Lipopolysaccharides of *Campylobacter jejuni* Serotype O:19: Structures of O Antigen Chains from the Serostrain and Two Bacterial Isolates from Patients with the Guillain–Barré Syndrome†

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ABSTRACT: An O antigenic polysaccharide was liberated from the lipopolysaccharide of high M_r from *Campylobacter jejuni* serotype O:19 by acetic acid hydrolysis of the ketosidic linkage to lipid A. The structure of the polysaccharide was established in several one- and two-dimensional ^1H and ^{13}C NMR experiments, fast atom bombardment mass spectrometry and methylation linkage analysis of the permethylated glycan and its degradation products. It is concluded that the glycan is a derivative of hyaluronic acid in which the β-D-glucuronic acid residues in the alternating sequence [–4)-β-D-GlcA-(1→3)-β-D-GlcNAc-(1]$_n$ are present as amides of 2-amino-2-deoxyglycerol. Parallel experiments were performed on O antigens liberated from lipopolysaccharides of high M_r from bacterial isolates that had been obtained from two patients who subsequently developed the Guillain-Barré syndrome. Within the limits of structural analysis by NMR spectroscopy and methylation linkage analysis, both these O antigens were identical to that from the serostrain.

The Guillain-Barré syndrome (GBS),[1] although of relatively infrequent occurrence, is the most common cause of acute generalized paralysis which usually occurs 1–3 weeks after bacterial or viral infections (Ropper, 1992). However, the role of preceding infections on the development of the syndrome remains unknown. One of the best documented conditions associated with the subsequent onset of GBS is that of previous *Campylobacter jejuni* infection. In Japan a strikingly high proportion of such reported *C. jejuni* isolates has been classified as those of serotype O:19 (Kuroki et al., 1991), a serotype of otherwise infrequent occurrence. In continuation of studies of LPS structures from *C. jejuni* serotypes (Aspinall et al., 1992, 1993b) to provide a molecular basis for serotype diversity and specificity, LPS were isolated from the serotype 19 serostrain and from two O:19 typed isolates, OH 4382 and OH 4384, from GBS patients (Aspinall et al., 1993a), and were examined by SDS–PAGE. The low M_r LPS components, with detection by silver staining, differed in electrophoretic mobility. In the preceding paper (Aspinall et al., 1994), we report the characterization of the core oligosaccharide (OS) regions from each low M_r LPS in confirmation of the structural differences implied by SDS–PAGE and show that the core OS from isolate OH 4384 differed, albeit slightly, from that of the O:19 serostrain, whereas that from isolate OH 4382 was of markedly shorter chain length. In contrast (Aspinall et al., 1993a), SDS–PAGE of LPS samples with detection by immunoblotting with homologous antisera showed the characteristic banding patterns of high M_r LPS with O antigenic chains, with similar spacing but nonidentical mobilities. We report herein structural characterization of the O antigenic polysaccharide and show that, despite attachment to nonidentical core regions, the O antigen chains from the O:19 serostrain and the two isolates are indistinguishable.

EXPERIMENTAL PROCEDURES

Except as listed below, experimental procedures were those described in the preceding paper (Aspinall et al., 1994), wherein is reported the isolation of the O antigenic polysaccharide (PS **19**-P1) from the serotype O:19 LPS and, in similar fashion, O glycans 4382-P1 and 4384-P1, respectively, from isolates OH 4382 and OH 4384.

Hyaluronic acid, purchased as the sodium salt (Sigma), was decationized for NMR experiments.

^1H and ^{13}C NMR Spectroscopy. Homonuclear 2D ^1H TOCSY, TQF-COSY, inverse 2D ^1H–^{13}C HMQC, and inverse 2D ^1H–^{13}C HMBC experiments were performed on a Bruker AMX 500 spectrometer. Homonuclear 2D ^1H COSY and NOESY experiments were performed on a Bruker AM 300 spectrometer as described in the preceding paper (Aspinall et al., 1994).

Other Analytical Procedures. Glycose analysis was performed by the method of Sawareker et al. (1967) with GC analysis using program A. Enantiomeric configurations were established by conversion of hydrolyzates into 2-(*R*)-butyl glycosides (Gerwig et al., 1978) and GC analysis of acetylated derivatives using program D.

Methylated O Glycans, Linkage Analysis, and Degradation by Methanolysis. Methylated O glycans were prepared by the method of Ciucanu and Kerek (1984). Permethylated

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[1] Abbreviations: 1D, one-dimensional; 2D, two-dimensional; CI, chemical ionization; COSY, correlated spectroscopy; EI, electron impact; FAB/MS, fast atom bombardment mass spectrometry; GBS, Guillain-Barré syndrome; GC/MS, gas chromatography–mass spectrometry; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; GroN, 2-amino-2-deoxyglycerol; HMBC, heteronuclear multiple bond correlated spectroscopy; HMQC, heteronuclear multiple quantum correlated spectroscopy; LPS, lipopolysaccharide; NOESY, nuclear Overhauser enhancement spectroscopy; OS, oligosaccharide; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TOCSY, total correlated spectroscopy; TQF, triple quantum filtered.

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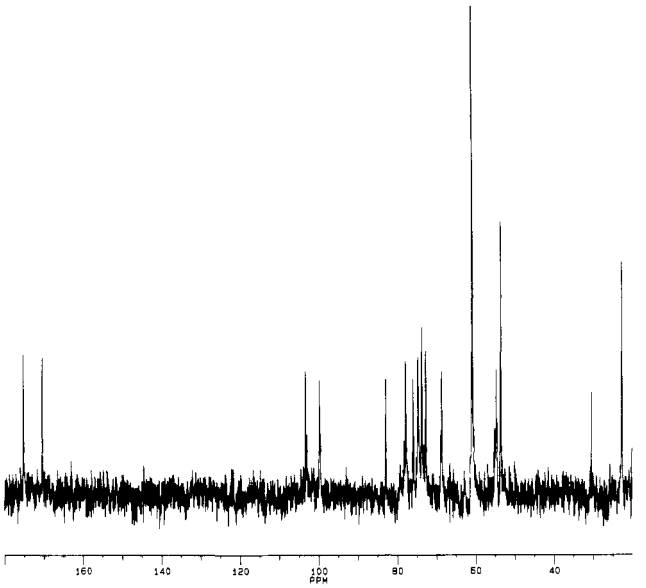

FIGURE 1: One-dimensional ^{13}C NMR spectrum of O antigenic polysaccharide PS **19**-P1.

derivatives were hydrolyzed as described in the preceding paper (Aspinall et al., 1994) with subsequent conversion into partially methylated alditol acetates for GC/MS analysis in both EI and CI modes using program B. The lactone component in the mixture was reduced with $NaBD_4$ and the mixtures was reacetylated for analysis in the EI mode. Permethylated O glycans were depolymerized by heating with trifluoroacetic acid (2 M in methanol) at 100 °C for 20 h, and the product was acetylated (pyridine–acetic anhydride) and the resulting disaccharide methyl glycoside(s) was examined by GC/MS (program F) and by positive ion FAB/MS.

RESULTS

Characterization of LPS of High M_r from C. jejuni O:19 Serostrain. Material obtained from the aqueous supernatant layer from phenol–water extraction of bacterial cells was submitted to mild acid hydrolysis. Insoluble lipid A-like material was removed by centrifugation and soluble O polysaccharide designated PS **19**-P1 was purified by GPC, giving a single band eluting at the column void volume. From hydrolysis of the glycan under standard conditions followed by reduction ($NaBD_4$) and acetylation, only two major products, GlcNAc and 2-amino-2-deoxyglycerol (GroN), were detected on analysis by GC/MS; trace amounts of the following constituents (in total <10%), Glc, Gal, and LD-Hep (in relative proportions corresponding to those in the core OS), were present. The quantities of these minor sugar constituents were insufficient to accommodate the presence of appreciable amounts of other glycans. Similarly, the small ribose content together with negligible UV absorption at 260 nm precluded the presence of significant contamination by RNA. In a separate experiment GlcNAc was shown to be the D enantiomer by conversion into 2-(*R*)-butyl glycoside acetates.

The ^1H and ^{13}C NMR spectra of PS **19**-P1 indicated the presence of a second main sugar constituent. The ^{13}C NMR spectrum (Figure 1) pointed to a rather simple disaccharide repeating unit of 17 carbon atoms, including 2 anomeric carbon atoms at δ 100.6 ($J_{C,H}$ 168 Hz) and 104.1 ($J_{C,H}$ 165 Hz), two carbonyl resonances at δ 175.9 and 171.1, one of which together with a signal at δ 23.7 (CH_3) was due to the *N*-acetyl group of the GlcNAc residue, two nitrogen-bearing carbon atoms at δ 55.6 and 54.5, and three overlapping unsubstituted

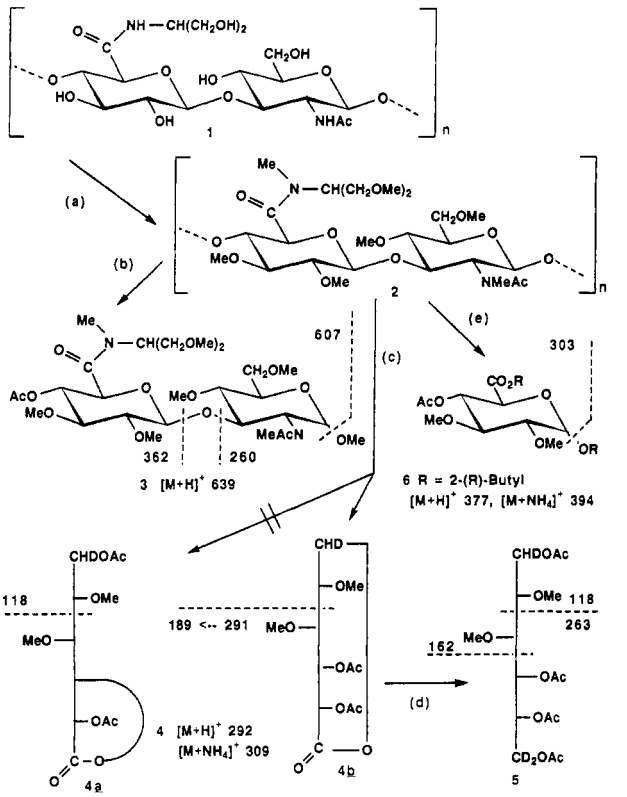

FIGURE 2: Chemical transformations in the characterization of PS **19**-P1 (**1**) with citation of key mass spectral data: (a) permethylation; (b) methanolysis of **2**, followed by acetylation to give **3**; (c) linkage analysis of **2** with formation *inter alia* of a [1-^{2}H]-Ac_2-2,3-Me_2-hexonolactone (**4a** or **4b**); (d) reduction ($NaBD_4$) of **4** followed by acetylation to give [1,6,6'-$^2H_3$]-1,4,5,6-Ac_4-2,3-Me_2-glucitol (**5**); (e) hydrolysis of **2**, followed by glycosidation and esterification in (*R*)-2-butanol and acetylation to give **6**.

primary hydroxyl groups at δ 61.7 (JMODXH). The ^1H NMR spectrum was in accord with these conclusions and showed two overlapping β-anomeric protons at δ 4.51 ($J_{1,2}$ 7.5 Hz) and 4.50 ($J_{1,2}$ 7.5 Hz), and the *N*-acetyl group at δ 2.04. Since eight carbon atoms could be accounted for by the β-GlcNAc (residue B) and three carbon atoms by the 2-amino-2-deoxyglycerol, the remaining six carbon atoms were suspected to be those of a hexuronic acid residue.

The key evidence for the identity of the hexuronic acid and its occurrence in amide linkage to 2-amino-2-deoxyglycerol was obtained from linkage analysis of PS **19**-P1 coupled with FAB/MS data. Four derivatives were formed in the linkage analysis and characterized by GC/MS in both the EI and CI modes: (i) 1,3-Me_2Gro2NAc and (ii) *N*,1,3-Me_3Gro2NAc from the amino-2-deoxyglycerol unit with incomplete *N*-methylation; (iii) the partially methylated alditol acetate from *N*,4,6-Me_3GlcNAc; and (iv) a compound not recognized initially, but whose pseudo-molecular ions, $[M + H]^+$ at m/z 292 and $[M + NH_4]^+$ at m/z 309, corresponded to those of a [^{2}H]Ac_2Me_2-hexonolactone originating from a hexuronic acid residue. As far as we are aware, alditol acetates of this type have not been reported previously as hexuronic acid derivatives for GC detection in linkage analyses. It was considered most likely that this compound would be a five-membered ring derivative formed as a [1-^{2}H]-2,4-Me_2-hexono-6,3-lactone from a 3-linked hexuronic acid residue. However, reduction of the lactone with $NaBD_4$ gave a derivative (Figure 2, compound **5**) that was coincident on GC and whose mass spectrum was identical, other than in isotopic labeling, to the partially methylated alditol acetate prepared from 2,3-Me_2Glc. The hexonic acid derivative could have

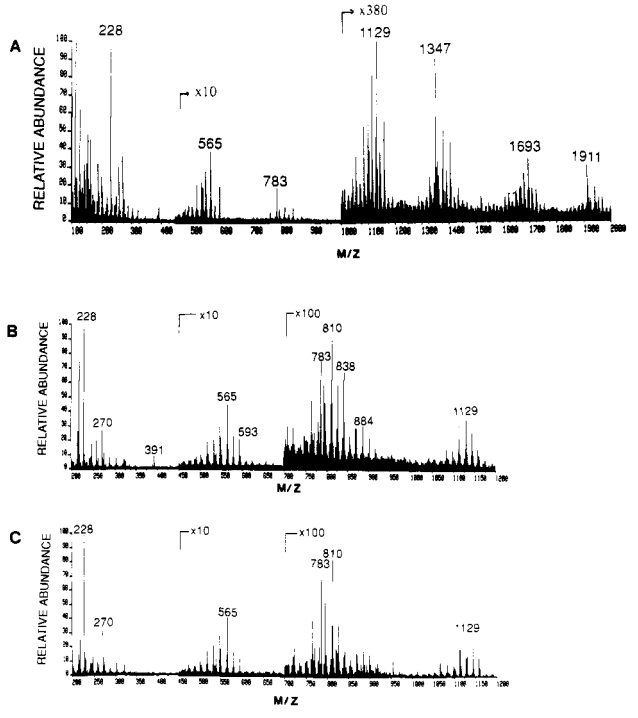

FIGURE 3: Positive ion FAB/MS spectra of permethylated polysaccharides: (A) PS **19**-P1 (**2**), (B) 4382-P1, and (C) 4384-P1.

Table 1: FAB/MS Analysis of Glycosyloxonium and Double Cleavage Ions from Permethylated O Polysaccharides PS **19**-P1, 4382-P1, and 4384-P1

composition of fragment ion	fragment ions (m/z) from permethylated glycans			
	glycosyloxonium ions		double cleavage ions	
	series 1	series 2	series 1	series 2
HexNAc	260		246	
HexA6(NGro)		334 → 302		
	320			
HexNAc, HexA6(NGro)	579	579	565	565
$HexNAc_2HexA6(NGro)$	824		810	
$HexNAc[HexA6(NGro)_2]$		898 → 866	884	
$HexNAc_2[HexA6(NGro)]_2$	1143	1143	1129	1129
$HexNAc_3[HexA6(NGro)]_2$	1388		1374	
$HexNAc_2[HexA6(NGro)]_3$		1462 → 1430	1448	
$HexNAc_3[HexA6(NGro)]_3$	1707	1707	1693	1693
$HexNAc_4[HexA6(NGro)]_3$	1952		1938	
$HexNAc_3[HexA6(NGro)]_4$		2026 → 1994	2012	
$HexNAc_4[HexA6(NGro)]_4$	2271	2271	2257	2257
$HexNAc_5[HexA6(NGro)]_4$			2502	
$HexNAc_5[HexA6(NGro)]_5$			2821	2821

been either a four-membered ring 6,4-lactone (**4a**) or a seven-membered ring 6,1-lactone (**4b**). However, GC/MS in the EI mode showed no prominent fragment ion at m/z 118 that would be characteristic of [1-^{2}H]-2,3-Me_2-alditol acetates such as **4a**, but instead an abundant fragment ion was observed at m/z 189 that could have arisen from endocyclic cleavage of the C_2–C_3 bond in **4b** followed by loss of 102 amu (acetic anhydride or acetic acid and ketene in succession).

The positive ion FAB/MS of permethylated PS **19**-P1 (Figure 3 and Table 1) showed a multitude of fragment ions, most but not all of which may be assigned compositions and accommodated in homologous series. The members of these series were separated by increments of 564 amu, corresponding to the addition of HexNAc (245) and HexA[NGro] (319) residues. Two series of glycosyloxonium ions (Dell, 1986) could be discerned starting from nonreducing end groups and followed by successive mass increments of 319 (HexA[NGro]) and 245 (HexNAc) amu in the repeating unit. Series 1 started with a terminal HexNAc unit at m/z 260 (of low abundance) and its identity was supported by the detection of terminal GlcNAc in the linkage analysis. Series 2 started with a terminal HexA[NGro] unit at m/z 334 and was followed by successive increments of 245 and 319, but with low abundance for ions with odd numbers of residues, i.e., those with glycosyloxonium ions arising from HexA[NGro] units, while those ions with even numbers of residues could not be distinguished from their isomers in Series 1. However, secondary ions formed from HexA[NGro] glycosyloxonium ions with loss of methanol, e.g., those at m/z 302, 866, 1430, and 1994, were relatively abundant. In addition, many double cleavage ions (Dell, 1986) were seen with the corresponding increments, e.g., with m/z 320, 565, etc., up to 2821 with five repeating units. One of the more prominent of the double cleavage ions was that at m/z 565.2932 (calculated for $C_{25}H_{45}O_{12}N_2$: 565.2972), whose accurate mass determination corresponded to a glycobiosyl unit of Me_2,NMe-HexNAc-(Me_2,NMe-Gro)-Me_2HexA or its isomer (Me_2,NMe-Gro)-Me_2HexA-Me_2,NMe-HexNAc. The increment of 319 was consistent with that of a HexA residue in amide linkage with the 2-amino-2-deoxyglycerol. The series of rather prominent ions at m/z 783, 1347, and 1911 could imply a third increment of 218 amu that could originate from a nonamidated HexA unit, either originally present but not supported by other evidence or arising from another type of mass spectral cleavage. The single most prominent ion at m/z 228 was a secondary ion arising through β-elimination from all glycosyloxonium ions and double cleavage ions involving chain scission at 3-linked HexNAc residues.

Degradations of the permethylated glycan provided further evidence for its structure (**2**) and hence for the parent glycan (**1**) as an amidated derivative. Methanolysis of the methylated glycan was carried out to obtain oligomers containing the substituted GlcA6(NGro) residues. The product was acetylated and GC/MS (EI mode) showed a single volatile component for which structure **3** (Figure 2) may be advanced on the basis of fragment ions at m/z 362 (glycosyloxonium ion) and 260 (J_2 ion from HexNAc). FAB/MS showed a protonated molecular ion $[M + H]^+$ at m/z 639 and no ions at higher mass, together with glycosyloxonium ions at m/z 362 and 607 as shown in Figure 3. The remaining aspect of structure to be defined was the absolute configuration of the GlcA residue. Linkage analysis of permethylated PS **19**-P1 had shown that, in contrast to the parent glycan, monomeric components were formed readily on acid hydrolysis. The hydrolysis products were converted, by glycosidation in (*R*)-2-butanol (Vinogradov et al., 1991) followed by acetylation, into a mixture of chiral glycosides including acetylated (*R*)-2-butyl ester (*R*)-2-butyl glycosides that were identical by GC/CIMS to the derivatives formed similarly from permethylated hyaluronic acid and from a sample of methyl 4-*O*-acetyl-2,3-di-*O*-methyl-α-D-glucopyranosiduronic acid (Aspinall & Barron, 1972). These results defined the O glycan PS **19**-P1 as having a covalent structure with disaccharide repeating units, →4)-β-D-GlcA6(NGro)-(1→3)-β-D-GlcNAc-(1-, as in hyaluronic acid, but with the GlcA residues amidated with 2-amino-2-deoxyglycerol. The proposed structure (**1**) was further substantiated in 2D NMR spectroscopic analyses.

Identity of LPS of High M_r from C. jejuni O:19 and Isolates OH 4382 and OH 4384. O Polysaccharides 4382-P1 and 4384-P1 from the isolates were prepared as described for PS **19**-P1. Parallel experiments performed on these glycans

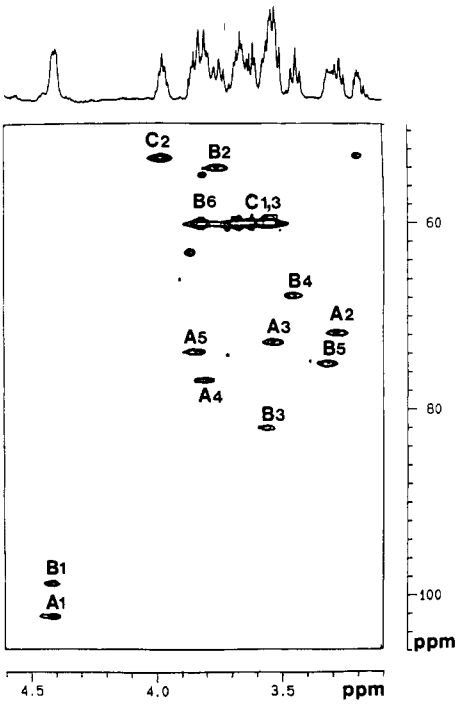

FIGURE 4: Two-dimensional ^1H–^{13}C HMQC spectrum of O polysaccharide 4382-P1.

included 1D ^1H and ^{13}C NMR spectroscopy, compositional analysis, and studies on the permethylated derivatives involving FAB-MS (Figure 3), linkage analysis, and methanolysis followed by acetylation to give disaccharide **3** (Figure 2). The results showed that by these criteria the three O glycan samples were indistinguishable. More detailed examinations were carried out on the available quantities of glycans that remained.

Two-Dimensional NMR Spectroscopy of PS 19-P1 and Isolate 4382-P1. The analysis started with an inverse ^1H–^{13}C HMQC experiment on 4382-P1 (Figure 4) giving (i) an assignment of protons attached to N-bearing carbon atoms (H-2 of GlcNAc at δ 3.85 and H-2 of Gro2N at δ 4.08), (ii) a differentiation of the three overlapping methylene resonances of CH_2OH groups at C-6 of GlcNAc and C-1 and C-3 of Gro2N, and (iii) confirmation of the differentiation of the partially overlapping anomeric proton signals from the GlcNAc and GlcA6(NGro) residues. Full proton assignments for 4382-P1 and PS **19**-P1 were established through proton connectivities starting from the H-1 resonances in 2D 300-MHz ^1H–^1H COSY (Figure 5) and 500-MHz TOCSY (Figure 6) and 2D ^1H TQF-COSY (not shown) experiments. Assignments of resonances of the mutually coupled protons H-5, H-6, and H-6′ of GlcNAc and measurements of associated coupling constants were achieved in the TQF-COSY experiment. The results are summarized in Table 2 and confirm *β-gluco* configurations for both residues, each in the $^4C_1$ conformation.

The inverse ^1H–^{13}C HMQC experiment (Figure 4) also led to assignment of carbon resonances in the 4382-P1 sample, with results given in Table 3. The downfield signals at δ 83.7 and 78.6 corresponded to those of the linkage positions at C-3 of GlcNAc and C-4 of the GlcA6(NGro) residues, respectively. Assignment of the carbonyl resonance at C-6 of the GlcA residues in 4382-P1 and proof of its substitution in amide linkage was achieved through a long-range inverse ^1H–^{13}C HMBC experiment. Intraresidue connectivities were shown (Figure 7A) from C-6 to H-5 of the GlcA residue and from C-6 of the GlcA residue to H-2 of the Gro2N residue, thereby

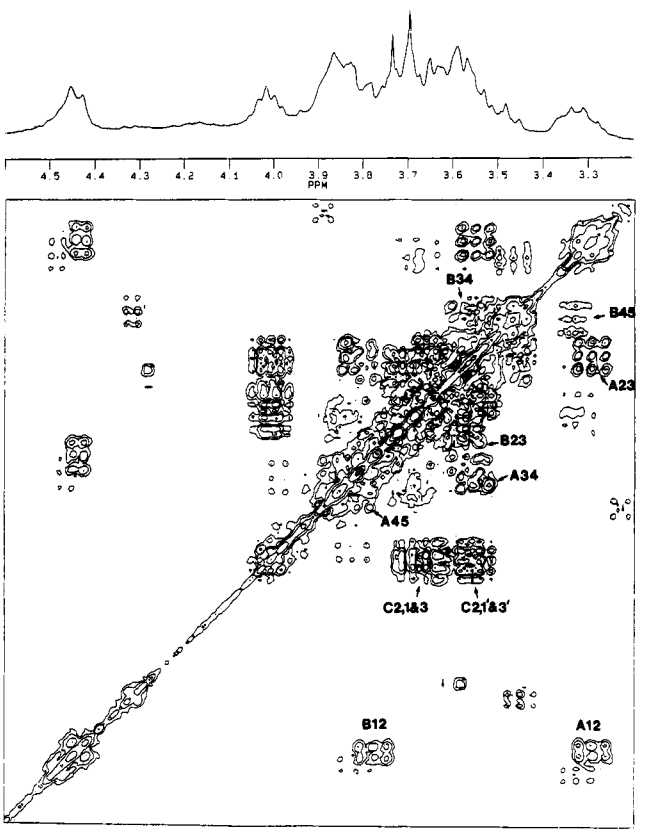

FIGURE 5: Two-dimensional 300-MHz ^1H–^1H COSY spectrum of O polysaccharide PS **19**-P1.

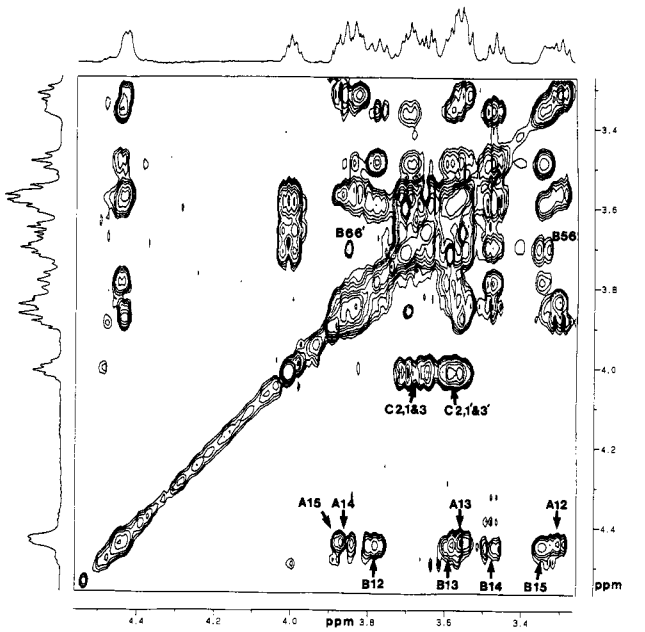

FIGURE 6: Two-dimensional 500-MHz ^1H–^1H TOCSY spectrum of O polysaccharide 4382-P1.

unequivocally establishing the Gro2N amide linkage to the GlcA residue. In addition, interresidue connectivities were observed across glycosidic bonds from C-1 of GlcA6(NGro) to H-3 of GlcNAc and from C-1 of GlcNAc to H-4 of GlcA6-(NGro).

Proton assignments from COSY and TOCSY experiments were also utilized in the 2D 300-MHz NOESY experiment (Figure 7B). Intraresidue NOE interactions were observed in the GlcA6(NGro) residue between H-2 and H-4 and between H-3 and H-5 and in the GlcNAc residue between

Table 2: ^{1}H NMR Chemical Shift Data (ppm) and Vicinal Coupling Constants (Hz) for O Glycans PS **19**-P1, 4382-P1, and 4384-P1

sugar residue[a]	H-1 ($J_{1,2}$)[b]	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6'}$) ($J_{5,6}$)	H-6 ($J_{6,6'}$)
β-GlcA (A)	4.50 (7.5)	3.37 (7.8)	3.59 (~8)	3.87 (~8)	3.93	
β-GlcNAc (B)	4.51 (7.5)	3.85 (~8)	3.64 (~8)	3.52 (~8)	3.39 (~5) (~4)	3.92 (~12), 3.74 [2.04][c]
Gro2N (C)	3.74 (m), 3.63 (m)	4.08 (5.5)	3.74 (m), 3.63 (m)			

[a] Residues A, B, and C are as shown in Figure 3. [b] Coupling constants $J_{n,n+1}$ are in parentheses. [c] CH_3 of *N*-acetyl.

Table 3: ^{13}C NMR Chemical Shift Data (ppm) for O Glycans PS **19**-P1, 4382-P1, and 4384-P1

sugar residue[a]	C-1 ($J_{C,H}$)[b]	C-2	C-3	C-4	C-5	C-6	C=O	CH_3
β-GlcA (A)	104.1 (165)	73.5	74.5	78.6	75.5	171.1		
β-GlcNAc (B)	100.6 (168)	55.6	83.7	69.5	76.7	61.7	175.9	23.7
Gro2N (C)	61.7	54.5	61.7					

[a] Residues A, B, and C are as shown in Figure 3. [b] C–H couplings (Hz) are in parentheses.

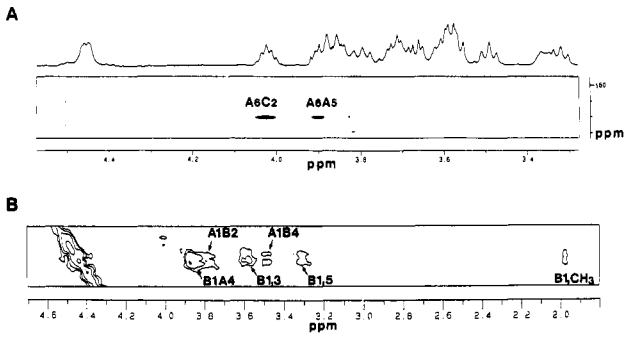

FIGURE 7: (A) Partial 2D ^{1}H–^{13}C HMBC spectrum of O polysaccharide 4382-P1. (B) Partial 2D 300-MHz ^{1}H NOESY spectrum of O polysaccharide 4382-P1.

Table 4: ^{1}H NMR Chemical Shift Data (ppm) and Vicinal Coupling Constants (Hz) for Hyaluronic Acid[a]

sugar residue[a]	H-1 ($J_{1,2}$)[b]	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5
β-GlcA (A)	4.55 (~9)	3.35 (8.5)	3.66 (8.9)	3.81 (8.8)	4.01
β-GlcNAc (B)	4.58 (~9)	3.84 (8.4)	3.75 (8.9)	3.55 (8.5)	3.51

[a] Residues A and B correspond to those in the O antigen (**1**) in Figure 1. [b] Coupling constants $J_{n,n+1}$ are in parentheses.

H-1 and H-3, H-5, and CH_3, between H-2 and H-4, and between H-3 and H-5; in both instances in confirmation of $^4C_1$ conformations. An interresidue NOE was observed between H-1 of GlcNAc and H-4 of GlcA6(NGro). However, for the GlcA6(NGro)→GlcNAc linkage, interresidue NOE cross peaks were observed between H-1 of GlcA6(NGro) and H-4 and H-2 of GlcNAc but not with H-3 at the linkage position. For purposes of comparison, 2D COSY and NOESY experiments were performed with hyaluronic acid under the same conditions at 300 MHz and in D_2O solution. Much of the COSY spectrum was superimposable on that of O:19 P1, and the proton assignments are given in Table 4. The intraresidue NOE interactions for both residues, between H-1 and H-3 and H-5, between H-3 and H-5, and between H-2 and H-4 for GlcA and between H-2 and H-4 of GlcNAc, were in accord with $^4C_1$ ring conformations. An interresidue NOE was observed between H-1 of GlcNAc and H-4 of GlcA, but none was detected between H-1 of GlcA and the GlcNAc residue. Kvam et al. (1992), in an extensive NMR study of hyaluronic acid derivatives, were unable to identify unequivocally interresidue cross-peaks in NOESY experiments at 200 MHz due to partial overlap of signals. In a very recent publication, Livant et al. (1992) have reported NMR studies at 600 MHz on a tetrasaccharide from hyaluronic acid, and at the higher field strength they were able to define the expected interresidue cross-peaks at all the glycosidic linkages in each of the anomers at the reducing terminus. In our experiments we conclude that the interresidue cross-peak of H-1 of GlcA6-(NGro) with H-4 of GlcNAc, but *not* at the linkage site, points to a significantly different chain conformation in the *C. jejuni* serotype O-19 O antigen chains from the generally similar conformations of hyaluronic acid of the various rigid multiply hydrogen-bonded networks proposed in the solid state from X-ray diffraction (Arnott et al., 1983) and in solution from NMR studies (Heatley & Scott, 1988). No detailed conformation can be proposed for the O antigen, but it is very likely that the conformational difference from hyaluronic acid will be reflected in immunological properties.

DISCUSSION

The O antigen region of the LPS of high M_r from *C. jejuni* serotype O:19 has been shown to consist of a disaccharide repeating unit identical to that in hyaluronic acid except for the occurrence of the β-D-GlcA residues as amides of 2-amino-2-deoxyglycerol (NGro). Amidated hexuronic acid residues are encountered in other bacterial O antigens (Lindberg, 1991), but to our knowledge 2-amino-2-deoxyglycerol has only been reported as a constituent in those from *Shigella boydii* type 8 (Lvov et al., 1983), and *Vibrio cholerae* strain H11 (non-O1) (Vinogradov et al., 1992) in attachment to α-D-GalA residues.

The present investigation was prompted by the observation that in Japan a rather high proportion of previous *Campylobacter* infections leading to the subsequent onset of GBS were those of organisms typed as *C. jejuni* serotype O:19. The detection of such infections in two siblings, both of whom later developed GBS within a short period of each other, provided an excellent opportunity to compare LPS as the distinctive surface components of the separate isolates and to establish their degree of similarity or difference. In the present studies the O antigen chains of the O:19 serostrain and of the isolates OH 4382 and OH 4384 proved to be indistinguishable despite the observations that the LPS preparations were nonidentical in SDS–PAGE (Aspinall et al., 1993a). The lack of identity of the LPS samples may be attributed to structural differences in the core OS regions as shown in the low M_r LPS, in which core OS is linked to lipid A but is not further extended by the apposition of O antigen chains. The common generalizations regarding LPS structures in Gram-negative bacteria, exemplified by those from *Salmonella* species (Rietschel et al., 1990), is that lipid A and core OS regions are conserved, with structural variations between species or between serotypes within a species being exhibited in the O antigen chains. In rather few instances have the exact sites of attachment of O antigen chain to core OS been established unambiguously. The best documented example is that for many *Salmonella* spp. where rough mutants giving rise to incomplete core OS lack one or more of the required enzymes, so that the site for attachment of O antigen repeating units is missing. In the examples under investigation the presence of O antigen chains as integral regions in the high M_r LPS preparations is implied, if not unambiguously proven, in SDS–PAGE where the characteristic banding patterns are

displaced with respect to each other but show similar spacing between the bands. Assuming that O chains are linked to the corresponding residues in each core OS segment, the further implication is that the point of attachment may be in the inner core heptose/KDO region and not in the outer core region as in *Salmonella* LPS.

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