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Identification of the lipopolysaccharide O-chain of *Escherichia hermannii* (ATCC 33651) as a D-rhamnan*[†]

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ABSTRACT

The structure of the antigenic O-polysaccharide portion of the lipopolysaccharide produced by *Escherichia hermannii* (ATCC 33651) has been determined by composition and methylation analysis, together with ^{1}H- and ^{13}C-n.m.r. spectroscopy, to be a (1→3)-β-D-rhamnopyran with ~25% of the residues 2-acetylated.

INTRODUCTION

E. hermannii is an atypical biogroup of *E. coli*[1], the clinical significance of which in human disease has not been investigated. Some of the strains of the organism were identified falsely as the pathogen *E. coli* O157:H7 by the use of rabbit antisera[2,3] and by serological cross-reaction with *Brucella* antisera[4]. Chemical and serological analyses revealed that the cross-reactivities could be related to the occurrence of epitopes that involved *N*-acyl derivatives of 4-amino-4,6-dideoxy-α-D-mannopyranosyl residues in the respective LPS O-chains of *E. hermannii* strains[4], *E. coli* O157 (ref. 5), *B. abortus*[6], and *B. melitensis*[7].

Whereas all of the clinically isolated strains of *E. hermanni* which reacted with polyclonal *E. coli* O157 antisera had unbranched LPS O-chains composed of pentasaccharide repeating-units that contained (1→2)- and (1→3)-linked 4-acetamido-4,6-dideoxy-α-D-mannopyranosyl residues[4], it was of interest to determine if all strains had the same antigenic O-chain relationship. Our investigations have revealed that the three strains of *E. hermannii* in the American Type Culture Collection did not show the serological cross-reactions described above and that the LPS O-chains had different structures.

E. hermannii strains ATCC 33650 and 33652 have LPS O-chains of repeating tetrasaccharide units composed of D-glucose, D-galactose, and D-rhamnose (1:1:2) (ref. 8), whereas that of strain ATCC 33651 is now reported to be composed of (1→3)-linked β-D-rhamnopyranosyl residues some of which are 2-acetylated.

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RESULTS AND DISCUSSION

Fermenter-grown cells of *E. hermannii* (ATCC 33651) were extracted by a modified phenol–water procedure[9], and the LPS were obtained as precipitated gels by repeated ultracentrifugation of the separately dialysed and concentrated phenol and water layers. The soluble LPS obtained from the phenol and aqueous phases in yields of 1.6 and 6.6%, respectively (based on the dry wt. of the cells), were essentially identical and the work reported here was carried out on the LPS from the aqueous phase.

Mild hydrolysis of the LPS afforded an insoluble lipid A (25%) and chromatography on Sephadex G-100 of the concentrated aqueous hydrolysate gave an O-polysaccharide fraction (K_{av} 0.25, 81%), no detectable core oligosaccharide, and a low-molecular-weight fraction (K_{av} 0.99, 9%) that contained 3-deoxy-D-*manno*-octulosonate (Kdo) and phosphate.

The O-polysaccharide had $[\alpha]_D$ $-92°$ (*c* 2.2, water), and g.l.c. of the alditol acetates derived from the products of hydrolysis[10] indicated the composition of rhamnose (83%), galactose (5%), glucose (4%), 2-amino-2-deoxyglucose (2%), and L-*glycero*-D-*manno*-heptose (1.5%). Determination of acetate[11] showed it to account for 5.2% of the O-chain composition. Preparative p.c. of the hydrolysate of the O-chain afforded D-rhamnose (R_{GAL} 1.98), $[\alpha]_D$ $-9°$ (*c* 1.1, water), which was converted into methyl α-D-rhamnopyranoside {m.p. and mixture m.p. 106°, and $[\alpha]_D$ $+63°$ (*c* 0.9, water)}, and g.l.c. (program *A*) of the derived alditol penta-acetate gave a single peak (T_{GA} 0.61) with a retention time the same as that of rhamnitol penta-acetate. That all of the rhamnose was D was established by capillary g.l.c. of the acetates of the derived (+)-2-butyl glycosides[12].

Methylation analysis of the O-chain and g.l.c.–m.s. (program *B*) of the reduced ($NaBD_4$) and acetylated product gave a single peak (T_{GM} 0.97) identified as 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylrhamnitol-*1-d*, indicating the O-chain to be composed exclusively of (1→3)-linked D-rhamnopyranosyl residues, a conclusion that was supported by the n.m.r. data.

The ^{1}H- and ^{13}C-n.m.r. spectra of the O-polysaccharide were complex owing to the partial *O*-acetylation (Figs. 1A and 2A). Comparison of the integrated intensities of the ^{1}H signals for OAc (δ 2.2) and Me-6 (δ 1.3) indicated that the polymer was 20–25% *O*-acetylated.

As expected, the *O*-deacetylated O-polysaccharide, which had $[\alpha]_D$ $-98°$ (*c* 1.5, water), gave greatly simplified n.m.r. spectra (Figs. 1B and 2B). The ^{1}H-n.m.r. spectrum contained a single resonance for H-1, which confirmed the presence of a single type of monosaccharide residue, and the full assignment of the signals was made from a 2D COSY[13] experiment. The ^{13}C-n.m.r. spectrum of the *O*-deacetylated O-chain contained six resonances consistent with a (1→3)-β-D-rhamnopyranose structure where the $J_{C-1,H-1}$ value (161 Hz) was indicative of the β-D configuration[14]. The assignments made from a ^{1}H–^{13}C chemical shift-correlation experiment corresponded to literature data for (1→3)-linked β-L-rhamnopyranosyl residues[15] (Table I).

Application of homo- and hetero-nuclear chemical shift-correlation techniques

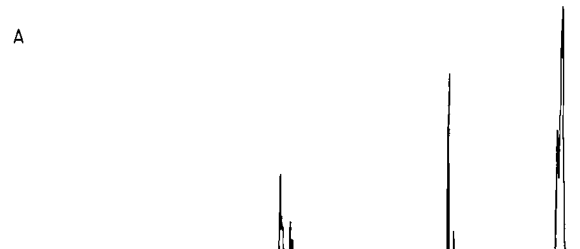

Fig. 1. ^{1}H-N.m.r. spectra of the LPS O-polysaccharides of *E. hermannii* (ATCC 33651): *A*, native polysaccharide; *B*, *O*-deacetylated polysaccharide.

TABLE I

^{1}H- and ^{13}C-n.m.r. resonance assignments for the →3)-β-D-Rha*p*-(1→ residues in the *O*-deacetylated LPS O-polysaccharide of *E. hermannii* (ATCC 33651)

Proton	*Chemical shift (p.p.m.)*	*Coupling constant (Hz)*	*Carbon atom*	*Chemical shift (p.p.m.)*
H-1	4.810	s[a]	C-1	97.62 (161 Hz)[b]
H-2	4.261	2.7	C-2	68.96
H-3	3.874	9.7	C-3	78.95
H-4	3.537	9.2	C-4	71.41
H-5	3.461	5.9	C-5	72.95
H-6	1.342	–	C-6	17.79

[a] Unresolved singlet, $W_{1/2} < 3$ Hz. [b] $^1J_{C,H}$ value (Hz).

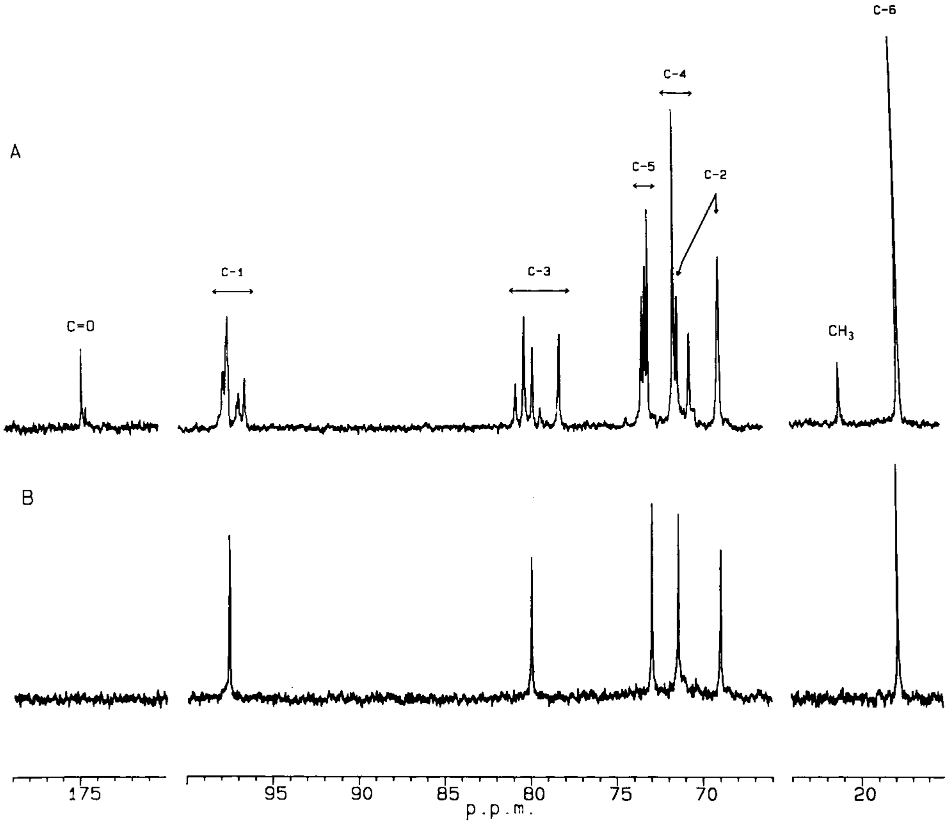

Fig. 2. Proton-decoupled ^{13}C-n.m.r. spectra of the LPS O-polysaccharides of *E. hermannii* (ATCC 33651): *A*, native polysaccharide; *B*, *O*-deacetylated polysaccharide. Resonance assignments associated with the D-rhamnosyl residues were made by ^{13}C–^{1}H chemical shift correlation, using CHORTLE.

to the O-chain revealed the *O*-acetyl substituents to be located exclusively at position 2 of 20–25% of the D-rhamnopyranosyl residues, as indicated by the low-field position of the H-2 resonances (δ 5.65, ~0.2 H) of the 2-acetylated D-rhamnopyranosyl residues[16]. The ^{13}C assignments (Fig. 2A) indicate that, for each block of ten D-rhamnopyranosyl residues, 2–3 were 2-acetylated.

D-Rhamnose-containing bacterial polysaccharides have been described as components of *Xanthomonas campestris*[17], *Pseudomonas cepacia*[18], *P. syringae* pv. *morsprunorum*[19], *P. aeruginosa* IID 1008[20], *P. maltophilia* 555 (ref. 21), *Myxobacterium* 402 (ref. 22), and other bacteria, including the two strains of *E. hermannii* (ATCC 33650 and 33652). Apparently, an antigenic LPS O-chain monopolymer of D-rhamnose residues with a monosaccharide repeating unit has not been reported hitherto.

EXPERIMENTAL

Production of lipopolysaccharide and O-polysaccharide. — *E. hermannii* (ATCC 33651) was fermenter-grown in a brain–heart infusion, the saline-washed cells (yield, ~4 g/L wet weight) were extracted by a modified hot phenol–water procedure[9], the concentrated and dialysed water and phenol phases were ultracentrifuged (105 000 *g*, 10 h, 4°), and a solution of each precipitated LPS gel in water was lyophilized. 1% Solutions of LPS in aqueous 1.5% acetic acid were heated for 2 h at 100°, the precipitated lipid A was removed by low-speed centrifugation, the clear supernatant solution was lyophilized, and the product was eluted from a column (2 × 90 cm) of Sephadex G-100 with 0.05M aqueous pyridinium acetate (pH 4.5).

Analytical methods. — Quantitative methods for glycose analysis, methylation, and g.l.c.-m.s. used were those described[23]. G.l.c. was performed on a Hewlett–Packard 5710A chromatograph fitted with a flame-ionization detector, a glass column (180 × 0.2 cm) packed with 3% SP2340 on Supelcoport, and the temperature programs *A*, 180° for 2 min, →240° at 4°/min; and *B*, 180° for 2 min, →240° at 1°/min. Retention times are relative to those of D-glucitol hexa-acetate (T_{GA}) or 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (T_{GM}). Hydrolyses were carried out in sealed glass tubes with 2M trifluoroacetic acid for 6 h at 100° followed by concentration to dryness. Glycoses were determined by g.l.c. of their alditol acetate derivatives[10], using *myo*-inositol as an internal standard. The configurations of aldoses were established by capillary g.l.c of their acetylated derived (+)-2-butyl glycosides. *O*-Deacetylation was effected with M NaOH (1 h, 100°) containing a trace of $NaBH_4$, followed by dialysis, and lyophilization of the retentate.

N.m.r. spectroscopy. — Measurements were made on solutions in D_2O (25 mg/mL), using a Bruker AM500 spectrometer equipped with an Aspect 3000 computer. ^{1}H-Decoupled ^{13}C-n.m.r. spectra (125 MHz) were recorded at 47° for a 25-kHz spectral width, by use of a $\pi/2$ pulse and a 32k data set. Chemical shifts are expressed in p.p.m. relative to the methyl carbon of internal acetone (1%, δ 31.07). $^1J_{C,H}$ values were measured using gated decoupling. ^{1}H-N.m.r. spectra (500 MHz) were recorded at 47° with a spectral width of 2.5 kHz and a $\pi/2$ pulse, with acetone as the internal reference (0.1%, δ 2.225).

^{1}H-Homonuclear-correlated 2D-n.m.r. experiments COSY[13] and relay COSY[24] were performed at 47°, using standard Bruker software. Spectra were measured using data sets (t_1, t_2) of 256 × 2048 points and a recycle delay of 1.4 s, and 32 transients were collected for each value of the incremental delay. For the relay COSY experiments, a fixed delay of 32 ms was employed. The data matrices were zero-filled to 1024 × 2048, and resolution enhancement in both domains was done by a nonshifted sine-bell function prior to Fourier transformation.

Heteronuclear ^{13}C–^{1}H shift-correlation experiments were done using the CHORTLE (carbon–hydrogen correlation from one-dimensional polarization transfer spectra by least-squares analysis) technique[25].

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