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Conformations of *Neisseria meningitidis* serogroup A and X polysaccharides: The effects of chain length and O-acetylation

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ABSTRACT

Neisseria meningitidis is a major cause of bacterial meningitis worldwide especially in Africa. The capsular polysaccharide (CPS) is the main virulence factor and the target antigen for polysaccharide and conjugate vaccines. The high burden of serogroup A disease in the Meningitis Belt of sub-Saharan Africa led to the introduction of MenAfriVac®, which has successfully reduced the number of cases of group A disease. However, several outbreaks caused by other serogroups have been reported, including those due to serogroup X. The capsular polysaccharides of serogroups A and X are both homopolymers of amino sugars (α-D-ManNAc and α-D-GlcNAc) containing phosphodiester linkages at C-6 and C-4, respectively. The similarity of the primary structures of the two polysaccharides suggests that serogroup A vaccination may provide cross-protection against serogroup X disease. Molecular dynamics simulations of a series of serogroup A and X oligosaccharides reveal that the MenA CPS behaves as a flexible random coil which becomes less conformationally defined as the length increases, whereas serogroup X forms a more stable regular helical structure. The presence of the MenX helix is supported by NMR analysis; it has four residues per turn and becomes more stable as the chain length increases. Licensed MenA vaccines are largely O-acetylated at C-3: simulations show that these O-acetyl groups are highly solvent exposed and their presence favors more extended conformations compared to the more compact conformations of MenA without O-acetylation. These findings may have implications for the design of optimal conjugate vaccines.

1. Introduction

The encapsulated bacterium *Neisseria meningitidis* is the causative agent of meningococcal disease, which results in meningitis, septicemia, and pneumonia. Humans are the only known host of *N. meningitidis*, which asymptomatically inhabits the nasopharynx of approximately 10% of the general population [1]. Meningococcus is easily spread through respiratory droplets; thereafter infection occurs if the bacteria cross the mucosal membranes into the blood stream [2]. If left untreated, meningococcal disease has a high mortality rate [3]. Meningitis epidemics occur regularly on the African continent in an area extending from Senegal to Ethiopia, termed “the Meningitis Belt” [4,5].

The bacterial capsular polysaccharide (CPS) of meningococcus is the main virulence factor and vaccination with the CPS is an effective method of limiting the spread of the disease [6]. The first successful vaccines against *N. meningitidis* infection were polysaccharide vaccines developed in the 1970s [7,8] which are being replaced by the more immunogenic polysaccharide-protein tetravalent conjugate vaccines (Menactra, Menveo, and Nimenrix) that are more effective in children [9].

The *N. meningitidis* CPS is used to categorize the bacteria into twelve serogroups, of which six (A, B, C, W, X and Y) cause virtually all meningococcal disease worldwide. Prior to vaccination, over 90% of meningococcal disease in the Meningitis Belt was caused by serogroup A (MenA). After introduction of a cost-effective MenA conjugate vaccine (MenAfriVac®, developed by the Meningitis Vaccine Project [10]) in 2010, MenA infections have been virtually eliminated in vaccinated populations [11–14]. However, recent outbreaks of serogroup X (MenX) disease [6,15] suggest the possibility of replacement of serogroup A by MenX [12–16]. This has prompted the development of a new pentavalent conjugate vaccine (NmCV-5) against serogroups A, C, Y, W, and X [17].

The CPS of the meningococcal serogroups fall into three structurally related pairs: CPSs of serogroup A and X are phosphodiester-linked homopolymers of hexose amino sugars, serogroup B and C are homopolymers of sialic acid, and serogroup Y and W are polymers of hexose-sialic acid, as listed below.

A: →6)-α-D-Man*p*NAc(3/4OAc)-(1→OPO_3 →

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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 focus of this study is on the MenA and MenX pair, which differ in their linkage position (4-linked in MenX and 6-linked in MenA) and are epimeric at the C-2 position: the N-acetyl group is axial in MenA (*manno*) and equatorial in MenX (*gluco*). Furthermore, the MenA CPS is O-acetylated predominantly at C-3 (70–95%) with low levels at C-4. O-acetylation is considered to be important for immunogenicity [18] and WHO guidelines recommend that MenA vaccines have at least 61.5% O-acetylation [19].

Oligosaccharide length is an important consideration for the development of synthetic conjugate vaccines [20]: the oligosaccharide used must be sufficiently long to be representative of the CPS conformation, but short enough for synthesis to be practical. For the synthetic MenX vaccine in particular, it is important to determine the minimal epitope of the saccharide required for immunological activity against the MenX CPS [21]. However, there is limited data on the oligosaccharide length necessary for an immunogenic MenA and MenX glycoconjugate. A trimer of a carba-sugar analogue of MenA elicited a poor immune response in mice in comparison to oligosaccharides of 6–15 RU [22]. However, a MenA tetramer conjugate was shown to be antigenic in a competitive inhibition assay with rabbit antisera, although immunogenicity is still to be proven [23]. Fiebig et al. showed that an average degree of polymerization of 10 units (avDP10) MenA conjugate had similar immunogenicity in mice as compared to the avDP15 standard [24]. For MenX, Morelli et al. synthesized monomer, dimer, and trimer glycoconjugates and found that only the trimer was able to induce IgG antibodies against MenX CPS, however, this was at low levels in comparison to the avDP15 MenX control that induced a large antibody response [21]. Although many variables affect the immunogenicity of conjugate vaccines, it is not clear what the minimum repeating unit (RU) length should be for MenA and MenX oligosaccharide-based vaccines.

Vaccine efficacy data indicates that the chemical structural similarity of bacterial CPSs alone is not able to reliably predict cross-protection between closely related strains [25–27]. Small changes in a carbohydrate structure can lead to large changes in conformation and dynamics and thus affect antigen binding and immunogenicity. Experimental techniques such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy are only able to provide partial insights into carbohydrate conformation [28,29], whereas computational methods can provide key theoretical insights into polysaccharide conformation. In particular, molecular dynamics (MD) simulations provide atomistic information on dynamic polysaccharide motion in solution on the nanosecond timescale that is inaccessible to physical experiments. MD is a powerful predictor of carbohydrate conformation which can be used to compare related antigenic carbohydrates: our recent work on *N. meningitidis* serogroups Y and W demonstrated key conformational differences that provide a rationale for the unusual cross-reactivity observed [30].

Here we employ MD simulations to explore the conformational preferences of MenA and MenX oligosaccharides. Our established methodology [31,32] employs an incremental approach whereby we first simulate the respective disaccharide repeating units in order to determine the preferred individual linkage conformations. As saccharide rings are relatively rigid structures, the conformations of oligosaccharide chains are primarily determined by the dihedral orientations in these glycosidic linkages. The preferred dihedral values are then used to build longer oligomers for MD simulation in order to generate predictions of the conformation of the CPS. We simulate 2, 6, and 10 repeating units (RU) to determine the effect of chain length, considered an important factor for the immunogenicity of glycoconjugate vaccines [33,34]. Evaluation of predicted structures is primarily performed by cluster analysis which provides insight into the relative preference of the polymer for particular conformations. The conformational clusters can be compared between structurally similar repeating units of different saccharides to determine the extent of the role that CPS conformation plays in explaining observed immunological cross-reactivity. We use NMR NOESY correlations as corroboration for the predicted conformations. Lastly, we model MenA with 3-O-acetylation to observe the effect of this substituent on CPS conformation.

2. Results and discussion

In the discussion below, we first present the conformations of MenA, then MenX, and finally the effect of 3-O-acetylation on MenA. For each serogroup, we first discuss the linkage conformations [α(1- > 6) for MenA and α(1- > 4) for MenX] and then move on to conformational analysis of the 2RU, 6RU, and 10RU oligomers. In the case of MenX, NMR evidence is used to corroborate our findings.

2.1. MenA CPS backbone conformation

The flexible α(1→6) linkage in MenA is described by five dihedral angles: φ, ψ, ω, ε and χ (shown on a representative disaccharide linkage in Fig. 1a–b). The dihedral time series from the 400 ns simulation of 6RU of MenA (Fig. 1c) shows that these dihedrals vary in flexibility. The φ and ε dihedrals are the least flexible, each having a single conformation with average values of φ = 341° and ε = 179°, respectively. The χ primary alcohol dihedral is constrained, with only brief transitions away from the primary *gg* conformation (χ = 174°). In contrast, the two C-O-P-O dihedrals (ψ and ω) are much more flexible and have strongly correlated motion in the 6RU MenA simulation with transitions between two main conformations: a primary conformation with ψ, ω ≈ 77°,77° and a secondary conformation with ψ, ω ≈ 273°, 282° (Fig. 1c). The primary dihedral linkage conformation orients the adjacent sugar ring in an extended conformation (Fig. 1a), whereas the secondary conformation results in a hairpin bend conformation around the phosphodiester linkage (Fig. 1b).

As conformational changes of the α(1→6) linkage occur predominantly due to transitions in ψ and ω, we simplify the conformational preferences of the α(1→6) linkage in our simulations of 2RU, 6RU and 10RU oligosaccharide strands with plots of the ψ vs ω dihedral angles only. The glycosidic linkage in the 2RU strand is predominantly in the hairpin bend conformation (ψ, ω ≈ 274°, 283°, Fig. 2a), with a second major population of the extended conformation (ψ, ω ≈ 77°,78°). Upon increasing the length of the oligosaccharide strand, the conformational preferences shift so that 6RU (Fig. 2b) has an approximately equal weighting of hairpin bends and extended conformations, as well as a range of intermediate conformations. For the 10RU simulation (Fig. 2c), the extended conformation population increases, as does the population of intermediate conformations. As repeating unit length changes, the conformational preferences of the MenA α(1→6) linkages change, therefore, the conformations of very short chains are not likely to be representative of the behavior of the native MenA CPS.

Our simulations reveal that the MenA CPS is a highly dynamic random coil with no regular conformational epitope. Clustering of the last 250 ns of the 400 ns MenA 6RU simulation reveals a wide range of conformational families, as shown in Fig. 3. The 6RU chain switches dynamically between compact (Fig. 3, A1, 34%), extended (Fig. 3, A2, 16%) and a variety of intermediate conformations (Fig. 3, A3, A4 and A5). This behavior of the MenA oligosaccharide correlates with reported ^{3}J ^{31}P-^{13}C coupling values that suggest a preference for extended anti conformations for the φ and ε dihedral values [35].