrom Q (see also 13). D-Glucose and D-galactose were further estimated with fungal glucose oxidase (EC 1.1.3.4)(14), and with galactose dehydrogenase (EC 1.1.1.48) from Pseudomonas fluorescens (15). The phosphorus determinations were carried out according to Lowry et al. (16).

Isolation and analyses of LPS core oligosaccharides. The LPS samples were degraded by heating (2 h at 100°C) in 1% aqueous acetic acid, the insoluble lipid A was removed by centrifugation, and the core oligosaccharides were chromatographed through Biogel P2 (see 17 for details). For dephosphorylation, the materials were treated (4 days at 4°C) with 50% aqueous HF, and passed over Biogel again (see 18).

The aldose and phosphorus analyses of the core oligosaccharides were carried out as described above. The materials - before, or after dephosphorylation - were also methylated with methyl sulfinyl carbanion in dimethyl sulfoxide (19,20). The methylated products were purified by passage over Sephadex LH20 (13), hydrolyzed with formic/sulfuric acid (20), and the methylated monomers were identified by g.l.c./m.s. as the alditol acetates (or, occasionally, as the aldose acetates)(21,22). The retention times were recorded with the VARIAN aerograph (see above), and the mass spectrometry was carried out with a combined FINNIGAN g.l.c. (model 9500)/m.s. (model 3200E-003) instrument, employing electron impact ionization (for further details see also 14,18).

RESULTS

Using the ten Enterobacteriaceae LPS core specific bacte-

*Plaque forming units.

TABLE 1. CELL WALL LIPOPOLYSACCHARIDE (CORE OLIGOSACCHARIDE) ANALYSES, AND SENSITIVITY TO BACTERIOPHAGE ØX174 IN MUTANTS OF *ESCHERICHIA COLI* C.

STRAIN	APPROXIMATE MOLAR RATIO IN CELL WALL LPS (CORE OLIGOS.)[A]				SENSITIVITY TO ØX174		
	GAL[B]	GLC[C]	HEP[D]	P	RELATIVE EOP[E]	ADSORPTION RATE ($\times 10^{-10}$)[F]	ID_{50} OF LPS[G]
E.COLI C, WT	2(2)	3(3)	3(3)	7(3)	-	65	16(3)
C61[H]	2(2)	3(3)	2(2)	8?(2)	1.3	97	8(1)
C71[I]	2(2)	2(2)	3(3)	5(3)	0.5	33	2-3(15)
C23[I]	1	3	2	5	$<10^{-8}$	8	>1000(>400)
C23.1[J]	-(-)	2(2)	3(3)	5(3)	$<10^{-8}$	5	>1000(>400)
C64[I]	-(-)	-(-)	N(2?)	M(2?)	$<10^{-8}$	6	>1000

[A] IN ADDITION, QUALITATIVE ANALYSES SHOWED THE PRESENCE OF 3-DEOXY-OCTULOSONIC ACID IN ALL LPS'S. [B] D-GALACTOSE. [C] D-GLUCOSE. [D] ALDITOL ACETATE CO-CHROMATOGRAPHING IN G.L.C. WITH L-GLYCERO-D-MANNO-HEPTITOL. [E] RELATIVE EFFICIENCY OF PLATING ON MUTANT/WT. [F] ML/MIN, AT 37°C IN STARVATION BUFFER (11); ROUGHLY THE SAME RELATIVE RATES WERE OBTAINED IN A GROWTH MEDIUM. [G] AMOUNT OF HOMOGENIZED LPS/ML STARVATION BUFFER (OR MERCK BROTH) WHICH CAUSES 50 PERCENT INACTIVATION OF 1000 PFU/ML (AFTER 1 H AT 37°C). [H] SPONTANEOUS MUTANT OF *E.COLI* C. [I] OBTAINED AFTER MUTAGENIZATION OF *E.COLI* C WITH ACRIDINE ORANGE. [J] SPONTANEOUS MUTANT OF *E.COLI* C23.

TABLE 2. METHYLATION/GAS-LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY OF *ESCHERICHIA COLI* C WILD-TYPE AND MUTANT CELL WALL LPS CORE OLIGOSACCHARIDES.

ALDITOL DERIVATIVE[A]	T[B] LIT.[C]	T[B] FOUND	PRIMARY FRAGMENTS FOUND (M/E): 45	89	117	161	189	205	233	APPROXIMATE MOLAR RATIO[D] FROM LPS CORE IN *E.COLI*: C, WT	C71	C23.1
2.3.4.6-GLC	1.00[E]	1.00[E]	+		+	+		+		0.8	-	0.7
2.3.4.6-GAL	1.25	1.25	+		+	+		+		0.7	0.9	-
2.4.6-GLC	1.95	1.90[F]	+		+	+			+	1.0[D]	1.0[D,F]	1.0[D]
3.4.6-GLC	1.98	1.90[F]	+			+	+	+	+	-	1.0[F]	-
2.3.4.6.7-HEP[G]	2.13	2.18	+	+	+	+		+		0.7[G]	0.9	0.8
3.4.6-GAL	2.50	2.45	+			+	+	+	+	0.9	1.1	-
4.6-GLC	4.02	3.96	+			+				0.9	-	-
2.4.6.7-HEP	4.40	4.29	+	+	+			+	+	0.7	0.9	0.7
2.4.6-HEP	13.0	13.3			+				+	1.0	1.0	0.9

[A] 2.3.4.6-GLC = 1,5-DI-O-ACETYL-2,3,4,6-TETRA-O-METHYL-D-GLUCITOL ETC. [B] RETENTION TIME RELATIVE TO 2.3.4.6-GLC (T = 1.00) AND 2.3-GLC (T = 5.39) ON ECNSS-M (21,22). [C] (18,21, 22). [D] PEAK RATIO RELATIVE TO 2.4.6-GLC = 1.0, AS CALCULATED FROM METHYLATIONS BEFORE AND AFTER DEPHOSPHORYLATION (SEE TEXT). [E] STANDARD. [F] 2.4.6-GLC AND 3.4.6-GLC DO NOT SEPARATE ON ECNSS-M AS THE ALDITOL ACETATES. THEY WERE SEPARATED AS THE ALDOSE ACETATES, CO-CHROMATOGRAPHING WITH STANDARDS (AND GIVING THE SAME MASS SPECTRA). [G] THE SAME AMOUNT OF METHYLATED BRANCH HEPTOSE WAS OBTAINED FROM NATIVE AND FROM DEPHOSPHORYLATED OLIGOSACCHARIDE, INDICATIVE OF ABSENCE OF PHOSPHATE SUBSTITUTION ON THIS SUGAR.

RIOPHAGES (3,8,23) LISTED IN MATERIALS AND METHODS, LPS DEFECTIVE MUTANTS OF E.COLI C WERE SELECTED AND TYPED. FIVE REPRESENTATIVE MUTANTS OF DIFFERENT PHAGE TYPES WERE STUDIED:

COMPARING WITH E.COLI C WT, THE MUTANTS WERE TESTED FOR ADSORPTION RESISTANCE TOWARDS ØX174, AND THEIR CELL WALL LPS'S WERE ISOLATED AND ASSAYED FOR ØX174 INACTIVATION (4,5), AS WELL AS ANALYZED FOR ALDOSES AND PHOSPHORUS (SEE TABLE 1).

IN ADDITION, MOST LPS'S WERE TREATED WITH MILD ACID AND THE CORE OLIGOSACCHARIDES WERE ISOLATED (17). THEY WERE ANALYZED FOR THE SAME CONSTITUENTS (TABLE 1), AND THREE OF THEM WERE ALSO SUBJECTED TO METHYLATION/G.L.C./M.S. (19-21). SINCE METHYLATION OF PHOSPHATE-SUBSTITUTED HEPTOSE RESIDUES REMAINS INCOMPLETE, AND BECAUSE, ON THE OTHER HAND, DEPHOSPHORYLATION WITH HF (18) WAS FOUND TO CAUSE SOME LOSS OF GALACTOSE ALSO, BOTH THE NATIVE AND THE DEPHOSPHORYLATED OLIGOSACCHARIDES WERE METHYLATED AND THE RESULTS SUITABLY COMPUTED (SEE TABLE 2).

DISCUSSION

THE RESULTING STRUCTURES OF THE HEXOSE-HEPTOSE REGIONS IN THE E.COLI C WT AND MUTANT LPS CORES ARE SHOWN IN TABLE 3. FROM ANALOGY TO THE OTHER ENTEROBACTERIACEAE LPS CORE STRUCTURES SO FAR INVESTIGATED (E.G. 18,24), SEPARATE HEXOSE AND HEPTOSE REGIONS, AS WELL AS (1→3)-LINKAGES OF THE CHAIN HEPTOSES CAN BE ASSUMED. THE METHYLATION ANALYSIS OF THE C23.1 CORE OLIGOSACCHARIDE (TABLE 2) THUS YIELDS THE STRUCTURE GIVEN IN TABLE 3. THE STRUCTURES OF THE C71 AND WT CORES THEN FOLLOW CONSECUTIVELY FROM THEIR RESPECTIVE METHYLATION ANALYSES, AND THE C23 AND C61 STRUCTURES CAN FINALLY BE DEDUCED FROM THEIR SUGAR COMPOSITIONS ALONE (TABLE 1).

THE DATA PRESENTED IN TABLES 1 AND 3 ALSO SHOW THAT LOSS OF

TABLE 3. CELL WALL LIPOPOLYSACCHARIDE CORE STRUCTURES[A] IN *ESCHERICHIA COLI* C AND ITS MUTANTS.

STRAIN	HEXOSE–HEPTOSE REGION OF LPS CORE OLIGOSACCH.[A]
E.COLI C, WT	Gal1 —(α)[B]→ 2Gal1 → 2Glc1 → 3Glc1 → 3Hep1 → 3Hep1 → ?7 (Hep[C]); 2Glc1 ↑3 Glc
C61	Gal1 —(α)[B]→ 2Gal1 → 2Glc1 → 3Glc1 → 3Hep1 → 3Hep1 →; 2Glc1 ↑3 Glc
C71	Gal1 —(α)[B]→ 2Gal1 → 2Glc1 → 3Glc1 → 3Hep1 → 3Hep1 → ?7 (Hep[C])
C23[B]	Gal1 —[B]→ 2Glc1 → 3Glc1 → 3Hep1 → 3Hep1 →; 2Glc1 ↑3 Glc
C23.1	Glc1 → 3Glc1 → 3Hep1 → 3Hep1 → ?7 (Hep[C])

[A] IN ADDITION TO WHAT IS SHOWN, THE NATIVE CORE OLIGOSACCHARIDES CARRY DIFFERENT AMOUNTS OF PHOSPHATE-CONTAINING SUBSTITUENTS (SEE TABLE 1), AND TERMINATE IN REDUCING 3-DEOXY-OCTULOSONIC ACID (COMPARE 18,24; SEE TABLE 1). ALL SUGARS OCCUR AS PYRANOSES. [B] SINCE, IN *S.TYPHIMURIUM*, ØX174 IS SPECIFIC FOR α(1→2)-LINKED TERMINAL HEXOSES (5), AN α-LINKAGE IS PROPOSED FOR THE TERMINAL GALACTOSE. NO SUCH PREDICTION IS POSSIBLE FOR THE SUB-TERMINAL GALACTOSE, BECAUSE *E.COLI* C23 EXHIBITS SOME DOUBTFUL, RESIDUAL ADSORPTION OF ØX174 (TABLE 1). [C] IT IS NOT KNOWN IF THE BRANCH HEPTOSE IS LOCATED ON THE DISTAL, OR ON THE PROXIMAL CHAIN HEPTOSE.

THE *E.COLI* C WT CORE TERMINAL GALACTOSE RESIDUE LEADS TO ØX174 ADSORPTION RESISTANCE (COMPARE C61 AND C23), WHEREAS LOSS OF THE BRANCH HEPTOSE (C61), OR OF THE BRANCH GLUCOSE (C71) DOES NOT HAVE THIS EFFECT. THE FACT THAT THE RESISTENT MUTANTS C23, C23.1, AND C64 ALL SHOW LPS PHOSPHATE DEFECTS ALSO (TABLE 1) RAISES THE QUESTION, IF NOT THESE PHOSPHATE LOSSES MAY BE THE

REASON FOR THE RESISTANCE. HOWEVER, E.COLI C71 - WHICH IS SENSITIVE - CONTAINS THE SAME AMOUNT OF PHOSPHATE AS DOES C23.1 - WHICH IS ØX174 RESISTANT; AND SIMILAR EXPERIMENTS WITH SALMONELLA TYPHIMURIUM MUTANTS (5) ALSO IMPLIED LPS SUGAR, AND NOT PHOSPHATE RESIDUES IN ØX174 ATTACHMENT:

IN S.TYPHIMURIUM, LOSS OF LPS CORE TERMINAL, (1→2)-LINKED N-ACETYL-α-D-GLUCOSAMINE REDUCES, AND ADDITIONAL LOSS OF SUBTERMINAL, ALSO (1→2)-LINKED α-D-GLUCOSE ABOLISHES ØX174 ADSORPTION (5,24). IN CONJUNCTION WITH THE RESULTS PRESENTED HERE, THIS SHOWS THAT ØX174 IS NOT SPECIFIC FOR THE CORE TERMINAL SUGAR ITSELF, BUT POSSIBLY FOR THE CONFIGURATION (AND THE RESULTING CONFORMATION) AROUND THE TERMINAL GLYCOSIDIC LINKAGE: IN ALL CASES INVESTIGATED - WITH THE DOUBTFUL EXCEPTION OF E.COLI C23 (TABLES 1 AND 3) - ØX174 WAS FOUND TO ADSORB TO BACTERIA ONLY, THE LPS CORES OF WHICH TERMINATE IN (1→2)-LINKED HEXOSES (PROBABLY ALWAYS AXIAL1→2EQUATORIAL LINKAGES).

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