AN UNUSUAL ACIDIC POLYSACCHARIDE PRODUCED BY A ROUGH STRAIN OF ESCHERICHIA COLI.

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SUMMARY. - Lipopolysaccharide and an acidic polysaccharide were extracted with phenol-water from a rough strain of Escherichia coli (LP1092). The polysaccharide portion of lipopolysaccharide contained galactose, glucose, L-glycero-D-mannoheptose, small amounts of mannose and an unusually high proportion of 3-deoxy-D-manno-octulosonic acid; this polysaccharide was shown to represent the complete coli R2 core. The acidic polysaccharide, which functioned as a K antigen, contained large amounts of a 2-keto-3-deoxy sugar acid. On colorimetric and chromatographic evidence this acid appeared to be 3-deoxy-D-manno-octulosonic acid.

It is well established that rough strains of Gram-negative bacteria are, in general, susceptible to the bactericidal action of normal human serum (1). The incubation of log phase cultures of rough Escherichia coli strains in serum usually results in a reduction in the viable count to less than 1% of the inoculum size within one hour (unpublished observation). An E.coli strain isolated from a case of urinary tract infection in this Department appeared to be an exception to this rule. Strain LP1092 agglutinated in 3.5% w/v NaCl and in 0.2% w/v acriflavine and produced morphologically rough colonies on nutrient agar plates. In addition, its sensitivity pattern to rough-specific bacteriophages (Table 1) was that expected from a rough E.coli strain possessing a complete lipopolysaccharide R2 core structure (2,3,4). Strain LP1092 was killed by serum but only after a delay of 1-2 hours (Fig. 1) when assayed by a technique described previously (5); this delayed killing effect is characteristic of certain smooth E.coli strains (6). Moreover, in contrast to F576 (R2) cells live LP1092 cells did

TABLE 1:

Bacteriophage sensitivity* patterns of E.coli LP1092 and the E.coli R1, R2 and R3 lipopolysaccharide core prototype strains of Schmidt et al (2,3). +, confluent lysis; -, no lysis.

Strain No.	Core Type	Phages FO	Br2	Br10	Fp1	6SR	C21	T3	T4
LP1092	?R2	+	+	+	+	+	-	+	+
F470	R1	-	-	+	+	+	+	+	+
F576	R2	+	+	+	+	+	-	+	+
F653	R3	-	+	+	+	+	-	+	+

* determined by applying drops of phage containing approximately 10^8 p.f.u./ml onto surface-inoculated nutrient agar plates. Phages were kindly supplied by Dr. G. Schmidt and Dr. R. Wilkinson.

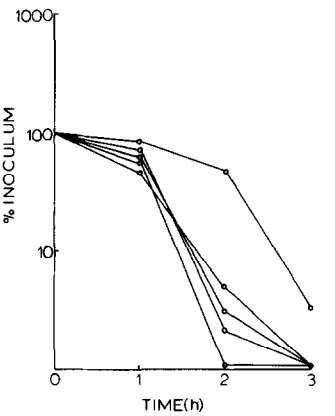

Fig. 1. The response of E.coli LP1092 to five samples of fresh normal human serum obtained from different invididuals. Washed log phase bacteria (1 x 10^6) in 1ml of tris - HCl pH 8.4 were added to 3ml of serum and viable counts performed after 0,1,2 and 3h incubation at 37°C.

not agglutinate in rabbit antisera prepared against heated (100°C; 1h) LP1092 or F576 cells, although they did agglutinate in antisera prepared against unheated LP1092 cells. These results suggested that E.coli LP1092, although

TABLE 2:

Serological reactivity and sugar composition of lipopolysaccharides from E.coli LP1092 and F576.

lipopoly-saccharide from strain	Passive haemagglutination*			Molar Ratio **				
	anti-F470	anti-F576	anti-F653	Mannose	Galactose	Glucose	L-glycero-D-mannoheptose	3-deoxy-D-manno-octulosonic acid.
LP1092	<20	5120	160	0.17	1.11	1.36	1.00	2.58
F576	<20	5120	320	0.25	1.00	1.18	1.00	1.05

* expressed as the reciprocal of the highest dilution of antiserum causing complete agglutination of lipopolysaccharide-coated sheep red cells.

** determined by gas-liquid chromatography of alditol acetates using a Pye Unicam 104 series chromotograph fitted with columns containing 3% ECNSS-M on 100/120 mesh Gas-Chrom Q.

rough, might synthesise a K-antigen; the cell surface antigens of this strain were therefore investigated further.

LP1092 cells were cultivated in nutrient broth and extracted with phenol-water (7). Water-soluble polysaccharides were precipitated in cold ethanol and fractionated with cetavlon according to Procedure III of Westphal and Jann (7). Two major ribonucleic acid-free fractions, one representing lipopolysaccharide (25mg/g dry cell mass) and the other acidic polysaccharide (20mg/g), were obtained.

The results of passive haemagglutination tests (2) confirmed that lipopolysaccharide from LP1092 contained the R2 core (Table 2). Also, the neutral sugar composition of LP1092 and F576 lipopolysaccharides, as determined by gas - liquid chromatography of alditol acetates (8,9) of sugars released by hydrolysis in 0.1N HCl (100°C; 48h), were very similar. Both polymers contained small amounts of mannose in addition to galactose, glucose and L-glycero-D-mannoheptose (Table 2). The amount of 2-keto-3-deoxy sugar acid (assumed to be 3-deoxy-D-manno-octulosonic acid) in the preparations was determined by the thiobarbiturate reaction (10,11); the ammonium salt of 3-deoxy-D-manno-octulosonic acid, prepared by the condensation of D-arabinose and oxalacetic acid (12), was used as a standard. LP1092 lipopolysaccharide contained greater amounts of 3-deoxy-D-manno-octulosonic acid than is normally found in lipopolysaccharides from rough strains of E.coli (Table 2).

The acidic polysaccharide was examined colorimetrically for the presence of hexuronic acid (13), neuraminic acid (14) and hexosamine (15) residues; none were detected. The unhydrolysed preparation did react very strongly, however, in the thiobarbiturate test (10, 11) with a λmax of 547nm (Fig.2); as also found by Ellwood (11), N-acetylneuraminic acid did not react in this test. The acidic polysaccharide appeared to consist of 90-100% 2-keto-3-deoxy sugar acid. The acidic polysaccharide was hydrolysed in 0.1N H_2SO_4 (100°C; 30 min) and hydrolysates subjected to descending paper chromatography in butan-2-ol +

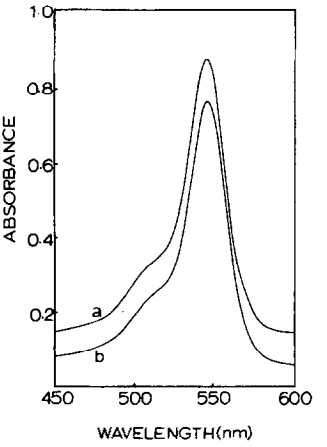

Fig. 2. Absorption spectra for (a) 30µg acidic polysaccharide from E.coli LP1092, and (b) 30µg 3-deoxy-D-manno-octulosonic acid, in the thiobarbiturate reaction.

TABLE 3:

Reactivity of lipopolysaccharide and acidic polysaccharide from E.coli LP1092 in antisera raised against heated ("R" antisera) and live ("RK" antisera) LP1092 cells.

	Passive haemagglutination *	
Preparation	"R" antisera	"RK" antisera
Lipopolysaccharide	640	10240
Acidic polysaccharide	<20	5120

* expressed as the reciprocal of the highest dilution of antiserum causing complete agglutination of coated sheep red cells.

acetic acid + water (8+1+1,v/v) and butan-1-ol + pyridine + 0.1N HCl (5+3+2, v/v); chromatograms were developed according to Anderson (16). In both systems, the hydrolysate chromatographed as a single pink thiobarbiturate-reactive spot with an identical mobility to acid-treated samples of synthetic

3-deoxy-D-manno-octulosonic acid. Although standards of other 2-keto-3-deoxy sugar acids were not available, they may be readily differentiated from 3-deoxy-D-manno-octulosonic acid in the solvent systems used (11). These results suggest, therefore, that the major component of acidic polysaccharide from E.coli LP1092 is 3-deoxy-D-manno-octulosonic acid.

Acidic polysaccharide from LP1092 behaved as a K-antigen in passive haemagglutination tests; sheep red cells coated with acidic polysaccharide reacted in rabbit antisera raised against live LP1092 cells but not in antisera prepared with heated cells (Table 3). In addition, acidic polysaccharide was able to non-specifically inhibit the agglutination of sheep red cells by anti-red cell serum in the system of Glynn and Howard (17); this ability is characteristic of many K-antigen preparations.

K-antigens are produced by a large number of E.coli strains, and the majority examined have been polysaccharides with acidic properties due to the presence of hexuronic acid (18), N-acetyl-neuraminic acid (19) or glycerol phosphate (20) residues. There appears to be no report in the literature, however, of acidic polysaccharides containing 3-deoxy-D-manno-octulosonic acid. The relatively large amounts of the sugar acid associated with the lipopolysaccharide could result from contamination with acidic polysaccharide, or it might suggest that there is some degree of linkage between the polymers. Further studies will be necessary in order to determine whether or not the acidic polysaccharide is responsible for the delayed serum killing effect, although it is interesting to note that it does not appear to affect the phage sensitivity of E.coli LP1092.

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