The common core of *N*-linked glycans: rigidity through branching?

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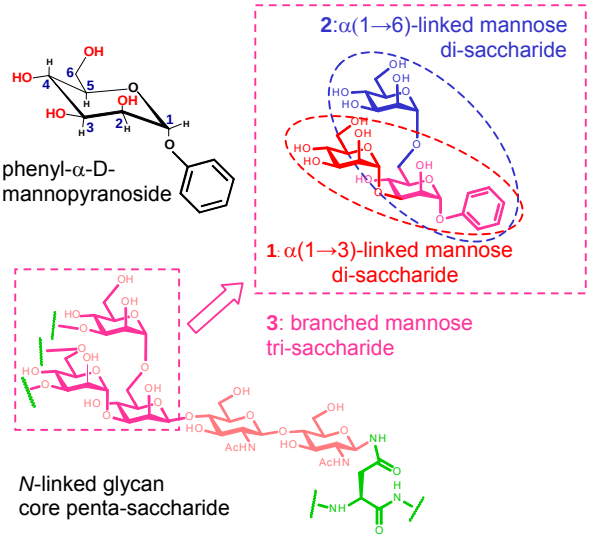
Introduction

Proteins are commonly modified by the addition of carbohydrate moieties to form glycoproteins; that is, side chains of some amino acids are covalently linked to glycans: mono- oligo- or poly-saccharides. Recognition of glycans plays a vital role in cell adhesion, signalling and trafficking. As such, glycans are essential for development in higher organisms and play an important role in disease. The vast majority of glycoproteins are *N*-linked, with glycans attached via the amide nitrogen of asparagine side chains. Development in multicellular organisms relies on *N*-linked glycans; studies with knockout mice show that although individual cells lacking the machinery for *N*-glycosylation are viable, embryos do not survive[1]. The common core penta-saccharide ($Man_3GlcNAc_2$, Scheme 1) of *N*-linked glycans is a highly conserved structural motif, but the underlying reasons for its success in this central role remain unclear. Elegant studies, using NMR and modelling techniques, have suggested the existence of *strong conformational biases*[2], and a fundamental role for carbohydrates as powerful and precise scaffolding units for multivalent presentation of key recognition motifs is emerging.

Here, we target the conserved core of *N*-linked glycoproteins (Scheme 1). The structure is broken down into separate regions for investigation: initial studies, described in this report, concentrate on the mannose branching region (boxed). The aim is to examine the innate structural preferences of this region and how the presence of multiple mannosyl units affects these preferences. First, we investigate the conformational landscape of the mannose monosaccharide phenyl-α-D-mannopyranoside (α-phMan, top left in Scheme 1). Then, the di- (**1**, **2**) and finally the tri-saccharide model structures (**3**) derived from the branching region are examined. Scheme 1 shows the structures of the target saccharides, which are tagged with a phenyl chromophore for near UV spectroscopic access.



Scheme 1

This project represents a significant extension of the recent focus in our laboratory on characterisation of the inherent conformational preferences of small carbohydrates (mono- and di-saccharides), their derivatives and hydrated complexes, all isolated at low temperature in the gas phase[3,4]. We are using the experimental techniques of jet-cooling and multiple laser spectroscopy in combination with electronic structure theory calculations to help build up a data base of basic conformational information for carbohydrate building blocks.

α-PhMan Monosaccharide

The resonant 2-photon ionisation (R2PI) and ultra-violet hole burn (UVHB) spectra of αphMan shown in Figure 1 reveal the presence of three distinct conformers, one major and two minor, labeled as A, B and C. The infra-red hole burn (IRHB) spectra of each conformer are displayed in Figure 2.

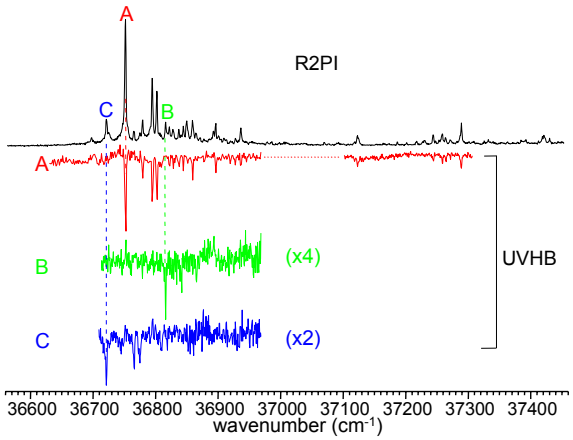


Figure 1. R2PI and UVHB spectra of α-phMan.

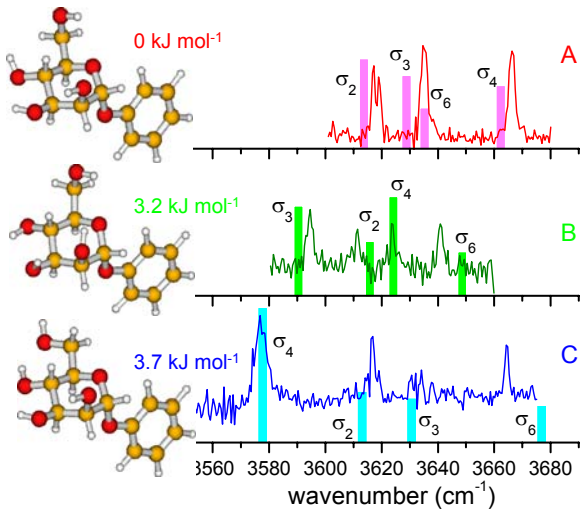


Figure 2. Measured IRHB spectra of α-phMan together with the conformers and calculated spectra showing the assignments.

Conformational assignment is effected through comparison of the measured IRHB spectra with those calculated for low-energy candidate conformations identified from computation. Figure 2 shows the conformational assignments for each of the three conformers of αphMan together with their relative energies computed at the MP2/6-311++G(d,p)//B3LYP/6-31+G(d) level of theory.

The normal mode analysis provided by the calculations allows the assignment of each observed vibration to the stretching motion of a particular OH group. The influence of hydrogen bonding on these groups can then be analyzed. The numbering of the OH groups is shown in Scheme 1. The easiest effect to

measure is a shift of the OH stretching mode towards lower wavenumber, the strongest interactions leading to the largest shifts. A clear example of this correlation is the evolution of the spectral shift of the feature labeled σ4. It appears at ~3665 cm^{-1} in conformer A, where the distance between H4 and its nearest neighbour, O6, is 3.2 Å. In conformer B, this band shifts by ~40 cm^{-1} to lower wavenumber, reflecting the weak hydrogen bond present between OH4 and O3 in this conformer (computed H4-O3 distance of 2.5 Å). In conformer C, the hydrogen bond between OH4 and O6 is much shorter, 2.0 Å, and the σ4 band shifts to lower wavenumber by an additional ~45 cm^{-1}.

Di- and Tri-Saccharides

The R2PI spectra of the tagged α-linked mannose di- and tri-saccharides **1**, **2** and **3** are shown in Figure 3. The features in the spectra are significantly broader than those of the R2PI of the monosaccharide (see Figure 1). This may be due to the increased number of low-frequency modes in these larger and more flexible oligosaccharides. The UVHB spectrum of the α(1→3)-linked disaccharide **1** (not shown) indicate the presence of a single conformer, only. The R2PI spectrum of the α(1→6)-linked disaccharide **2**, which has two separate broad features, suggests the population of two distinct conformers in the gas phase while for that of the branched tri-saccharide **3**, suggests the presence of only a single conformer. Further hole-burning experiments on **2** and **3** are necessary to substantiate this conjecture and to effect conformational assignment of these molecules.

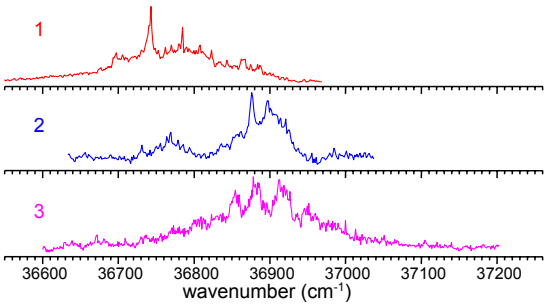


Figure 3. R2PI spectra of the mannose di-saccharides 1 and 2 and the branched tri-saccharide 3.

Assignment of the 1-3 Linked Mannose Di-saccharide

The experimental IRHB spectra of the α(1→3)-linked disaccharide **1** is shown in Figure 4, together with the assigned conformer and its computed IR spectrum. The IRHB spectrum of the mono-saccharide conformer **B** is shown in the middle frame of the Figure for comparison. Ring **a** of di-saccharide **1** resembles mono-saccharide conformer **B** in that its ring hydroxy groups, OH2-OH4, are in basically the same orientations in the two molecules. The locations of the corresponding σ2-σ4 stretching modes are virtually the same in the two spectra, indicating little cooperative influence on these modes in the disaccharide. However, the orientation of the hydroxy methyl group of ring **a** in the di-saccharide differs from that of the mono-saccharide conformer **B** because in the di-saccharide, OH6a acts as a hydrogen-bond *acceptor* from OH2 of ring **b**. As a consequence, OH6a is not a hydrogen-bond donor and the σ6a band is at higher wavenumber than the σ6 band of the monosaccharide conformer **B**, while the OH2b band is located at relatively low wavenumber, ~3515 cm^{-1}.

It is revealing to compare the IRHB spectrum of the α(1→3)-linked di-saccharide **1** with that measured previously for the β(1→4)-linked disaccharide benzyl β-lactoside[3], whose IRHB spectrum and conformational assignment are shown in the bottom frame of Figure 4. Like the mannose di-saccharide **1**, the lactoside populates a single conformer in the free-jet expansion. Unlike the mannose di-saccharide, however, its conformation is stabilised by a *cooperative network* of hydrogen bonding *encircling* the di-saccharide, which includes hydrogen bonds linking the sugar rings on two sides of the lactoside. As a result, the vibrational bands in the β(1→4)-linked lactoside are, on average, at lower wavenumber than those of the α(1→3)-linked mannose di-saccharide **1**. Especially noticeable are the three strongly-shifted (to lower wavenumber) bands of the lactoside compared to the single shifted band of the di-mannoside.

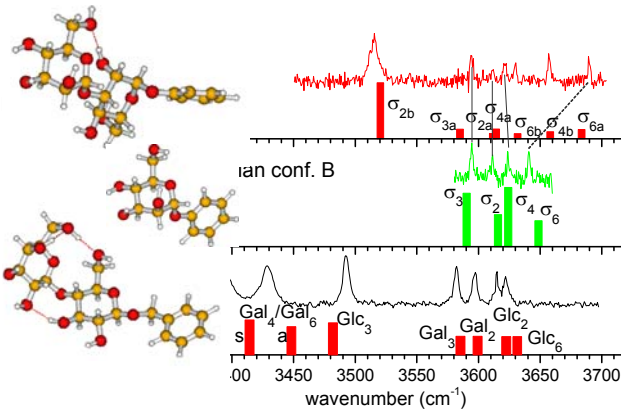


Figure 4. IHRB spectra of the mannose di-saccharides 1 (top) compared to those of conformer B of the mono-saccharide α-phMan (middle) and the di-saccharide benzyl β-lactoside (bottom).

Conclusions

Significant progress has been made towards characterizing the conformational landscape of the mannose branching region of *N*-linked glycans, isolated in the gas phase. Three conformations of α-phMan have been identified and assigned. The single conformer identified for the α(1→3)-linked mannose di-saccharide **1** has also been assigned. Preliminary results have also been obtained on the α(1→6)-linked di-saccharide **2** and on the branched tri-saccharide **3**. Results suggest that the α-linked mannose di-saccharides do not have as many strong inter-ring interactions as the previously examined β(1→4)-linked benzyl lactoside and that the mannose di-saccharide conformations are not as influenced by co-operativity as the lactoside. While the conformational landscape of the mannose di-saccharides appears to be simpler than that of the monosaccharide (in that fewer conformers are populated in the gas phase), there is clearly a certain flexibility of the mannose di-saccharides. Further experiments are underway to complete the characterization of the gas-phase conformations of the mannose branching region and to determine whether the branching does indeed promote rigidity.

Acknowledgements

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