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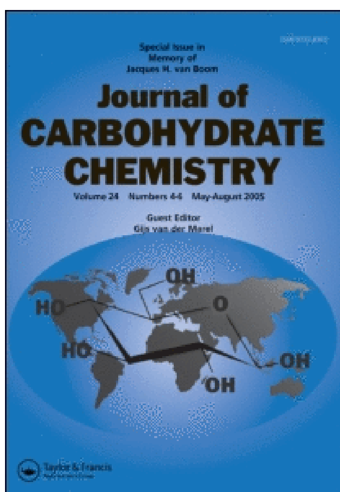

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Structure of the Type VI Group B *Streptococcus* Capsular Polysaccharide Determined by High Resolution NMR Spectroscopy

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STRUCTURE OF THE TYPE VI GROUP B *STREPTOCOCCUS* CAPSULAR POLYSACCHARIDE DETERMINED BY HIGH RESOLUTION NMR SPECTROSCOPY

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ABSTRACT

The structure of the capsular polysaccharide of the recently characterized type VI Group B *Streptococcus* (GBS) has been established using one- and two-dimensional homo- and heterocorrelated ^{1}H and ^{13}C NMR spectroscopy. The native polysaccharide contains D-glucose, D-galactose and sialic acid in the molar ratio 2:2:1. The structure of its repeating unit is:

→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*-(1→
3
↑
1
β-D-Gal*p*
3
↑
2
α-Neu5Ac

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INTRODUCTION

Group B *Streptococcus* (GBS) is a major cause of neonatal sepsis and meningitis.[1,2] GBS is classified into serotypes on the basis of type-specific capsular polysaccharides and strains isolated from infected infants can usually be classified into one of four major capsular types Ia, Ib, II and III[2] the capsular structures of which have been established.[3] Later it was found that many previously isolated non-typable strains also have polysaccharide capsules distinct from those of the four major serotypes. Additional serotypes IV and V were isolated[4] and the structure and immunochemical properties of their capsular polysaccharides elucidated.[5,6] More recently another serotype (VI) was reported and its pathogenicity and immunochemical properties characterized.[7] The type VI polysaccharide contained terminal sialic acid residues like all other reported GBS serotypes whereas it did not contain *N*-acetylglucosamine.[7] We now report the structure of the type VI polysaccharide.

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RESULTS AND DISCUSSION

It was shown previously that the type VI GBS capsular polysaccharide was composed of glucose, galactose and *N*-acetylneuraminic acid in the molar ratio 2:2:1.[7] In support of these findings, the ^{1}H NMR spectrum of the polysaccharide contained four signals associated with the anomeric protons of the constituent hexoses at 4.76 ppm ($J_{1,2}$= 7.7 Hz), 4.71 ppm ($J_{1,2}$= 7.9 Hz), 4.52 ppm ($J_{1,2}$= 8.1 Hz) and 4.51 ppm ($J_{1,2}$= 8.5 Hz), as well as signals at 2.75 and 1.81 ppm characteristic for the equatorial and axial H-3 of the α-*N*-acetylneuraminic acid. These data indicated that type VI GBS polysaccharide was composed of pentasaccharide repeating units consisting of four hexopyranoses having the β-glycosidic configuration and α-*N*-acetylneuraminic acid.

In order to facilitate structural elucidation, the type VI GBS capsular polysaccharide was desialylated by means of mild acidic hydrolysis prior to its analysis by high resolution NMR spectroscopy. Sialic acid cleaved upon hydrolysis was separated from the core polysaccharide by dialysis. The resulting core polysaccharide contained equimolar amounts of D-glucose and D-galactose. Its ^{1}H NMR spectrum contained, in agreement with the composition, four signals associated with anomeric protons at 4.72

ppm ($J_{1,2}$= 8.2 Hz), 4.69 ppm ($J_{1,2}$= 9 Hz), 4.53 ppm ($J_{1,2}$= 8.3 Hz), and 4.51 ppm ($J_{1,2}$= 9.2 Hz). Large values of the $J_{1,2}$ coupling constants indicated that all sugar units had a β-glycosidic configuration.

The hexose components of the repeating unit of the core polysaccharide were designated **a**, **b**, **c** and **d** according to the sequence of their anomeric proton signals in the ^{1}H NMR spectrum (Table 1). 2D COSY and 2D TOCSY experiments enabled the assignment of the other proton signals, thus allowing the identification of the corresponding monosaccharide residues. The low-field location of the H-4 resonance of units **b** and **d** (H-4**b** and H-4**d**) at 3.93 and 4.20 ppm, respectively together with small values of the respective $J_{3,4}$ coupling constants (~3 Hz) characterized these units as having the β-D-galactopyranosyl configuration. Consequently, units **a** and **c** should have the β-D-glucopyranosyl configuration, which was confirmed by the characteristic[13] pseudo-triplet shape of their H-3 signals due to the equal $J_{2,3}$ and $J_{3,4}$ coupling constants (≈ 9 Hz) located at 3.79 ppm (unit **a**) and 3.65 ppm (unit **c**). In order to provide unambiguous assignment of signals in the highly overlapped region of the proton spectrum between 3.6 - 3.9 ppm, TOCSY and NOESY experiments were carried out. NOESY spectra provided additional proof of the β-glycosidic configuration of the monosaccharide units since all anomeric proton signals gave intensive cross-peaks with the respective H-3 and H-5 signals. Complete assignment of the signals in the ^{1}H NMR spectrum of the core polysaccharide is given in Table 1.

The sequence of the sugar residues in the repeating unit of the core polysaccharide was established on the basis of the observed interresidual cross-signals in the NOESY spectrum. It was observed that H-1**a** gave an intense interresidual cross-peak with H-3**d** and a low intensity cross-peak with H-4**d**. H-1**b** gave a cross-peak with H-3**a** while H-1**c** gave cross-peaks with both H-6 and H-6' of unit **a**, and finally H-1**d** gave a cross-peak with H-4**c**. On the basis of these results, the following structure of the repeating unit of the core polysaccharide was proposed

```
          c                a                d
→4)-β-D-Glcp-(1→6)-β-D-Glcp-(1→3)-β-D-Galp-(1→
                           3
                           ↑
                         b 1
                         β-D-Galp
```

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TABLE 1. ^{1}H NMR chemical shift data for the type VI GBS polysaccharide (PS) and desialylated core polysaccharide (Core)

Unit	H-1	H-2	H-3	H-4	H-5	H-6
PS **a'**	4.71	3.59	3.81	3.64	3.64	4.21, 3.88
Core **a**	4.72	3.59	3.79	3.65	3.65	4.21, 3.89
PS **b'**	4.76	3.62	4.12	3.95	3.70	3.74, 3.71
Core **b**	4.69	3.59	3.69	3.92	3.72	3.79, 3.76
PS **c'**	4.52	3.34	3.64	3.65	3.59	3.97, 3.81
Core **c**	4.53	3.35	3.65	3.67	3.60	3.99, 3.81
PS **d'**	4.51	3.69	3.83	4.19	3.73	3.79, 3.74
Core **d**	4.51	3.71	3.84	4.20	3.74	3.79, 3.76
PS **e'**			1.81(ax) 2.75(eq)	3.68	3.85	3.62

Additional Neu5Ac signals: NAc 2.05, H-7 3.60, H8 3.88, H9 3.87, and H9' 3.64 ppm.

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The ^{13}C NMR spectrum of the core polysaccharide contained four signals in the anomeric region confirming that it was composed of tetrasaccharide repeating units. Complete assignment of the ^{13}C NMR signals was carried out using HMQC and HMQC-TOCSY techniques and the observed chemical shifts are listed in Table 2. The interglycosidic connectivities suggested by the NOESY experiment have been fully confirmed by an independent HMBC experiment that provided correlations between H-1**a** and C-3**d**, H-1**b** and C-3**a**, H-1**c** and C-6**a**, and H-1**d** and C-4**c**.

The ^{1}H NMR spectrum of the native sialylated type VI GBS polysaccharide showed in addition to the signals observed in the spectrum of the core polysaccharide, the signals at 1.81 and 2.75 ppm due to the axial and equatorial H-3 protons of sialic acid as well as the signal at 2.05 ppm integrating for three protons due to its *N*-acetyl group. Complete assignment of ^{1}H NMR signals of the native type VI GBS polysaccharide was performed using the above mentioned standard two-dimensional methods as well as one-dimensional analogs of the 3D NOESY-TOCSY, 3D TOCSY-

TABLE 2. ^{13}C NMR chemical shift data for the type VI GBS (PS) and its desialylated derivative (Core)

Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
PS **a'**	104.30	73.70	84.74	68.70	75.13	69.52
Core **a**	104.37	73.84	84.82	68.78	75.18	69.62
PS **b'**	103.80	70.53	76.37	68.35	75.88	61.85
Core **b**	104.16	72.10	73.43	69.45	76.18	61.94
PS **c'**	103.70	73.70	75.13	79.04	75.60	60.87
Core **c**	103.73	73.66	75.18	79.07	75.62	60.92
PS **d'**	103.40	70.90	82.98	69.10	75.88	61.85
Core **d**	103.43	70.99	82.92	69.08	75.88	61.94
PS **e'**	174.70	100.70	40.42	69.10	52.53	73.70

Additional Neu5Ac signals: NAc(CO) 175.90, NAc(CH_3) 22.95, C7 68.90, C8 72.66, and C9 63.41 ppm.

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NOESY, and 4D TOCSY-NOESY-TOCSY experiments as described by Uhrín *et al.*[12] Assignment of ^{13}C NMR signals was carried out using HMQC and HMBC techniques. ^{1}H and ^{13}C NMR chemical shifts of the type VI GBS polysaccharide are presented in Tables 1 and 2.

Sialylation caused a downfield shift of the anomeric proton signal of the galactosyl residue **b** of the core polysaccharide. Thus, for the native polysaccharide, the H-1 resonance of the side-chain galactose (unit **b'**) precedes the H-1 signal of the branching-point glucose (unit **a'**). The positions of the anomeric signals of the backbone glucose and galactose were not affected by sialylation and they were designated **c'** and **d'**, respectively. The *N*-acetylneuraminic acid residue was designated **e'**.

Sialylation caused a strong downfield shift of the resonance of H-3**b'** from 3.68 ppm in the core polysaccharide to 4.12 ppm in the native polysaccharide. A similar effect was observed also on the C-3**b'** resonance (73.43 versus 76.37 ppm). On the basis of these observations, it was concluded that in type VI GBS polysaccharide, sialic acid

is linked at position O-3 of the side-chain galactose. Thus, the structure of the repeating unit of the native type VI GBS polysaccharide is:

```
           c'                 a'               d'
→4)-β-D-Glcp-(1→6)-β-D-Glcp-(1→3)-β-D-Galp-(1→
                               3
                               ↑
                           b'  1
                            β-D-Galp
                               3
                               ↑
                           e'  2
                           α-Neu5Ac
```

The position of sialylation was independently confirmed by observation of the NOE cross-peak between H-3ax of the sialic acid and H-3**b'** as well as by a cross-peak between C-2**e'** and H-3**b'** in the HMBC spectrum. The structure of the native type VI GBS polysaccharide closely resembles that of the type III GBS capsular polysaccharide,[3] the only difference being that the α-Neu5Ac-(2→3)-β-D-Gal side-chain in type III GBS polysaccharide is linked to O-4 of β-D-GlcNAc.

Despite this high degree of structural similarity, the type VI GBS polysaccharide did not cross-react with the serum prepared against type III GBS,[7] thus demonstrating that terminal sialic acid is non-immunodominant. However, further immunological experiments in which the desialylated type VI polysaccharide was shown to bind very poorly to native type VI polysaccharide-specific antibody,[7] indicated that even though non-immunodominant in the classical sense, sialic acid is important to the formation of the native type VI epitope. This has also been reported for other type-specific GBS polysaccharides and has been attributed to the presence of sialic acid-controlled conformational epitopes.[14-16]

MATERIALS AND METHODS

Materials: The origin of the type VI GBS strain as well as the procedures for extraction and purification of its capsular polysaccharide have been previously described together with the physico-chemical characterization and monosaccharide composition of the polysaccharide.[7]

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Methods: Determination of the absolute configuration of the constituent glycoses was performed by a modification of a method described by Leontein *et al.*,[8] in which the (-)-2-butyl glycosides of the glycoses were trimethylsilylated and analysed by capillary GLC using a Varian Saturn II GC-MS instrument equipped with a DB-17 capillary column (0.25 mm x 30 m, film thickness 0.25 μm) in the temperature program 150 °C to 210 °C at 2 °C/min.

The desialylated core polysaccharide was prepared by 1% acetic acid hydrolysis of the native polysaccharide for 1 h at 80 °C followed by dialysis and lyophilization.

Instrumental: The NMR experiments were performed on a Bruker AMX 600 spectrometer at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR). A 5 mm broad-band probe with ^{1}H coil nearest to the sample was used. The native and desialylated polysaccharides were run as D_2O solutions at concentrations of 5mg/mL and all spectra were recorded at 295 K in D_2O at pH 7.0. The methyl resonance of internal acetone was used as reference (31.07 ppm for ^{13}C and 2.225 ppm for ^{1}H). The experiments were carried out without sample spinning. Two-dimensional homo- and heterocorrelated experiments (COSY, TOCSY, NOESY, HMQC) were carried out as described previously.[9] In NOESY experiments, a 100 ms mixing time was used. HMQC-TOCSY was performed according to Lerner and Bax[10] and ^{1}H-detected multiple-bond correlation (HMBC) experiments were performed essentially by the method of Bax and Summers,[11] as previously decribed.[9] One-dimensional analogs of 3D NOESY-TOCSY, 3D TOCSY-NOESY, and 4D TOCSY-NOESY-TOCSY experiments were performed as described by Uhrín *et al.*[12]

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REFERENCES

1. C.J. Baker and M.S. Edwards, *Rev. N.Y. Acad. Sci.* **459**, 193 (1988).

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2. P. Ferrieri, *Rev. Infect. Dis.* **12**, S394 (1990).

3. H.J. Jennings, *Curr. Top. Microbiol. Immunol.* **150**, 97 (1990).

4. J. Jelínková and J. Motlová, *J. Clin. Microbiol.* **21**, 361 (1985).

5. J.L. DiFabio, F. Michon, J.-R. Brisson, H.J. Jennings, M.R. Wessels, V.-J. Benedí and D.L. Kasper, *Can. J. Chem.* **67**, 877 (1989).

6. M.R. Wessels, J.L. DiFabio, V.-J. Benedí, D.L. Kasper, F. Michon, J.-R. Brisson, J. Jelínková and H.J. Jennings, *J. Biol. Chem.* **266**, 6714 (1991).

7. C. von Hunolstein, S. D'Ascenzi, B. Wagner, J. Jelínková, G. Alfarone, S. Recchia, M. Wagner and G. Orefici, *Infect. Immun.* **61**, 1272 (1993).

8. K. Leontein, B. Lindberg and J. Lönngren, *Carbohydr. Res.* **62**, 359 (1978).

9. H. Baumann, A.O. Tzianabos, J.-R. Brisson, D.L. Kasper and H.J. Jennings, *Biochemistry*, **31**, 4081 (1992).

10. L. Lerner and A. Bax, *J. Magn. Reson.* **69**, 378 (1986).

11. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).

12. D. Uhrín, J.-R. Brisson, G. Kogan and H.J. Jennings, *J. Magn. Reson.* (in press).

13. G.P. Reddy, C.-C. Chang and C.A. Bush, *Anal. Chem.* **65**, 913 (1993).

14. H.J. Jennings, C. Lugowski and D.L. Kasper, *Biochemistry*, **20**, 4511 (1981).

15. H.J. Jennings, E. Katzenellenbogen, C. Lugowski, F. Michon, R. Roy and D.L. Kasper, *Pure Appl. Chem.* **56**, 893 (1984).

16. M.R. Wessels, A. Muñoz and D.L. Kasper, *Proc. Natl. Acad. Sci. USA*, **84**, 9170 (1987).

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