Hydrophilic and hydrophobic carbohydate interactions

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Introduction

Carbohydrate molecules are notoriously flexible and in aqueous or physiological environments their conformational structures can be influenced by interaction with neighbouring ions or molecules and particularly, by explicit hydration. This may in turn, influence their selective molecular recognition at protein-carbohydrate receptor sites, thought to involve their preferred, solution conformation(s). Determining and understanding what rules, if any, govern them and characterizing their three dimensional conformations presents a major challenge. Their NMR spectra are complex and are associated with time averaged, dynamical structures which can complicate the interpretation of NMR measurements. Structural determinations based upon nuclear Overhauser effects in solution or residual dipolar coupling in liquid crystal environments, coupled with molecular dynamics simulations, may not provide unique answers. Recently a new laser-based strategy has been developed, which allows comparisons to be made between the conformational structures of isolated carbohydrates and their micro-hydrated complexes stabilized at low temperatures in the gas phase.[1,2] The vibrational spectra of individual conformers and hydrated clusters, isolated in a cold molecular beam, can be identified and selected through the depletion in their ground state populations promoted by resonant absorption of tunable IR laser radiation. The depletions are most simply detected via the dip in their UV laser induced, resonant two-photon ionization signals; when coupled with mass spectrometry this allows size selected molecular complexes to be detected and interrogated spectroscopically. Comparisons with the results of ab initio calculations using electronic structure theory, allow assignment of their vibrational spectra and their intrinsic structures and confromations.

Results and discussion

Hydration, conformation and selectivity–glucose, galactose and mannose

Key factors controlling conformational preference and site selectivity in hydrated monosaccharides include the flexibility of their exocyclic hydroxymethyl groups (in glucose, galactose and mannose); their anomeric configuration; and the relative orientations (axial vs. equatorial) of their hydroxyl groups. These factors can

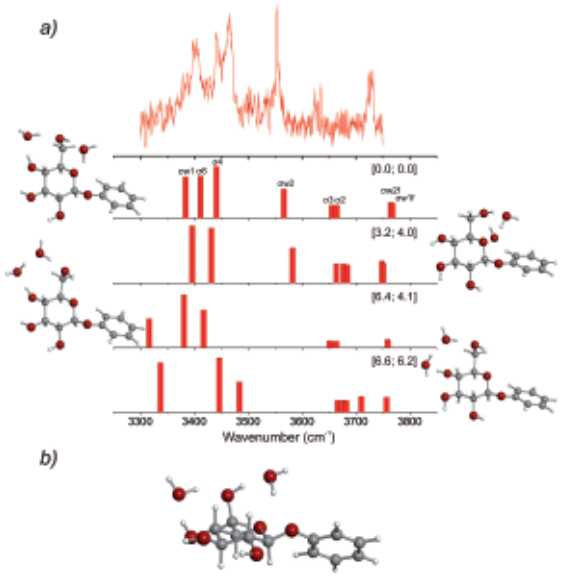

Figure 1. (a) IR ion dip spectra of hydrated β-phenyl glucose (βGlc) recorded in the βpGlc•$(H_2O)_2^+$ ion channel and the computed vibrational spectra, structures, relative energies and free energies (kJ mol^{-1}) of the four lowest energy βpGlc•$(H_2O)_2$ complexes; calculations conducted at the B3LYP/6-311+G*//MP2/6-311++G level. (b) Side-on view of the lowest energy, populated structure.**

operate separately or collectively, adapting the carbohydrate conformation to optimize the sequence of intra- and inter-molecular hydrogen-bonded interactions in the hydrated complex. The first (and second) bound water molecules are invariably located near the hydroxymethyl group, inserting into the weakest pre-existing hydrogen bond in the bare molecule. If necessary, the carbohydrate conformation changes in order to achieve this. An illustration of this is shown in Figure 1 which presents the experimental infrared ion dip (IRID) and computed vibrational spectra of doubly hydrated phenyl β-D-glucopyranoside, (βpGlc) recorded in the gas phase. The experimental spectrum is in excellent agreement with the computed global minimum energy structure. In order to best accommodate the bound water molecules located on each side of the (re-oriented)

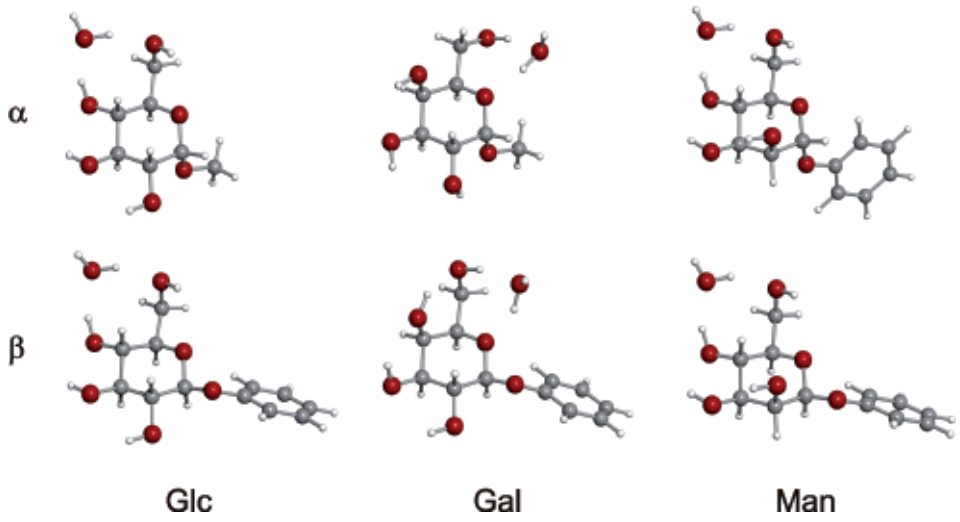

Figure 2. The preferred structures of the singly hydrated α- and β-anomers of phenyl (or methyl) glucoside, galactoside and mannoside.

hydroxymethyl group, the monosaccharide conformation is reshaped to create the extended, co-operatively H-bonded sequence, OH2→OH3→OH4→OH_{W1}→OH6→OH_{W2}→O5. The conformation of the hydroxymethyl group changes from G+g- to G-g+ while the orientation of the peripheral OH groups switches from counter-clockwise, 'cc' to clockwise, 'c'.

This behaviour follows general 'propensity rules', for example those identified for singly hydrated monosaccharides, see figure 2. When the OH4 group is oriented equatorially, as in Glc and Man, each of the hydrated sugars either adopts (α,βGlc, βMan), or retains (αMan) a cG-g+ conformation, to provide an optimized structure for reception of the bound water molecule at the (4,6) hydroxymethyl site - the weakest link in the original ccG+g- configuration - and maximise the length of the co-operatively hydrogen bonded chain, OH2→OH3→OH4→*W*→OH6→O5. In Gal the OH4 group is oriented axially, facilitating strong hydrogen bonding between OH4 and OH6 (in the cG-g+ conformer) or OH4 and OH3 (in the ccG+g- conformer), and in both anomers the water molecule inserts on the other side of the hydroxymethyl group, at the (6,5) site. In doubly hydrated βGlc (figure 1) the first water molecule occupies the (4,6) site and the second selects the (6,5) site, the next attractive vacancy. In addition, a side-on view reveals their preference for the more hydrophilic face of the carbohydrate. This is even more pronounced in triply hydrated mannose, Figure 3.

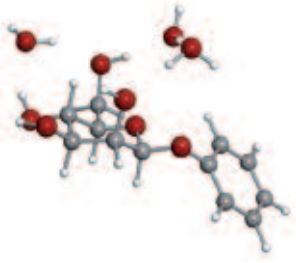

Figure 3. "Side-on" view of triply hydrated β-mannose.

N-Acetyl Glucosamine

Despite their essential role in virtually all living organisms, there have been very few spectroscopic or structural investigations of 2-deoxy amino sugars, such as *N*-acetyl glucosamine, (GlcNAc). The influence of the acetamido group in directing the preferred choice of hydration sites has now been revealed, and comparisons with singly and multiply hydrated glucose have led to an extension of the working rules governing selective hydration and conformational choice. Figure 4, which presents the experimental and computed IR spectra of βpGlcNAc•$(H_2O)_2$, provides an instructive illustration of the extended 'rules of the game'. Its experimental spectrum is associated predominantly, with the global minimum energy structure, although there is a minor component associated with its closest neighbour. Each one retains the preferred ccG+g- conformation of the bare monosaccharide and accommodates a bound water dimer (not two separate water molecules as in βpGlc•$(H_2O)_2$). The dimer is inserted between the acetamido and the OH3 groups, breaking the pre-existing OH3→O=C-NH bond to create the extended co-operative chain, OH4→OH3→OH_{W1}→OH_{W2}→O=C-NH. The water dimer lies above the pyranosyl ring in the global minimum structure, where the acetamido plane adopts a "perpendicular-NH down" configuration, figure 5(b) but in the neighbouring structure the plane rotates through ~180° into a "perpendicular-NH up" orientation and the bound water dimer lies below the pyranosyl ring, see figure 5(c).

Conclusions

In glycosides like glucose, galactose and mannose, regioselectivity is introduced and controlled primarily by the flexibility and orientation of the exocyclic hydroxymethyl group, and secondarily by the orientation (equatorial or axial) of the pyranosyl hydroxyl groups. In the hydrated 2-deoxyamino sugar, βpGlcNAc, bound water molecules are marshaled into place by the acetamido group, which now provides the dominant control. It can act both as a hydrogen bond donor and an acceptor and like the hydroxymethyl

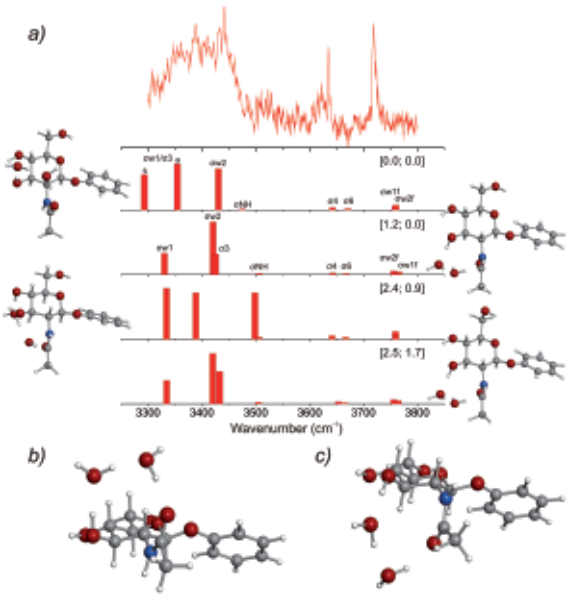

Figure 4. (a) IR ion dip spectrum recorded in the βpGlcNAc•$(H_2O)_2^+$ ion channel with the UV probe laser tuned to 36,809 cm^{-1} and the computed vibrational spectra, structures, relative energies and free energies (kJ mol^{-1}) of the four lowest energy βpGlcNAc•$(H_2O)_2$ complexes. (b,c) "side-on" views of the two, lowest energy populated structures, "NH-down" (b) and "NH-up" (c).

group in βpGlc or βpMan, it can fine-tune the preferred choice of binding site by rotating into alternative configurations: "in-plane", "perpendicular-NH up" or "perpendicular-NH down". These properties generate hydrate structures where the bound water molecules are located either above, or below, or above and below the pyranosyl ring. When neither an acetamido nor a hydroxymethyl group is present, for example in xylose or fucose, bound water molecules locate further round the pyranose ring, selecting peripheral sites associated with the weakest link in the chain. In general, the degree of control exerted by the groups around the pyranosyl ring lies in the order acetamido > hydroxymethyl > pyranosyl $(OH\text{-}O)_n$ chain. Multiply hydrated carbohydrates display supra-molecular structures which reflect their hydrophilic and hydrophobic topographies.

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References

1. P. Çarçabal, R. A. Jockusch, I. Hünig, L. C. Snoek, R. T. Kroemer, B. G. Davis, D. P. Gamblin, I. Compagnon, J. Oomens and J. P. Simons, *J. Amer. Chem. Soc.*, **127**, 11414-11425 (2005).
2. E. J. Cocinero, E. C. Stanca-Kaposta, E. M. Scanlan, D. P. Gamblin, B. G. Davis and J. P. Simons, *Chem. Eur. J.*, **14**, 8947-8955 (2008).