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Structure of the capsular polysaccharide of *Clostridium perfringens* Hobbs 5 as determined by NMR spectroscopy

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Abstract

The complete primary structure of the capsular polysaccharide of *Clostridium perfringens* Hobbs 5, an anaerobic bacterium implicated in food poisoning, was determined. The polysaccharide was isolated from *C. perfringens* Hobbs 5 cells, after deproteination, by ethanol precipitation and by ion-exchange chromatography. The polysaccharide was comprised of glucose, galactose, mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and glucuronic acid, in equimolar ratios. Sequence and linkage assignments of the glycosyl residues were obtained by NMR spectroscopy, specifically by the combination of two-dimensional homonuclear TOCSY and NOESY experiments and heteronuclear {^{1}H, ^{13}C} multiple-quantum coherence (HMQC, HMQC-COSY, HMQC-TOCSY and HMBC) experiments. Thus, the envelope polysaccharide of *C. perfringens* Hobbs 5 was found to be a polymer composed of a hexasaccharide repeating unit with the following structure:

$[\rightarrow 4)\text{Glc}p\beta(1\rightarrow 3)\text{Gal}p\text{NAc}\beta(1\rightarrow 4)\text{Glc}p\text{A}\,\beta(1\rightarrow 3)\text{Glc}\,p\text{NAc}\beta(1\rightarrow 2)\text{Gal}p\alpha(1\rightarrow 3)\text{Man}p\beta(1\rightarrow)]_n$

This structure is novel among bacterial cell-surface polysaccharides, and it is the first of many serotypically distinct capsular polysaccharides of *C. perfringens* to be described.

Keywords: 2D NMR spectroscopy; HMQC; TOCSY; NOESY; Polysaccharide structure; Antigen

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1. Introduction

Clostridium perfringens is an important histotoxic anaerobic bacterial species commonly involved in myonecrosis (gas gangrene) [1]. The bacterium has also been implicated in several clinical infections such as septicemia and uterine infections. The incidence of food-borne illness caused by *C. perfringens* ranks third behind *Salmonella* spp. and *Staphylococcus aureus* [2]. The principal serotype [3,4] specific antigens of *C. perfringens* are capsular heteropolysaccharides found associated with the cell envelope [5–9].

The capsule is antiphagocytic, and it may be an essential virulence factor during disease [10,11]. Although several hundred serological types of *C. perfringens* are known to exist, the capsular polysaccharides from only a few serotypes have been isolated, purified and their sugar compositions determined [5–7,9]. Certain strains of *C. perfringens* that were associated with food poisoning produced heat-resistant spores. These strains were used to prepare specific antisera that were later used for serological characterization. They were characterized by Arabic numerals and were designated as the Hobbs serotypes [12,13]. The immunochemistry of the *C. perfringens* capsule has not attracted much attention in recent years in spite of its obvious antigenic significance. In view of this we decided to isolate capsular polysaccharide from *C. perfringens* serotype Hobbs 5 and determine its primary structure.

The molecular weight of the polysaccharide, as determined by equilibrium ultracentrifugation of dilute solutions of the compound, was approximately 9.3 kDa [5]. Chemical methods that were used initially to elucidate the molar composition of this polysaccharide showed the following sugars: glucose (Glc), galactose (Gal), mannose (Man), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), and glucuronic acid (GlcA) in the ratio of 1.2:1.2:1.2:2:2:2.6 [5]. However, a ^{13}C NMR spectrum with sufficiently long interpulse delays (~ 5 s) clearly showed that the ratio of the six glycosyl residues was 1:1:1:1:1:1 (Fig. 1). Therefore, it was reasonable to hypothesize that the polysaccharide is composed of a repeating hexasaccharide unit. No further information regarding the primary structure of this polysaccharide was available when we decided to explore the applicability of multidimensional NMR spectroscopy for its complete characterization.

The strategy for the de novo sequencing of the Hobbs 5 capsular polysaccharide from *C. perfringens* by NMR spectroscopy was as follows [14–16]:

(1) Complete assignment of the ^{1}H NMR spectrum of the compound using, for example, a series of selective 1D TOCSY experiments.

(2) Complete assignment of the ^{13}C NMR spectrum using heteronuclear {^{1}H, ^{13}C} correlation experiments (HMQC and GHSQC).

(3) Sequencing the polysaccharide using a combination of two versatile methods that are capable of revealing interglycosidic contacts between adjacent sugar residues, viz. HMBC (tracing $^{3}J_{CH}$ interglycosidic couplings) and HMQC–NOESY (where {^{1}H, ^{1}H} NOEs, including the interglycosidic ones, are sorted according to ^{13}C chemical shifts).

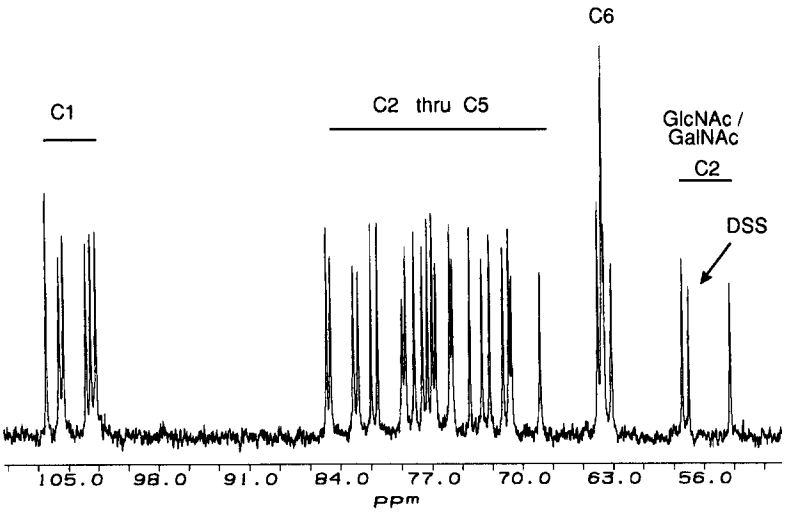

Fig. 1. 1D ^{13}C NMR spectrum of the *C. perfringens* Hobbs 5 polysaccharide recorded at 100 MHz and 70 °C. The carbonyl and methyl regions of the spectrum are not shown. The resonance at 56.96 ppm is due to DSS.

2. Experimental

Isolation of the capsular polysaccharide of C. perfringens *Hobbs 5.*—A culture of *C. perfringens* Hobbs 5 was provided by Dr. V.R. Dowell, Centers for Disease Control and Prevention, Atlanta, GA. The encapsulated strain was selected, maintained, and isolated as previously described [5]. Polysaccharide was extracted from acetone-dried cells with 0.85% NaCl and recovered by precipitation of the cell-free supernatant fraction with two volumes of ethanol [7]. The polysaccharide in 0.01 M 2-amino-2-hydroxymethyl-1,3-propandiol (Tris) was deproteinized with an equal volume of chloroform-1-butanol 9:1 (v/v) [17], and the polysaccharide was recovered by precipitation of the aqueous phase with ethanol as described above. The deproteinized polysaccharide, in 0.01 M Tris–0.02 M Na_2SO_4–0.2 M NaCl, was treated with hexadecyltrimethylammonium bromide (CTAB) to precipitate the contaminating nucleic acids [18]. After removing the precipitate by centrifugation, the supernatant was treated with ethanol to recover the polysaccharide. The polysaccharide was dissolved in distilled water, exhaustively dialyzed versus distilled water, and recovered by precipitation with ethanol. The polysaccharide was dissolved in 0.01 M Tris, pH 7.5, and then applied to a column (30 × 1.5 cm) of DEAE-Sephadex A-25 (Pharmacia), equilibrated with the same buffer. The sample was eluted with a linear gradient of 0–0.5 M NaCl in 0.01 M Tris. The polysaccharide eluted as a single symmetrical peak. The appropriate column fractions that contained carbohydrate were combined, dialyzed, and lyophilized [7]. The purified polysaccharide was stored desiccated at 23 °C. A portion (315 mg in 50 mL deionized water) was treated by ultrasonic irradiation for 2 h. The sample was centrifuged (15,000 × *g*), dialyzed against deionized water, and finally recovered by lyophilization (285 mg). This fraction of the Hobbs 5 polysaccharide was used for all subsequent analyses. The apparent mol. wt. of the polysaccharide was determined by gel-filtration chromatography on a Sepharose CL-6B (Pharmacia Fine Chemicals) column (90 × 1.5 cm). The column was equilibrated with 0.01 M Tris–0.1 M NaCl buffer (pH 7.2, and then it was calibrated with polysaccharide mol. wt. standards (Polymer Laboratories). The polysaccharide (6.1 mg) in 1.0 mL of Tris buffer was applied to the column. The column was eluted at a flow rate of 26 mL · h^{-1}. The polysaccharide eluted as a single polydisperse peak with an apparent mol. wt. of 71 kDa. This value is significantly different from the 9.3 kDa reported earlier for this polysaccharide [5].

Analytical procedures.—The presence of carbohydrate was detected by the phenol–sulfuric acid method [19]. The occurrence of uronic acid was determined by the procedure of Blumenkrantz and Asboe-Hansen [20]. The hexosamine content was estimated by the method of Smith and Gilkerson [21]. The constituent monosaccharides of the polysaccharide were identified and quantified by gas–liquid chromatography as their per-*O*-acetylated aldononitrile (PAAN) derivatives, as previously described [22,23].

NMR analysis.—An aliquot (15 mg) of the Hobbs 5 polysaccharide sample was repeatedly exchanged with D_2O. Afterwards the sample was dissolved in 0.5 mL D_2O (99.96% D; Cambridge Isotope Laboratories) and transferred into a 5-mm NMR tube (Wilmad 535-PP). Sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) was added as internal standard for chemical shift calibration. All NMR experiments were performed at a temperature of 57 °C, except as noted. This reduced the viscosity of the solution which resulted in the narrowing of the lines in the spectra. The 1D TOCSY [24,25] and the 2D ^{13}C-coupled HSQC [26] experiments were performed on a Bruker AM-500 spectrometer interfaced with an Aspect 3000 computer. The proton spectral width was 2500 Hz in both experiments and the carbon spectral width was 8804 Hz in the HSQC experiment. A DANTE pulse was used for selective excitation in the 1D TOCSY experiments and a composite pulse sequence was used for isotropic mixing (typically for 120 ms) [25]. The TQF-COSY experiment [27] was performed on a Bruker AMX-500 spectrometer with a spectral width of 1501.5 Hz in both dimensions. The other NMR experiments, viz., the HMQC [28–30], HMBC [31], HMQC–COSY, HMQC–TOCSY and HMQC–NOESY experiments [32–34] were performed on a Bruker AMX-600 spectrometer. Spectral widths were set at 3012 Hz in the proton dimension and 10563 Hz in the carbon dimension for all heteronuclear experiments. The mixing times for the HMQC–TOCSY and HMQC–NOESY experiments were 80 ms (MLEV-17; flanked by two 2.5 ms trim pulses) and 150 ms, respectively. The GHSQC experiment [35] was performed with a Varian UnityPlus 600 spectrometer; the two gradient strengths were 21 G/cm with durations of 2 ms and 0.5 ms. The data were processed off-line using the FELIX software package, version 2.05 (Biosym/Molecular Simulations, San Diego, CA), on a Silicon Graphics Per-

sonal IRIS workstation. Typically, a Lorentzian-to-Gaussian weighting function was applied in the t_2 domain and a squared sine-bell with zero-filling in the t_1 domain. Chemical shifts were referenced to internal DSS at 0.0 ppm for ^{1}H and ^{13}C spectra.

3. Results and discussion

^{1}H *and* ^{13}C NMR *assignments.*—Chemical analysis of the Hobbs 5 polysaccharide had shown [5] that the polysaccharide was composed of Glc, Gal, Man, GlcNAc, GalNAc and GlcA, in approximately equimolar amounts. The high-resolution 1D ^{1}H NMR spectrum (Fig. 2) of the *C. perfringens* Hobbs 5 polysaccharide showed six anomeric (H-1) resonances of equivalent areas which confirms that the polysaccharide is composed of a hexasaccharide repeating unit. Two of these H-1 signals, around 4.73 ppm, were partially overlapped. Selective 1D TOCSY experiments [36] were conducted on each anomeric resonance, in order to provide ^{1}H coupling networks (NMR signatures) for the different glycosyl residues. The mixing time was varied from 20 ms to 120 ms, in steps, for each residue. Typical results for the six residues (obtained at 120 ms mixing time) are shown in Fig. 3. (compare results with [15]). The spectra (Fig. 3a and d) resulting from irradiation of the H-1 resonances at 5.38 ppm and 4.56 ppm displayed the restricted Hartmann–Hahn transfer for a galactopyranosyl (Gal*p*) residue; assignment of spectrum (a) to Gal, that of (d) to GalNAc was based on the correlation of the H-2 signal in trace (d) with the C-2 at 53.75 ppm in the ^{13}C NMR spectrum (see Fig. 4). Selective excitation of the downfield of the two resonances at 4.73 ppm revealed magnetization transfer only to the broad signal at 4.23 ppm. This was strong evidence for the assignment of these signals to H-1 and H-2 ($^3J_{12} < 1.5$ Hz), respectively, of Man. The complete Man spectrum was obtained by TOCSY following selective irradiation of Man H-2 (Fig. 3b).

The TOCSY spectra in Fig. 3c, e and f all show the characteristics of glucopyranosyl residues. Selective excitation of the H-1 signal at 4.48 ppm showed Hartmann–Hahn transfer up to H-5 indicating that this spectrum belonged to the GlcA residue (Fig. 3f). The other two TOCSY spectra (Fig. 3c and e), arising from selective excitation of the anomeric resonances at 4.72 and 4.50 ppm, showed magnetization transfer throughout the ring to H-5 and were assigned to the GlcNAc and Glc residues, respectively. As above for Gal and GalNAc, the assignment of spectrum (c) to GlcNAc, that of (e) to Glc was based on the correlation of the H-2 signal in trace (c) with the C-2 at 57.5 ppm in the ^{13}C NMR spectrum (see Fig. 4).

While the selective 1D TOCSY experiments produced spectra that could be assigned to the different sugar units in the polymer, extensive resonance overlap in three of the individual 1D ^{1}H NMR spectra prevented unambiguous assignment for some of the pertinent sugar ring protons. For example, the 1D TOCSY spectra did not permit us to distinguish between Gal H-3/H-4 (Fig. 3a), GlcNAc H-4/H-5 (Fig. 3c), and Glc H-3/H-4 (Fig. 3e). Furthermore, magnetization induced into GalNAc H-1 did not show appreciable TOCSY transfer beyond H-4 to H-5/H-6/H-6′ of the GalNAc residues, due to the rather small $^3J_{45}$ (< 1.5 Hz) (see Fig. 3a, d); this prevented assignments for the H-5/H-6/H-6′ protons of the Gal and GalNAc residues. The intrinsically larger dispersion of chemical shifts in the ^{13}C spectrum of the polysaccharide (Fig. 1) provided an opportunity

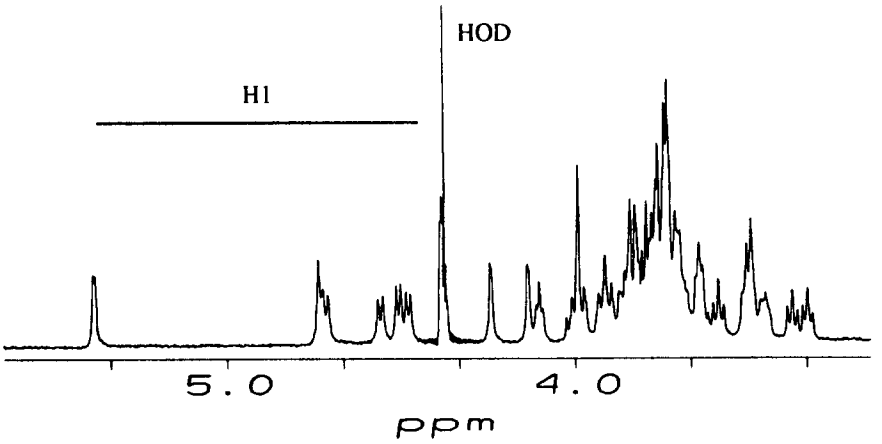

Fig. 2. 1D ^{1}H NMR spectrum of the *C. perfringens* Hobbs 5 polysaccharide recorded at 600 MHz and 57 °C.

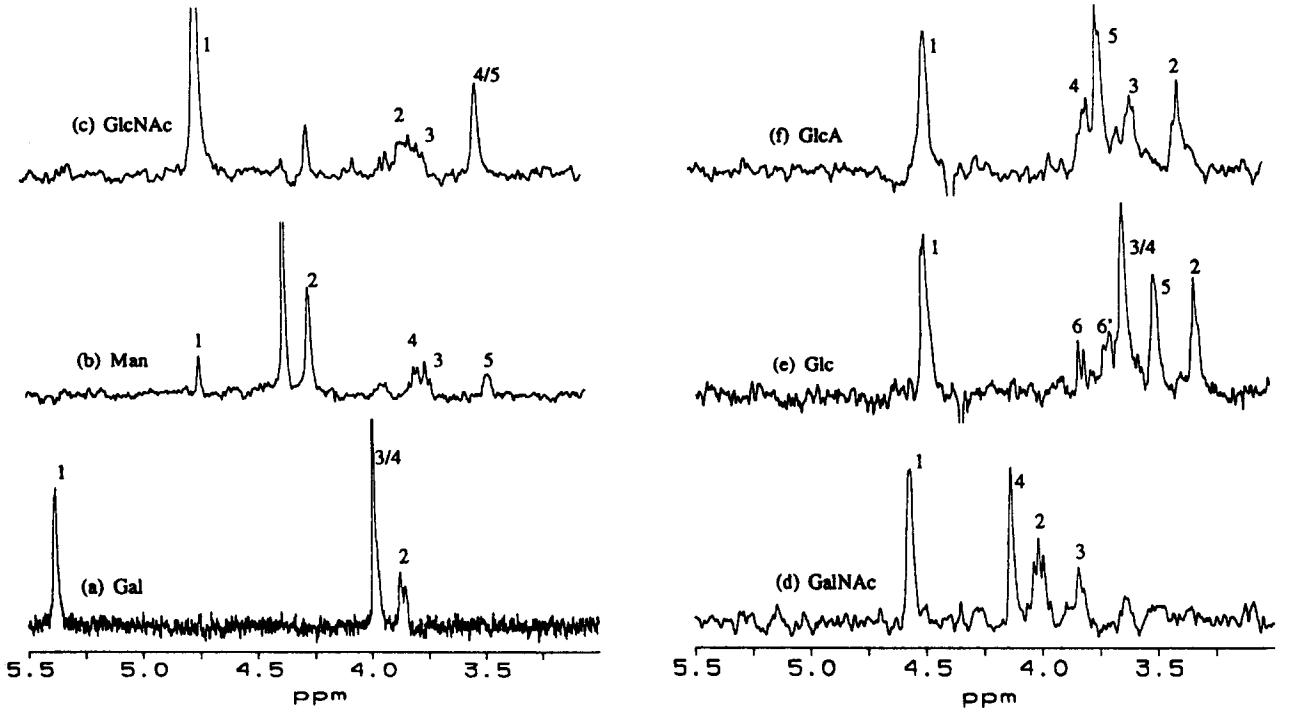

Fig. 3. 1D TOCSY spectra of the constituting glycosyl residues of the *C. perfringens* Hobbs 5 polysaccharide. The spectra (a) and (c)–(f) resulted from selective excitation of the anomeric protons, while for the Man residue (trace (b)) H-2 was irradiated. The isotropic mixing time for the spectra depicted was 120 ms. The numbers in the spectra refer to the ring proton assignments.

to resolve the remaining ambiguities in the ^{1}H assignments. Starting with the unequivocal ^{1}H assignments revealed by the 1D TOCSY experiments it was possible to assign a large number of the cross peaks in the HMQC spectrum (Fig. 4a, b). Confirmation of the hexosamine nature of the GlcNAc and GalNAc residues was obtained from the typical chemical shifts of their C-2 carbons (50–60 ppm) (Fig. 4b). Once the

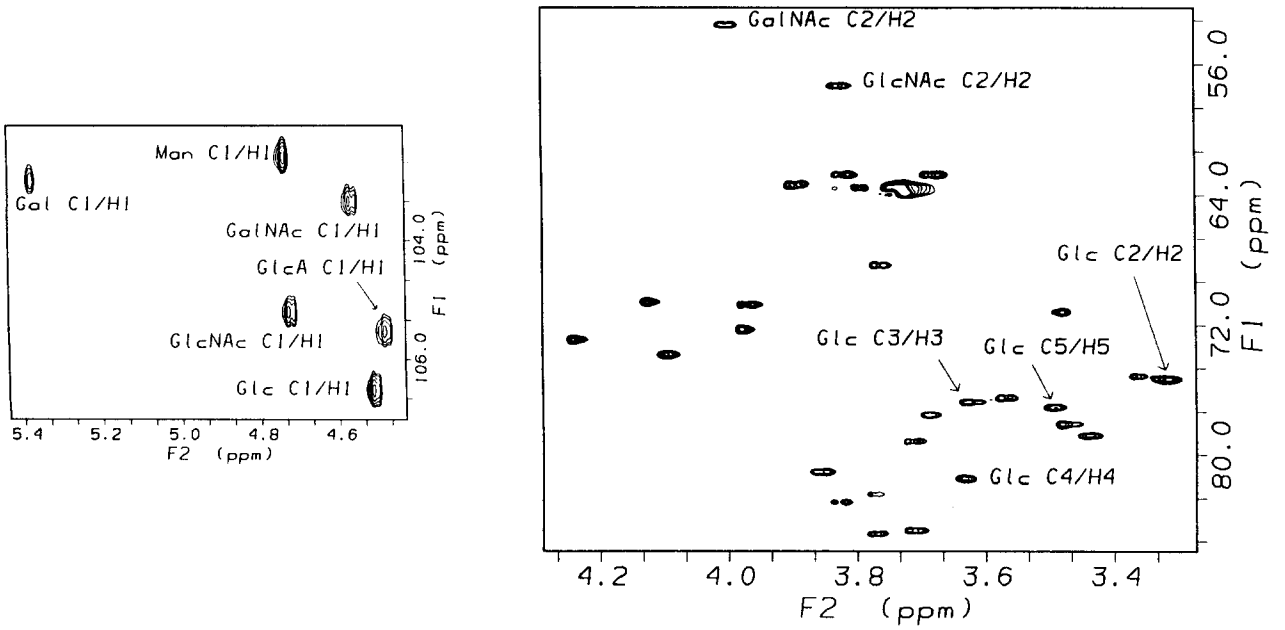

Fig. 4. {^{1}H, ^{13}C} one-bond correlation (HMQC) spectrum of the *C. perfringens* Hobbs 5 polysaccharide with heteronuclear decoupling in the t_1 dimension. (a) Expansion of the anomeric region; (b) Expansion of the non-anomeric region.

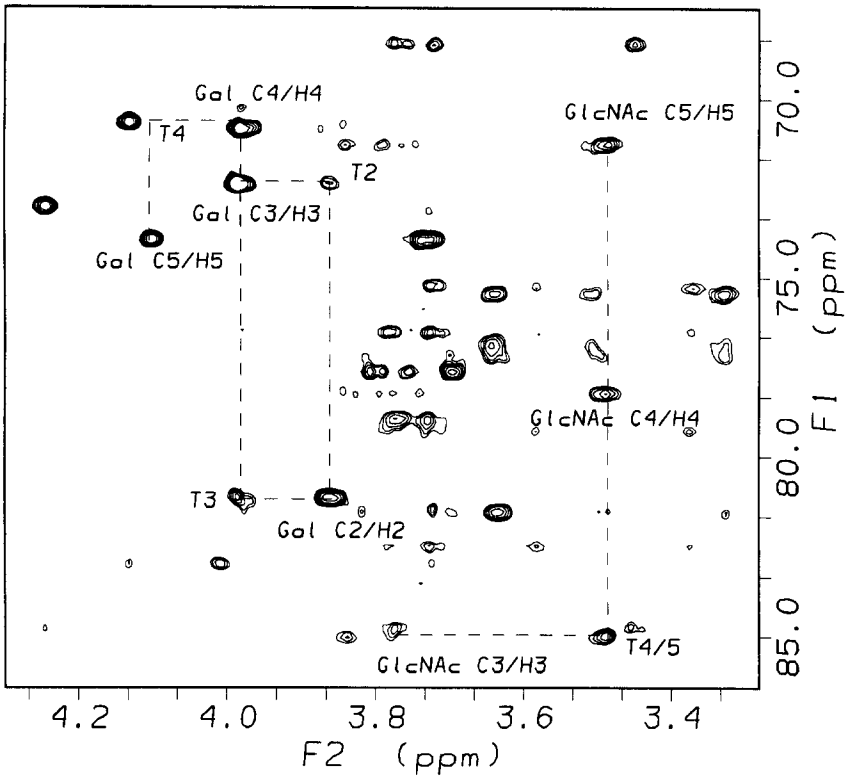

Fig. 5. 2D HMQC–TOCSY spectrum of *C. perfringens* Hobbs 5 polysaccharide with an isotropic mixing time of 110 ms. TOCSY subspectra starting from parent HMQC peaks were obtained for each of the six residues at appropriate contour levels. The data for gal and glcNAc are traced in the figure. TOCSY cross peaks are indicated by T.

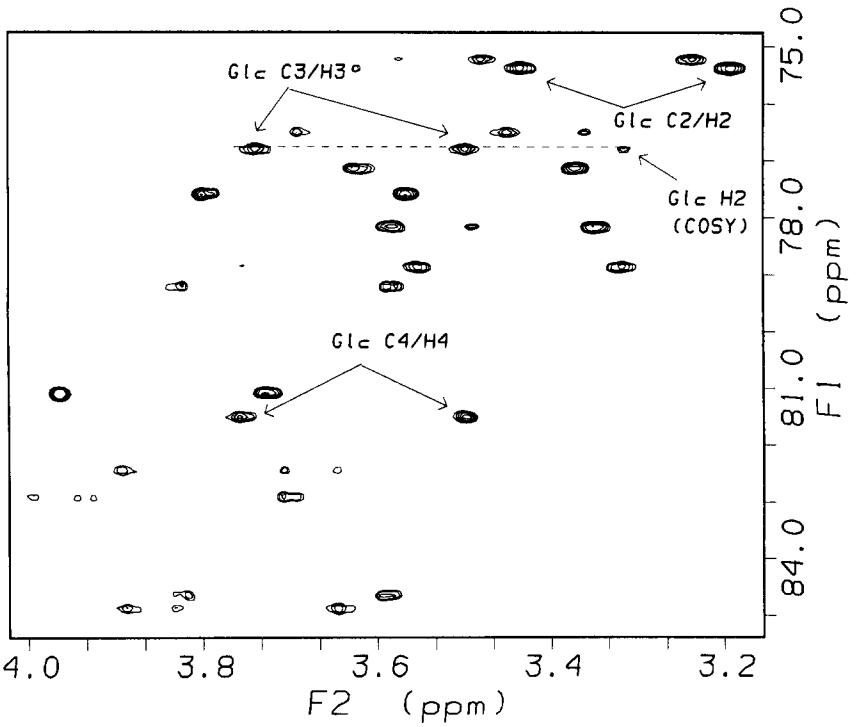

Fig. 6. Expansion of the 2D HMQC–COSY spectrum of the *C. perfringens* Hobbs 5 polysaccharide (acquired without ^{13}C-decoupling during acquisition). It is illustrated how this spectrum helped distinguish between the C-3 and C-4 of the Glc residue.

^{13}C spectrum of the polysaccharide had been partially assigned by HMQC, we attempted to resolve the aforementioned remaining ambiguities in the ^{1}H NMR assignments of the Gal, GlcNAc and Glc residues by using relayed HMQC experiments. This entailed following the basic HMQC pulse sequence with either a COSY or a TOCSY step. The HMQC–COSY and HMQC–TOCSY spectra permitted the correlation of the assignments from the parent HMQC spectrum to the scalar-coupled 'relayed' protons (and carbons) adjacent in the structure in the same spectrum.

The HMQC–TOCSY spectrum (Fig. 5) revealed the glycosyl subspectra starting from each parent HMQC peak; at relatively short mixing times, the intensities of the cross peaks diminished as the number of bonds to the site of the parent HMQC peak increased. For example, starting from the parent Gal C-2/H-2 resonance at 81.26/3.85 ppm, a strong correlation at ~ 3.97 ppm was observed; this was in keeping with the observation (Fig. 3a) that Gal H-3 and H-4 were superimposed at this position. By following this proton frequency, we traced two different carbon resonances at 72.48 and 70.94 ppm that display the same cross-peak pattern as Gal C-2. The parent HMQC cross peak at 72.48 ppm gave the strongest correlation to the proton at 3.85 ppm (Gal H-2); therefore, the latter chemical shift (72.48 ppm) was identified as belonging to Gal C-3. Similarly, we traced the intensity pattern to identify Gal C-4/H-4 and Gal C-5/H-5, even though the cross peak originating from the Gal C-4/H-4 parent peak to the proton at 4.09 ppm (H-5) was very weak, and magnetization transfer apparently did not proceed to Gal H-6/6′. A similar procedure led to the identification of the H-4 and H-5 protons of the GlcNAc residue that were overlapped in the proton dimension (~ 3.47 ppm) but whose carbon signals were clearly separated in their chemical shifts (78.33 and 71.38 ppm, respectively) (data not shown). This illustrated how the chemical shift dispersion in the ^{13}C dimension was exploited to obtain unambiguous proof for all the HMQC assignments.

At this stage, the only remaining ambiguity was in the assignment of the C-3/H-3 and C-4/H-4 correla-

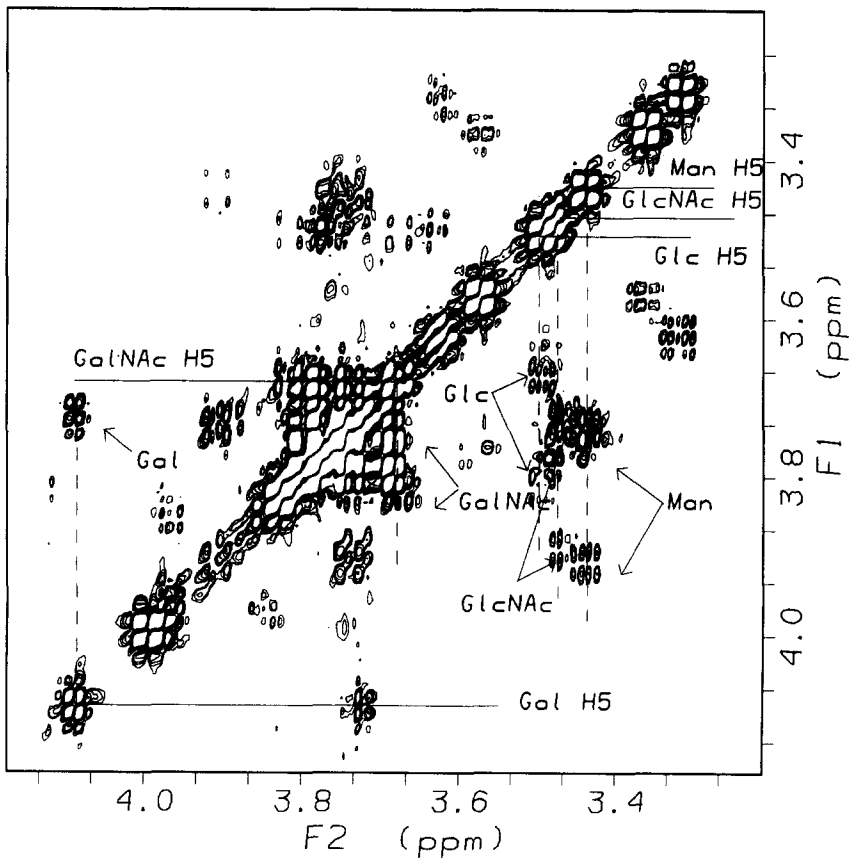

Fig. 7. Expansion of the 2D TQF–COSY spectrum of the *C. perfringens* Hobbs 5 polysaccharide recorded at 500 MHz and 57 °C. Arrows indicate H-5/H-6,6′ cross peaks (non-stereospecifically) for each residue. For the Gal residue, only one such correlation is seen. This implies that the chemical shifts of the H-6 and H-6′ protons for this residue coincide.

Table 1
^{1}H NMR chemical shifts [a] of the *C. perfringens* Hobbs 5 capsular polysaccharide measured at 57 °C, along with the coupling constants for the anomeric protons

	H-1	$^{3}J_{12}$	H-2	H-3	H-4	H-5	H-6/H-6′ [c]
Gal	5.378	3.5	3.850	3.972	3.963	4.090	3.72
Man	4.732	~ 1.5	4.236	3.705	3.759	3.434	3.75/3.92
GlcNAc [b]	4.718	8.6	3.819	3.764	3.471	3.475	3.72/3.89
GalNAc [b]	4.564	8.5	3.996	3.820	4.121	3.681	3.72/3.82
Glc	4.505	7.8	3.315	3.618	3.628	3.489	3.82/3.68
GlcA	4.476	7.8	3.359	3.564	3.767	3.706	–

[a] In ppm relative to internal DSS at δ 0.0; coupling constants $^{3}J_{12}$ in Hz.
[b] The GlcNAc and GalNAc *N*-acetyl methyl protons were observed at 2.016 and 2.008 ppm (not necessarily in that order).
[c] The H-6 and H-6′ proton signals of the Gal residue virtually coincide. The H-6/H-6′ assignments for the other glycosyl residues are non-stereospecific.

tions of the Glc residue. Although the corresponding carbons C-3 and C-4 were well separated (see HMQC spectrum, Fig. 4b), they could not be unambiguously assigned due to the exact overlap of the protons (3.63 ppm). We performed an HMQC–COSY experiment (Fig. 6) in order to discover the connectivity between one of the two parent HMQC cross peaks and the characteristic Glc H-2 multiplet at 3.32 ppm. Thus, starting from the parent HMQC peaks in question at 76.95/3.63 ppm and 81.65/3.63 ppm, we observed the COSY cross peak at 3.32 ppm (Glc H-2) only for the former, indicating that the signal at 76.95 ppm was the Glc C-3. This completed the assignment of the ^{1}H and ^{13}C spectra of the polysaccharide, with the exception of the H-6 and C-6 signals.

Protons H-6 and H-6′ for all residues were assigned non-stereospecifically by a TQF–COSY experiment; an expansion of the TQF-COSY spectrum is given in Fig. 7. Starting with the assignments for the H-5 protons, clear correlations were obtained to protons (H-6 and H-6′) for the Man, GlcNAc, Glc and GalNAc residues. Also, starting from the chemical shift of Gal H-5 at 4.09 ppm, we found that the chemical shift assignments of the Gal H-6/H-6′ coincided at 3.72 ppm. Finally, we assigned the C-6 carbon resonances for the various glycosyl residues from a GHSQC experiment based on the H-6/H-6′ proton assignments obtained from Fig. 7. This procedure was straightforward since the C-6 region of the ^{13}C spectrum (60–65 ppm) was well separated from the rest of the spectrum (70–110 ppm) (Fig. 1). The assignment of the C-6 signals was relatively straightforward (data not shown). Tables 1 and 2 contain the ^{1}H and ^{13}C chemical shifts for the capsular polysaccharide from *C. perfringens* Hobbs 5.

Configurations of the glycosidic linkages.—The values of the coupling constants ($^{3}J_{12}$) for the anomeric resonances (Table 1, column 2) were used to assign the configurations of each anomeric center. Values of less than 4 Hz were associated with an α-pyranosyl linkage (equatorial–axial orientation of H-1 and H-2), whereas values between 7 and 9 Hz were associated with β-pyranosyl linkages (both H-1 and H-2 in axial orientation) [37]. This reasoning could not be used for assigning the configuration of the Man residue. The Man H-1 signal at 4.73 ppm

Table 2
^{13}C NMR chemical shifts [a] of the *C. perfringens* Hobbs 5 capsular polysaccharide measured at 57 °C

	C-1	C-2	C-3	C-4	C-5	C-6
Gal	103.24	81.26	72.48	70.94	74.04	63.87
Man	102.82	73.12	84.88	68.51	79.03	63.51
GlcNAc [b]	105.40	57.50	85.08	78.33	71.38	63.30
GalNAc [b]	103.61	53.75	83.11	70.79	77.75	63.51
Glc	106.70	75.53	76.95	81.65	77.27	62.73
GlcA	105.72	75.34	76.71	82.62	79.39	176.64

[a] In ppm relative to internal DSS at δ 0.0.
[b] The GlcNAc and GalNAc carbonyl carbons were found to coincide at 177.51 ppm; their NAc methyl carbon signals appeared at 25.40 and 25.18 ppm (not necessarily in that order).

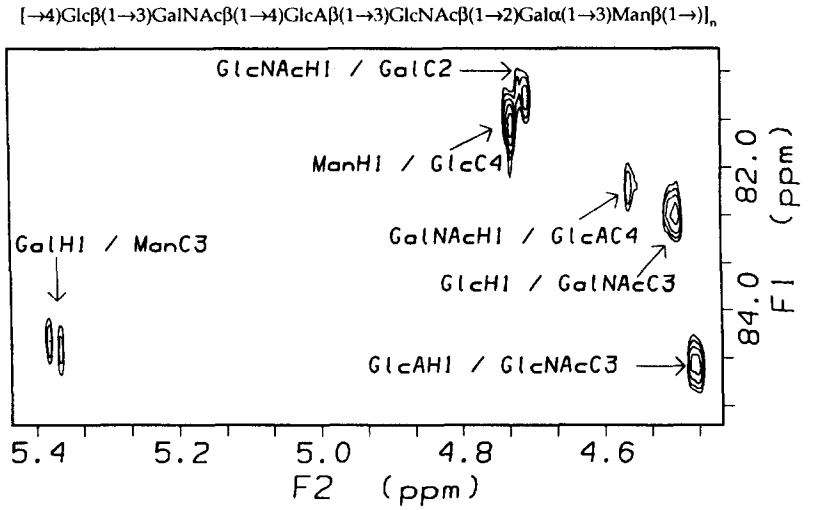

Fig. 8. Part of the {^{1}H, ^{13}C} multiple-bond correlation (HMBC) spectrum of the *C. perfringens* Hobbs 5 polysaccharide optimized for detection of a long-range coupling constant of 5 Hz. Starting from the anomeric proton resonance, the labels indicate the H-1 → C-x' cross-peaks for the linkage positions of the adjacent residue.

was characterized by a small $^3J_{12}$ (~ 1.5 Hz) due to the equatorial orientation of Man H-2. The anomeric configuration of the Man residue was ascertained from a ^{13}C-coupled 2D HSQC experiment (spectrum not shown) which revealed a $^1J_{CH}$ coupling constant of 162.3 Hz for Man H-1, indicating that Man occurs in the β configuration [38].

Sequencing of the glycosyl residues.—An HMBC spectrum of the polysaccharide (Fig. 8) revealed long range H-1 → C-x' and C-1 → H-x' connectivities for all six linkages. Specifically, the ^{13}C spectral region between 80.0 and 86.0 ppm contained only the six resonances for the C-x' carbons involved in glycosidic linkages. These carbon resonances had been assigned by HMQC, HMQC–COSY and HMQC–TOCSY experiments (see Table 2). This resulted in the assignment of the linkage positions for the various glycosyl residues. Thus, we deduced the complete sequence of the repeat unit of the Hobbs 5 polysaccharide from this portion of the HMBC spectrum.

The linkage positions were independently confirmed from an HMQC–NOESY spectrum of the polysaccharide (data not shown). For example, in the case of the linkage between the Man and Glc residues, the Man anomeric H-1/C-1 peak and the parent HMQC peak of Glc H-4/C-4 were found connected by symmetric cross peaks arising from the NOE interaction between Man H-1 and Glc H-4 on opposite sides of the Man → Glc linkage. A simple 2D NOESY spectrum would not have provided this information, because of the coincidence of the Glc H-3 and H-4 signals.

4. Conclusions

The primary structure of the *C. perfringens* Hobbs 5 capsular polysaccharide was determined by NMR spectroscopy to be the following:

[→4)Glc*p*β(1→3)Gal*p*NAcβ(1→4)-
Glc*p*Aβ(1→3)Glc*p*NAcβ(1→2)-
Gal*p*α(1→3)Man*p*β(1→)]$_n$

In this study we deduced for each residue present in the *C. perfringens* Hobbs 5 polysaccharide, its percent composition, its linkage position, its anomeric configuration, and its placement in the repeating sequence. This was accomplished by studying the native polysaccharide. This approach avoided the usual pitfalls (such as incomplete acid hydrolysis, differential loss of certain residues over others, nonspecific cleavage sites by acetolysis, low yields by partial acid hydrolysis, etc.) encountered in the study of complex polysaccharides by classical methods of structural analysis. The elucidation of the primary structure of this polysaccharide should generate interest in studying the antigenicity of the capsule of *C. perfringens*. This work will serve as the basis for the comparative analysis of the capsular polysaccharides from other serotypes of *C. perfringens*.

Acknowledgements

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