Carbohydrate Structure

A Conformational Carbohydrate Scaffold is Present in the Short-Chain Lipopolysaccharides of *Moraxella catarrhalis***

*Kristina Lycknert, Per Edebrink, and Göran Widmalm**

Moraxella catarrhalis is a pathogenic bacterium that causes otitis media and sinusitis in children. The lipopolysaccharides (LPS) of *M. catarrhalis* do not carry long polysaccharides with repeating units, but instead the O-antigen consists of

[*] Dr. K. Lycknert,[+] Dr. P. Edebrink,[+] Prof. Dr. G. Widmalm
Department of Organic Chemistry
Arrhenius Laboratory
Stockholm University
106 91 Stockholm (Sweden)
Fax: (+46) 815-4908
E-mail: gw@organ.su.se

[+] Present address:
AstraZeneca R&D Södertälje
151 85 Södertälje (Sweden).

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branched oligosaccharides. The three major serotypes A, B, and C account for approximately 95% of clinical isolates. The O-antigen structures of these three serotypes have recently been determined as well as reviewed.[1] The inner part of the LPS consists of →6)-β-D-Glc*p*N-(1→6)-β-D-Glc*p*N with phosphate groups at C1 and C4′ as well as acyl groups at C2, C3, C2′, and C3′.[2] In all the serotypes hitherto investigated this disaccharide is linked to a Kdo residue, which in turn is substituted at position 4 by another Kdo residue and at position 5 by short-chain O-antigenic polysaccharides. Thus, the inner part of the LPS consists of four sugar residues (denoted R^1). In all subsequent discussions the oligosaccharides will be referred to by the number of sugar residues belonging to the oligosaccharide moiety substituting position 5 of the Kdo residue. The oligosaccharide part of serotype A consists of eight sugar residues and is shown in Figure 1. Oligosaccharides of different sizes up to octasaccharides have been synthesized in which the R^1 group was chosen as a handle for conjugation to a protein carrier.[3] Chain elongations and changes of sugar residues constitute the differences between serotypes B and C and A. We herein describe the analysis of different oligosaccharides by NMR spectroscopy which has led to the proposal that a conformational carbohydrate scaffold is present as a novel motif in the LPS from *M. catarrhalis*.

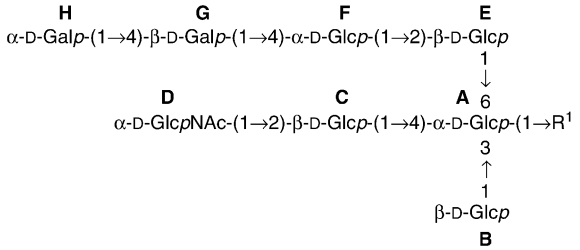

Figure 1. Structure of the oligosaccharide section that characterizes serotype A of the LPS from *M. catarrhalis*. In the native material the R^1 group represents lipid A and Kdo residues whereas in the synthetic oligosaccharides R^1 equals a 2-(4-trifluoroacetamidophenyl)ethyl group. The sugar residues are denoted by letters **A**–**H**. Serotype C is characterized by further chain elongation at position 4 of the *N*-acetylglucosamine residue. In serotype B the latter sugar is glucose.

Structural studies of the three serotypes showed anomalous ^{1}H NMR chemical shifts for certain sugar residues, and thus indicated that the three-dimensional structure of these oligosaccharides was of special interest. We first consider the oligosaccharides obtained by synthesis. The pentasaccharide is denoted OS-V, in which the sugar residues are **A**–**E**. The larger oligosaccharides are then designated OS-VI (residues **A**–**F**), OS-VII (residues **A**–**G**), and OS-VIII (residues **A**–**H**). The ^{1}H and ^{13}C chemical shifts observed in the NMR spectra of the oligosaccharides in D_2O at 25 °C were assigned by using 2D NMR techniques. Selected ^{1}H chemical shifts of the anomeric proton resonances as well as key resonances from residue **A** are plotted in Figure 2 as a function of oligosaccharide size. It is evident that significant changes in the chemical shift occur on going from OS-V to OS-VI, whereas addition of further sugar residues to the (1→6) branch does not lead to any notable changes. Thus, these results indicate that a conformational change occurs as a result of the addition of a single sugar residue to OS-V. The hexasaccharide consists of two sugar residues each at the (1→4) and (1→6) branches (Figure 3), and the above results suggest that this is the minimum size required to generate a characteristic three-dimensional structure of the *M. catarrhalis* oligosaccharides.

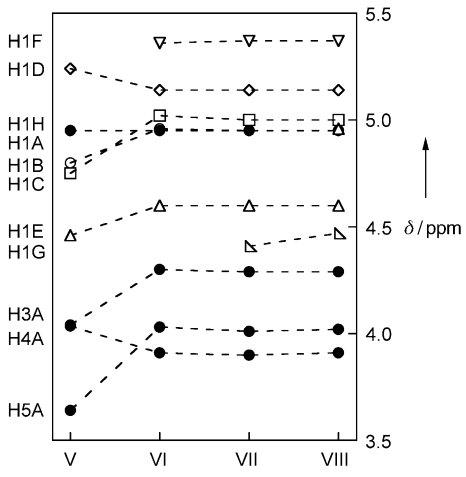

Figure 2. ^{1}H NMR chemical shifts (right axis) of selected protons (left axis) as a function of oligosaccharide size (OS-V to OS-VIII).

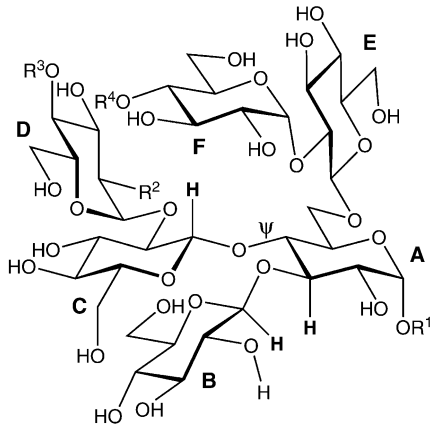

Figure 3. Schematic representation of the oligosaccharide structures of serotypes A–C of *M. catarrhalis*. R^1 = see Figure 1; R^2 = NHAc in serotypes A and C, whereas R^2 = OH in serotype B; R^3 = H in serotype A, whereas R^3 = galactosyl residues in serotypes B and C; R^4 = H or galactosyl residue(s). Key protons are indicated in bold (see Figure 4).

We now turn to the analysis of the oligosaccharides isolated during the structural studies. Whereas serotype A consists of one major octasaccharide (referring to the O-antigenic hexosyl region), both serotypes B and C consist of several glycoforms. In serotype B, mixtures with short-chain oligosaccharides were present that contained five to ten sugar residues as well as the Kdo residue at the reducing end.[4] Of particular interest is the fact that among the truncated structures, an oligosaccharide corresponding to a hexose containing a hexasaccharide unit with two sugar residues each at the (1→4) and (1→6) branches was present, that is, it

contained the terminal sugars corresponding to residues **D** and **F**. In addition, a pentasaccharide unit devoid of sugar residue **D** was also present in the mixture. Notably, significant changes in the ^{1}H chemical shifts of these two oligosaccharides were observed and characteristic ROE interactions were present in the 2D ^{1}H,^{1}H ROESY spectrum of the oligosaccharide mixture. In particular, a ROE interaction between H1 of residue **C** and H4 of **A** was present in the smaller oligosaccharide, whereas in the larger oligosaccharide H1 of residue **C** showed a pronounced ROE to H3 of **A**.[4] Similar NOE patterns were also observed for oligosaccharides OS-V to OS-VIII, with the latter compound showing NOE interactions from H1C to H3A and H1B to H3A (Figure 4). The

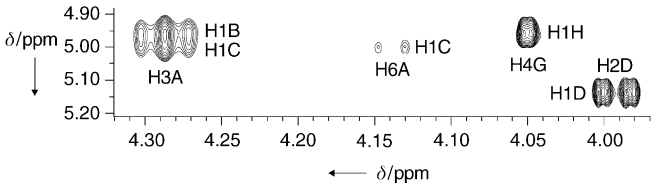

Figure 4. Selected region of a ^{1}H,^{1}H NOESY spectrum of OS-VIII in D_2O at 25 °C recorded at 14.1 T with a mixing time of 300 ms. The internuclear correlations of the anomeric protons in this region are annotated.

corresponding NOE interactions were observed in the oligosaccharide isolated from serotype A,[5,6] which indicated a folded conformation, that is, $\psi = \text{ca. } 180°$ at the (1→4) linkage.

From the above data obtained on oligosaccharides corresponding to different serotypes with different chain lengths we conclude that a minimum chain length of two sugar residues are necessary to obtain a specific three-dimensional structure, since in the absence of one sugar residue in either chain the altered conformation which displays large changes in the chemical shift is not present. The six hexose residues, therefore, make up a conformational scaffold that is needed to induce the proper three-dimensional structure for the *M. catarrhalis* LPS. The presence of additional sugar residues and different monosaccharides within the chains serve to make up the different serotypes of *M. catarrhalis*. These findings reveal a novel motif of oligosaccharide structures, and we propose that this is the structural basis for short-chain oligosaccharides in *M. catarrhalis*. Conformational epitopes and other conformational preferences have recently been discussed in the literature.[7,8] Our findings add to the understanding of the diverse plethora of possibilities explored by organisms in regard to carbohydrate structure and modifications thereof.

Future studies should aim to define the three-dimensional structure of the epitopes in detail, analyze the interactions of the oligosaccharides with monoclonal antibodies raised against the different serotypes, investigate the importance of the β-(1→3)-linked glucosyl residue on conformational preferences in the various oligosaccharides, and clarify the dynamics in these systems. Furthermore, it should be possible to produce a protective synthetic carbohydrate–protein-conjugate vaccine based on the common structural parts of the different serotypes in *M. catarrhalis*.

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Keywords: carbohydrates · conformation analysis · lipopolysaccharides · NMR spectroscopy · structure elucidation

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