

Notes

Chemical characterization of the lipopolysaccharides from enteropathogenic *Escherichia coli* O142 and O158

Anup Kr Datta* and Sumanta Basu†

Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Calcutta 700032

Received 27 March 1998; revised 22 July 1998

Lipopolysaccharides (LPS) from two enteropathogenic strains of *E. coli* O142 and O158 were isolated by hot phenol-water extraction procedure. Polyacrylamide gel electrophoretic pattern of the LPS showed the typical ladder like pattern of smooth type of LPS. The LPS of *E. coli* O158 was found to contain L-rhamnose, D-glucose and N-acetyl-D-galactosamine as major constituents together with D-galactose, N-acetyl-D-glucosamine, L-glycero-D-manno-heptose and 2-keto-3-deoxy-D-manno-octulosonic acid (KDO) whereas LPS from *E. coli* O142 contained L-rhamnose, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine as major constituents together with D-glucose, D-galactose, N-acetyl-D-glucosamine, L-glycero-D-manno-heptose and 2-keto-3-deoxy-D-manno-octulosonic acid (KDO). LPS was degraded by mild acid hydrolysis to yield a degraded polysaccharide fraction and an insoluble lipid-A fraction. The main fatty acids of the lipid-A fraction of the LPS were C12:O, C14:O, and 3-OH C14:O for O158 strain whereas *E. coli* O142 lipid-A consisted of C12:O, C14:O, 3-OH C14:O, and C16:O. The degraded polysaccharide fraction on gel permeation chromatography gave a high molecular weight O-chain fraction and a core oligosaccharide and a fraction containing degraded sugars. The chemical composition of LPS and its fragmented products are reported in this communication.

Escherichia coli is one of the most opportunistic pathogens¹ causing intestinal and extraintestinal diseases in both man and animals². The most pathogenic role of *E. coli* is to produce diarrhoeal diseases. Diarrhoea is primarily a disease of developing countries where it is associated with poor hygiene, over crowding and stress³. The enteropathogenic *E. coli* strains (EPEC) are known to be associated with infantile diarrhoea which continues to be one of the most common causes of morbidity and mortality among infants and children⁴. The surface of the Gram-negative bacteria, of which lipopolysaccharide is a major component, mediates its interaction with the host⁵. Lipopolysaccharides (LPS)

consist of three moieties : lipid-A, core oligosaccharide, and the O-specific polysaccharide chain. The O-specific side chain, the major LPS component responsible for its serological specificity consists of repeating oligosaccharide unit of variable composition and structure. Core structures show a lower degree of diversity but its role in immunogenicity and antigenicity and in biological activities of lipid-A has gained interest recently^{6,7}. Lipid-A part of the LPS confers stability to the outer membrane and renders it less permeable to lipophilic molecules. The composition and structure of lipid-A component of LPS reflect in many instances, the phylogenetic relationships of Gram-negative bacteria⁸. The present work describes the chemical composition of the LPS and its three components from *E. coli* O142 and O158, two EPEC strains of Indian origin.

Escherichia coli O142 and O158 were obtained from Central Research Institute, Kasauli, India. The bacteria were grown at 37°C in beef extract-peptone medium (150 ml, pH 7.2) containing beef extract (1%), peptone (1%), sodium chloride (0.5%) and agar (1.5%) in Roux bottles for 48 hr. The cells were harvested at an early stationary phase by gently shaking the cultures with 0.85% saline and isolated by centrifugation. The bacterial cells were then thoroughly washed with water and freeze dried.

LPS from *E. coli* O142 and O158 strain was isolated using hot phenol-water method⁹. The water extract was exhaustively dialysed and then freeze dried to yield the mixture of LPS and bacterial nucleic acid. The nucleic acid was removed by ultracentrifugation followed by precipitation with Cetavlon (cetyl trimethyl ammonium bromide), a cationic detergent. LPS was isolated from the supernatant after dialysis and freeze drying and was further purified by passing through a column of Sephadex G-100, eluted with pyridine-acetate buffer (pH 4.5) and the eluate was collected in 5 ml fractions which were monitored by Waters Associates Differential Refractometer (Model 403) fitted with a recorder. Appropriate fractions were combined and the buffer removed by rotary evaporation and lyophilisation.

LPS (100 mg) dissolved in 1.5% (v/v) acetic acid

*Corresponding author

†Expired on 8th March, 1998

(20 ml) was heated for 2 hr on a boiling water bath and following the removal of the precipitated lipid A (20 mg) from the cooled solution by centrifugation, the supernatant was lyophilised. Gel permeation chromatography of the purified fraction on a column of Sephadex G-50 gave a high molecular weight O-specific polysaccharide, a core oligosaccharide, and a fraction containing phosphate and 2-keto-3-deoxy-D-manno-octulosonic acid (KDO). The gel filtration properties of the eluates are expressed in terms of their distribution coefficients (K_{av}); $K_{av} = V_e - V_0 / V_t - V_0$, where V_e is the elution volume, V_0 is the void volume, and V_t is the total volume of the system (Table 1).

Neutral sugars were liberated by hydrolysis with 2 M trifluoroacetic acid at 120°C for 2 hr. The products were then converted to their alditol acetates and analysed by gas-liquid chromatography (GLC) on a gas chromatograph (Hewlett Packard model 5890 series II) equipped with a flame ionisation detector. Peaks were identified by cochromatography with authentic standards. The alditol acetates were resolved on a glass column (1.8 m × 6 mm) containing 3% ECNSS-M on Gas Chrom Q (100-120 mesh) at 170°C and HP-1 fused silica column (0.25mm × 30 m) at 160°C for 2 min followed by an increase of 20°C/min to 240°C. The head pressure of the column was 105 Kpa. Amino sugars were detected with a glass column packed with 3% Poly-A 103 on Gas Chrom Q (100-120 mesh) at 200°C after hydrolysis with 4 M HCl at 100°C for 6 hrs. Fatty acid analysis of the methyl esters was done with a metal column containing 3% SE-52 at 164°C. Paper chromatography was performed on Whatman No. 1 and No. 3 MM papers. The solvent systems were (I) 8:2:1 (v/v/v) ethyl acetate-pyridine-water, (II) 6:4:3 (v/v/v) *n*-butanol-pyridine-water (upper layer), and (III) 5:5:1:3 (v/v/v) ethyl acetate-pyridine-acetic acid-water. 2-Keto-3-deoxy-D-manno-octulosonic acid (KDO) was detected by thiobarbituric acid method after hydrolysing the samples with 1 M HCl at 100°C for 30 min. Gel filtration was performed on columns of Sephadex G-100 (70 × 1.5 cm) and Sephadex G-50 (70 × 1.5 cm) using a Gilson fraction collector (model 201), and the eluate was monitored with a Waters Associates Differential Refractometer (Model 403) fitted with a recorder. Optical rotations were measured by using a Perkin-Elmer 241 MC spectropolarimeter.

Table 1—Distribution coefficient K_{av} and yield of LPS from *E. coli* O142 and O158 strains in eluates from gel permeation chromatography on Sephadex G-50

K_{av}	O-specific PS	Core	KDO/others
K_{av} (O158)	0.04 (45% yield)	0.62 (10% yield)	0.90 (5% yield)
K_{av} (O142)	0.03 (42% yield)	0.58 (10% yield)	0.92 (5% yield)

SDS-PAGE was carried out using Tris-glycine buffer, pH 8.4 containing 10% sodium dodecyl sulphate with 13% acrylamide running gel and 4% stacking gel. After pre-electrophoresis at 25 mA the samples were separated at 18 mA until the dye reached the bottom of the gel. LPS bands were detected after periodic oxidation with silver nitrate staining of Tsai and Frasch¹⁰.

E. coli O158 and O142 strains were grown in large quantities on beef extract-peptone medium and the LPS isolated by hot phenol-water method yielded 8-10% of the dry bacterial weight. The crude LPS thus obtained was purified by ultracentrifugation, followed by cetavlon precipitation and finally by gel permeation chromatography. The purified LPS was found to be free from protein and nucleic acid. SDS-Polyacrylamide gel electrophoretic pattern of the purified LPS revealed that the long chain sugars as well as the short chain sugars are present in *E. coli* O142 and O158 LPS^{11,12}. *E. coli* O158 LPS contains sugars, namely, L-rhamnose, D-glucose and *N*-acetyl-D-galactosamine as the major constituents together with D-galactose, *N*-acetyl-D-glucosamine, L-glycero-D-manno-heptose and 2-keto-3-deoxy-D-manno-octulosonic acid (KDO).

The main fatty acids are C12:O, C14:O, 3-OHC14:O. *E. coli* O142 LPS contains sugars, namely, L-rhamnose, D-glucose, *N*-acetyl-D-galactosamine, and *N*-acetyl-D-glucosamine as the major constituents together with D-galactose, L-glycero-D-manno-heptose and 2-keto-3-deoxy-D-manno-octulosonic acid (KDO). The main fatty acids are C12:O, C14:O, 3-OHC14:O together with small amounts of C16:O. LPS from both *E. coli* O158 and O142 was degraded into lipid-A and polysaccharide fraction by mild acid hydrolysis. Lipid-A was removed by centrifugation and supernatant polysaccharide fraction was fractionated through a column of Sephadex G-50 giving three fractions. The sugar analysis of high molecular weight O-chain polysaccharide from *E. coli*

Table 2—Chemical composition of *E. coli* O158 lipopolysaccharide

Component	Chemical composition (w/w) %			
	LPS	O-PS	COS	Lipid-A
L-Rhamnose	13.05	19.07	-	-
D-Galactose	8.70	-	16.54	-
D-Glucose	15.10	40.62	48.50	-
N-acetyl-D-glucosamine	8.80	-	15.90	21.07
N-acetyl-D-galactosamine	23.80	39.56	-	-
L-glycero-D-manno-heptose	8.70	-	14.98	-
KDO	present	-	-	-
C12 :O	3.30	-	-	17.02
C14 :O	1.50	-	-	9.04
3-OH C14 :O	14.50	-	-	46.75

Table 3—Chemical composition of *E. coli* O142 lipopolysaccharide

Component	Chemical composition (w/w) %			
	LPS	O-PS	COS	Lipid-A
L-Rhamnose	13.08	21.70	-	-
D-Galactose	4.09	-	21.50	-
D-Glucose	6.75	-	37.90	-
N-acetyl-D-glucosamine	12.50	20.00	16.80	22.80
N-acetyl-D-galactosamine	32.50	58.00	-	-
L-glycero-D-manno-heptose	9.85	-	16.75	-
KDO	present	-	-	-
C12 :O	3.5	-	-	15.70
C14 :O	2.6	-	-	11.50
3-OH C14 :O	12.5	-	-	45.00
C 16 :O	0.9	-	-	4.01

O158 $\{[\alpha]_D = +85^\circ (C = 0.5, H_2O)\}$ and *E. coli* O142 $\{[\alpha]_D = +112^\circ (C = 0.5, H_2O)\}$ are shown in Table 2 and Table 3. These results revealed that L-rhamnose, D-glucose, N-acetyl-D-galactosamine constitute the O-chain polysaccharide from *E. coli* O158 LPS whereas L-rhamnose, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine constitute the O-chain polysaccharide from *E. coli* O142. The structure of the O-specific polysaccharide of *E. coli* O142 has already been reported¹³. The sugar analysis of the core oligosaccharide (COS) from *E. coli* O158, $\{[\alpha]_D = +23.69^\circ (C = 0.5, H_2O)\}$ and *E. coli* O142, $\{[\alpha]_D = +26.51^\circ (C = 0.5, H_2O)\}$ are shown in Table 2 and Table 3. The results showed that D-glucose, D-galactose, N-acetyl-D-glucosamine and L-glycero-D-manno-heptose are the major constituents of both core oligosaccharide (COS) fractions but differs in their mole ratios.

The continued prevalence of serious bacterial infections despite the development of antimicrobial agents has been followed by an active interest in chemical and immunochemical studies on bacterial lipopolysaccharide for the diagnosis and protection of Gram-negative bacterial infection. The pathogenicity of *E. coli* is a very complex phenomenon, in which the chemical nature of the bacterial cell surface plays an important role. A thorough and precise knowledge about the surface structures of *E. coli* is evidently important. LPS from enteropathogenic strain of *E. coli* O158 and O142 was studied to elucidate the composition of the outer most major surface structure of this serotype and also to understand the chemical basis of its serological behaviour. As O-specific polysaccharide is different in composition their

structure must be different. As O-specificity is responsible for serological specificity, any difference found in the chemical composition in the O-specific polysaccharide leads to the different chemical structural changes and serological behaviour of the bacterial strains. This is also confirmed in (Ouchterlony) double diffusion test. The antibody raised against O142 strains bind with the O142 LPS but does not binds with the O158 LPS. Similar results were obtained with antibody raised against O158 strains. Here Table 2 and Table 3 shows that *E. coli* O158 and O142 O-PS differs in their chemical composition. The fatty acid profile of the LPS is characteristic of the enterobacterial lipid-A. These results will help in the immunochemical and chemical characterisation of *E. coli* O158 and *E. coli* O142 LPS leading to the understanding of the minimal requirement to define O158 and O142 sero-specificity.

The authors thank to Profs N Roy, B P Chatterjee and Dr A K Guha for their constant help and encouragement during the work and to Lily Sarkar for microbiological work. AKD thanks CSIR, New Delhi, India for providing him with a junior research fellowship. The work was partially supported with financial assistance from grant No. SP/SO/D39/94, Department of Science and Technology, Government of India.

References

- 1 Orskov F & Orskov I (1992) *Can J Microbiol* 38, 699-704
- 2 Hacker J (1992) *Can J Microbiol* 38, 720-727
- 3 Albert M J, Faruque S M, Faruque A S, Neogi P K, Ansaruzzaman M, Bhuiyan N A, Alam K, & Akbar M S (1995) *J Clin Microbiol* 33, b 973-977

- 4 Snyder J D & Merson M H (1982) *Bull WHO* 60, 605-613
- 5 Lindberg A A, Karnell A & Weintraub A (1991) *Rev Infect Dis* 13, S279-284
- 6 Brade H, Brade L, & Rietschel E T (1988) *Zentrabl Bakteriolog Hyg Ser A* 268, 151-179
- 7 Vinogradov E V, Stuike-Prill R, Bock K, Holst O, & Brade H (1993) *Eur J Biochem* 218, 543-554
- 8 Mayer H (1994) in *The cell membrane: Its role in interaction with outside world* (Haber ed) E71-83, Plenum Press, New York
- 9 Westphal O, Luderitz O, & Bister F (1952) *Z Naturforsch* 7b, 148-155
- 10 Tsai C M & Frasch C E (1982) *Anal Biochem* 119, 115-119
- 11 Datta A K & Basu S (1997) *Biochem Archives* V 13, 29-35
- 12 Sengupta P & Basu S (1997) *Biochem. Archives* V 13, 61-67
- 13 Landerjo C, Weintraub A, & Wildmalm G (1997) *Eur J Biochem* 244, 449-453