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Cross-protection in *Neisseria meningitidis* serogroups Y and W polysaccharides: A comparative conformational analysis

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ABSTRACT

The capsular polysaccharide is the main virulence factor in meningococcus. The capsular polysaccharides for meningococcal serogroups Y and W are almost identical polymers of hexose-sialic acid, suggesting the possibility of cross-protection between group Y and W vaccines. However, early studies indicated that they elicit different levels of cross-protection. Here we explore the conformations of the meningococcal Y and W polysaccharides with molecular dynamics simulations of three repeating unit oligosaccharide strands. We find differences in Y and W antigen conformation: the Y polysaccharide has a single dominant conformation, whereas W exhibits a family of conformations including the Y conformation. This result is supported by our NMR NOESY analysis, which indicates key close contacts for W that are not present in Y. These conformational differences provide an explanation for the different levels of cross-protection measured for the Y and W monovalent vaccines and the high group W responses observed in HibMenCY-TT vaccinees.

1. Introduction

Infections by *Neisseria meningitidis* cause meningitis, bacteremia, and pneumonia and may result in permanent damage and death. Humans are the only host and the bacteria are easily transmitted by respiratory droplets, leading to outbreaks and epidemics. Five of the twelve meningococcal serogroups (A, B, C, Y, and W) are responsible for the vast majority of disease in children and adults, although recently serogroup X has caused seasonal outbreaks in Africa [1,2]. The non-specific symptoms, rapid onset and increasing antibiotic resistance make vaccination the most cost-effective way to control meningococcal disease.

The capsular polysaccharide (CP) is the main virulence factor in meningococcus: immunity to infection is conferred by antibodies to the CP. The first successful vaccine against meningococcal disease, developed in the 1970s, was a divalent A, C polysaccharide vaccine [3,4]. An increase in disease due to groups Y and W led to the introduction of tetravalent A, C, Y, W polysaccharide vaccines in 1982 [5,6]. Today the polysaccharide vaccines are being largely replaced by the more immunogenic tetravalent meningococcal conjugate vaccines that are also effective in young children (Menactra, Menveo and Nimenrix) [7]. The emergence of serogroup X has led to the development of a pentavalent conjugate vaccine (MCV-5) against groups A, C, Y, W and X [8].

Structurally, CPs of the six meningococcal serogroups fall into three pairs: the CPs for A and X are phosphodiester-containing homopolymers of amino sugars, B and C are homopolymers of sialic acid, whereas Y and W are almost identical polymers of hexose-sialic acid, as listed below.

A:	→ 6)-α-D-Man*p*NAc(3/4OAc)-(1→ OPO_3→
X:	→ 4)-α-D-Glc*p*NAc-(1→ OPO_3→
B:	→ 8)-α-D-Neu*p*NAc-(2→
C:	→ 9)-α-D-Neu*p*NAc(7/8OAc)-(2→
Y:	→ 6)-α-D-**Glc***p*-(1→ 4)-α-D-Neu*p*NAc(7/9OAc)-(2→
W:	→ 6)-α-D-**Gal***p*-(1→ 4)-α-D-Neu*p*NAc(7/9OAc)-(2→

The CP repeating units (RUs) of Y and W differ only in the stereochemistry of the C-4 hydroxyl group: equatorial in group Y (glucose) and axial in group W (galactose). This structural similarity of the antigens suggests the possibility of cross-protection between group Y and W vaccines, as reported between some structurally similar pneumococcal serotypes [9], but the phenomenon has not

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been evaluated because group Y and W vaccines were licensed together as part of the tetravalent polysaccharide vaccine. However, during the development of this vaccine, a small scale clinical trial tested monovalent and divalent polysaccharide Y and W vaccine formulations [10]. The study was performed in adult volunteers and monitored reactogenicity, binding, and bactericidal antibody formation. Both the group Y and W monovalent polysaccharide vaccines were able to elicit a strong immune response against their respective antigens as measured by bactericidal antibody after 4 weeks (100% responders to the group Y vaccine and 90% responders to the group W vaccine). The divalent vaccine, as expected, elicited bactericidal antibody against both serogroups. Interestingly, the monovalent vaccines elicited different levels of cross-protection. While 71% of volunteers who received the group Y vaccine had bactericidal antibody against group W bacteria after 4 weeks, only 30% of volunteers receiving the group W vaccine had bactericidal antibody against group Y bacteria after the same period. Further, possible cross-protection by the group Y vaccine against group W was suggested by a recent trial of a HibMenCY-TT conjugate vaccine. The vaccinees showed markedly higher seroprotection rates and antibody titre to group W compared to the control group, even though neither group had previously received the group W antigen [11]. The authors noted that the lack of response in controls indicates that the high group W responses observed in the HibMenCY-TT vaccinee group were not the result of natural immunity or a lack of assay sensitivity.

It has been generally assumed that the similarity in RU for the Y and W CPs would result in very similar molecular conformations. This assumption is supported by the only modelling study performed to date on the polysaccharides: Moore et al. built a single model for each of Y and W, using a simple minimization strategy in vacuum and predicted similar regular helical conformations for the Y and W polysaccharides (four residues per turn) [12]. However, this molecular mechanical approach does not reflect the fact that, in aqueous solution, polysaccharides are dynamic molecules that may explore a range of conformations. Further, C-4 stereochemistry is known to have a marked affect on the orientation and dynamics of the hydroxymethyl group in galacose (axial) and glucose (equatorial), which has a direct bearing on the $\alpha(2\rightarrow6)$ linkage in the Y and W polysaccharides. The primary alcohol in both glucose and galactose is in an equilibrium between three staggered rotameric conformations, termed gauche-trans (*gt*, $\omega\approx+60^\circ$), gauche-gauche (*gg*, $\omega\approx-60^\circ$), and trans-gauche (*tg*, $\omega\approx 180^\circ$), where the first letter refers to the trans or gauche orientation of the ω dihedral (*O5-C5-C6-O6*) and the second letter the orientation of the ω' dihedral (*C4-C5-C6-O6*). However, the relative populations of the rotamers differ significantly: glucose favours the *gg* conformation, galactose the *gt* conformation and the overall *gg*:*gt*:*tg* population rankings are estimated to be approximately 6:4:0 for glucopyranosides and 2:6:2 for galactopyranosides [13–15]. Extrapolation of these differing populations to the $\alpha(2\rightarrow6)$ linkages in Y and W indicates the possibility of different conformations for these CPs that could account for the different levels of cross-protection observed.

Here we investigate the conformations of the meningococcal Y and W CPs with molecular dynamics simulations in aqueous solution. The Y and W polysaccharides are partly O-acetylated at O-7/O-9 and WHO specifications for the polysaccharide vaccine are > 14.3% per RU [16]. As in the previous modelling study, we do not consider the effects of O-acetylation on conformation in this investigation. This position is supported by pre-clinical and clinical studies that show that the de-O-acetylated epitope is the primary target for bactericidal antibodies and that O-acetyl groups may be used by the pathogen to mask these protective epitopes [17]. Further, a survey of meningococci isolated in the UK showed that only 8% of serogroup W isolates but 79% of serogroup Y were found to express O-acetylated capsules [18]. During the Hajj serogroup W outbreak in 2000, all strains were de-O-acetylated [19]. Furthermore, it is not technically feasible to perform modelling studies with low levels of O-acetylation present at two positions.

Our established systematic approach to modelling bacterial polysaccharides [20,21] involves first calculation of the potential of mean force (PMF) for the glycosidic linkages in representative disaccharide units (Table 1) to determine the preferred conformations, and then progresses to molecular dynamics simulations of 3RU oligosaccharides in solution to establish the preferred conformations and dynamics of the strands. 3RU has been postulated to be sufficient length for a strong antibody response: Moore et al. showed complete inhibition of antibody binding for 3RU in both Y and W, with NMR shifts for 3RU being comparable to longer chains [12]. In addition, in previous work we compared simulations of 3RU and 6RU for pneumococcal serotypes 19A and 19F and found no significant differences in the longer chain conformations for these very flexible oligosaccharide strands: the hydrodynamic behaviour of the longer 6RU strands was consistent with the corresponding 3RU simulations [21].

We perform experimental NMR NOESY measurements on Y and W CPs to corroborate our final predicted conformations.

2. Results and discussion

As saccharide rings are relatively rigid, the conformation of an oligosaccharide chain is determined chiefly by the orientations of the glycosidic linkages. Therefore, for our conformational analysis of the CPs in meningococcal serogroups Y and W, we first characterise the preferred conformations of the $\alpha(1\rightarrow4)$ and $\alpha(2\rightarrow6)$ linkages and then progress to the preferred conformations and dynamics of oligosaccharide chains.

2.1. The $\alpha(1\rightarrow4)$ linkages

The Glc14N and Gal14N disaccharides are simplest representation of the $\alpha(1\rightarrow4)$ linkages in the Y and W CPs respectively (Table 1). The ϕ,ψ PMF surfaces calculated in aqueous solution for Glc14N (Fig. 1a) and Gal14N (Fig. 1b) are, unsurprisingly, very similar. The sole difference between these disaccharides is in the hexose C-4 stereochemistry (Fig. 1c) which, because C-4 is situated on the opposite side of the ring to the glycosidic linkage, has little effect on the preferred conformations of Glc14N and Gal14N. In both PMF surfaces, the central syn-syn well contains the global minimum, at $\phi,\psi=-41^\circ,-34^\circ$ in Glc14N and $\phi,\psi=-46^\circ,-41^\circ$ in Gal14N. Both PMFs have a secondary syn-syn minimum at $\phi,\psi=-29^\circ,39^\circ$ in Glc14N ($\Delta G=2.4$ kcal/mol) and $\phi,\psi=-25^\circ,44^\circ$ in Gal14N ($\Delta G=2.4$ kcal/mol), as well as a tertiary low-energy anti-ψ minimum, at $\phi,\psi=-39^\circ,-166^\circ$ in Glc14N ($\Delta G=3.7$ kcal/mol) and $\phi,\psi=-41^\circ,176^\circ$ in Gal14N ($\Delta G=3.4$ kcal/mol). The tertiary minimum shows some difference between the disaccharides: it is lower in energy and somewhat broader in Gal14N as compared to Glc14N.

The ϕ,ψ time series plots for the each of the three $\alpha(1\rightarrow4)$

Table 1
Representative disaccharides and corresponding abbreviations for the Y and W meningococcal CPs.

Linkage	Abbreviation	CP
α-D-Glc*p*-(1→4)-α-D-Neu*p*NAc	Glc14N	Y
α-D-Gal*p*-(1→4)-α-D-Neu*p*NAc	Gal14N	W
α-D-Neu*p*NAc-(2→6)-α-D-Glc*p*	N26Glc	Y
α-D-Neu*p*NAc-(2→6)-α-D-Gal*p*	N26Gal	W