Influence of the Anomeric Conformation in the Intermolecular Interactions of Glucose

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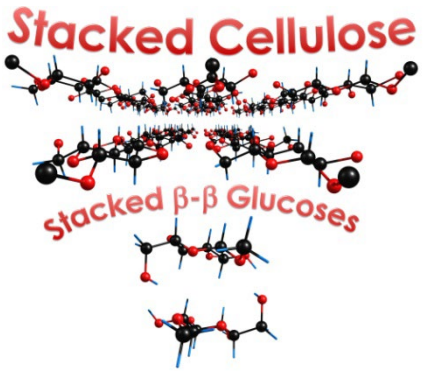
ABSTRACT Sugars play essential roles in the energy regulation as well as in the immunity system and signaling processes. These functions are governed by the intermolecular interactions between

sugar units and other biomolecules. In order to evaluate the leading intermolecular contribution occurring at two-body level characterizing these biological structures, this work reports dimeric studies of glucopyranose anomers (methyl-α/β-D-glucose, phenyl-α/β-D-glucose and D-glucose), combining experimental mass-resolved laser spectroscopy measurements and DFT theoretical calculations. The synergy between these two approaches allowed to reveal as the orientation of the anomeric substituent is fundamental in influencing the ability of glucopyranose to bind and form aggregates. The results clearly demonstrate that the already known intramolecular anomeric effect is inverted and enhanced during the intermolecular complexation, giving rise to significantly more stable β-anomers dimers. These findings contribute to rationalize the interactions competing in the condensed phase, where the same driving force is amplified or hindered by the surroundings.

TOC GRAPHICS



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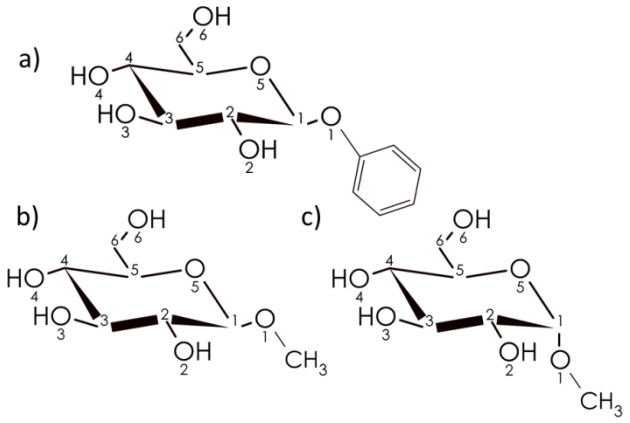
From the conformational point of view, sugars are a versatile family of molecules.[1] They present a number of hydroxyl substituents that may be in axial or equatorial position, leading to different epimers. In living beings they also aggregate forming polysaccharides that can be found isolated or attached to lipids and proteins, frequently decorating the extracellular side of the plasmatic membrane, playing a central role in the immune system of multicellular organisms.[2] Those polysaccharides contain a variable number of units with different degree of branching and a variable composition, which may also present modifications such as acetylations or sialyations. The number of possible combinations is enormous and accordingly to some authors exceeds those of amino acids or DNA bases, as the number of "letters" in the alphabet of sugars is several orders of magnitude larger than in the other two alphabets.[3]

The molecular-recognition process is based on the molecular complementarity, main feature that characterizes the binding between biomolecules. One example of this specificity related to anomeric carbohydrates is the interaction of sugars with synthetic receptors: in most cases, the artificial receptor binds preferentially an anomer (α or β) of a given sugar.[3] The reason behind such preference is not known, but certainly it is governed by the equilibrium between the intrinsic binding forces belonging to the direct interaction and the indirect contributions coming from the environment. Another explaining model is represented by the case of the cellulose and the starch. They present specific macroscopic properties, but it is very difficult to define how much is the contribution of each interaction and the effects that lead to them.

The questions at that point is: is there any specific interaction between anomers that most influences the sum of interactions exhibited by a macroscopic model? For this goal the study of model anomeric dimers, like β-β, β-α and α-α, is proposed in order to isolate the interactions occurring within these moieties and evaluate their relative stability. To get insights into the molecular aggregation process, we approached such mechanism studying dimers between methyl-α/β-D-glucopyranoside (α/β-MeGlc) and phenyl-β-D-glucopyranoside (β-PhGlc, see Scheme 1) using a laser desorption system combined with the pulsed supersonic expansion, inside the ionization chamber of a mass spectrometer (see Supporting Information, Experimental Methodology Section). The collisions during the expansion cool the molecules and this favors the formation of molecular complexes that can be afterwards studied using mass-resolved spectroscopic techniques.



Scheme 1. a) Phenyl-β-D-Glucopyranoside (β-PhGlc); b) Methyl-β-D-Glucopyranoside (β-MeGlc); c) Methyl-α-D-Glucopyranoside (α-MeGlc).

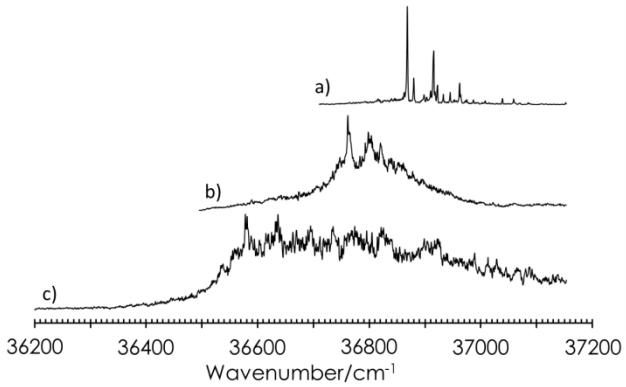


Figure 1. 1-color REMPI spectra of: a) β-PhGlc; b) β-PhGlc···β-MeGlc and c) β-PhGlc···α-MeGlc.

Figure 1 shows the resonance enhanced multiphoton ionization (REMPI, Scheme S2) spectra of β-PhGlc (a) and its complexes with α-MeGlc (b) and β-MeGlc (c). The spectrum of β-PhGlc presents several well-resolved bands that, as demonstrated in a previous work by Simons' group,[5] correspond to at least three isomers that differ in the relative orientation of the exocyclic hydroxymethyl moiety with respect to the rest of the molecule.

Due to the appearance of low-frequency intermolecular modes, structural changes upon electronic excitation or the excited state lifetime in the formation of complexes with α/β-MeGlc, the REMPI spectra are broadened. They present a red-shift from the origin band of β-PhGlc, especially in the case of β-PhGlc···α-MeGlc. Both spectra are characterized by several discrete features, built on top of a broad absorption.

Although the spectra in Figure 1 (see a full description in Figure S1) display lines not perfectly resolved, the recording of the mass-resolved IR spectra of the complexes could be possible using the IR/UV double resonance technique (see Schemes S3 in the Supporting Information).

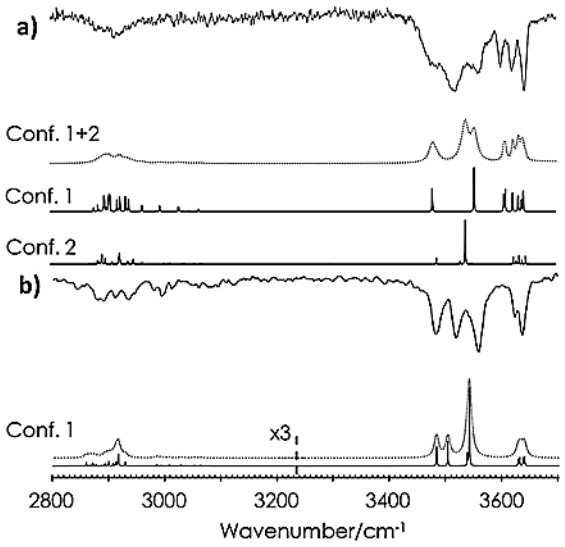


Figure 2. Mass-resolved IR/UV spectra of: a) β-PhGlc···α-MeGlc and b) β-PhGlc···β-MeGlc. Simulated spectra for the assigned structures are also reported for comparison. Correction factors of 0.9385 in the OH region and of 0.9525 in the CH region were used to account for anharmonicity. The corresponding structures, computed at the M06-2X/6-311++G(d,p) level, are shown in Figure 3.

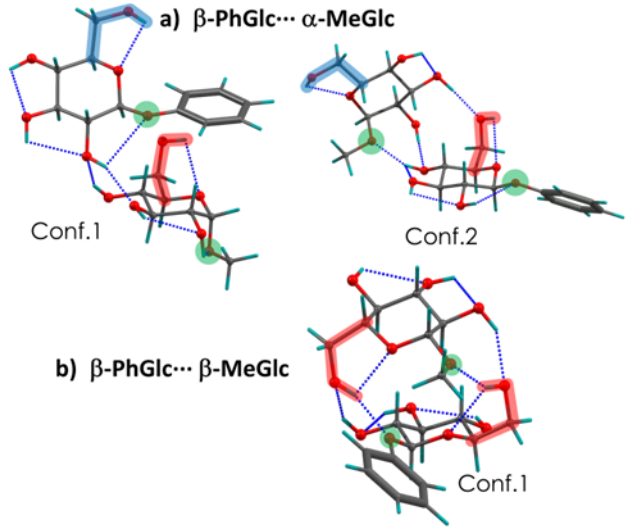


Figure 3. Structures of the dimers assigned to the experimental spectra: a) the two β-PhGlc···α-MeGlc isomers; b) the one β-PhGlc···β-MeGlc.

Figure 2 shows the mass-resolved IR/UV traces obtained for β-PhGlc···α-MeGlc (a) and β-PhGlc···β-MeGlc (b) in the stretching region, covering from the C-H (~2800-3000 cm^{-1}) up to the O-H stretches (3400-3700 cm^{-1}). Several bands appear in both regions of the spectrum, consequence of the twenty C-H and the eight O-H bonds present in the complex. The bands corresponding to the C-H stretches usually appear as a group of transitions with several combination bands, congesting the spectrum and precluding the extraction of structural information.

Conversely, the OH stretching region is usually more informative and provides important structural information. Three well-resolved bands appear at the blue-end of the spectrum of β-PhGlc···α-MeGlc (a), together with three broad absorptions. The complex has eight OH groups and therefore, the observed transitions may hide contributions from several vibrations. None of the bands lie in the region of the free OH stretches (~ 3710 cm^{-1}) and consequently all the hydroxyl groups may be involved in inter/intramolecular interactions of different strength, which result in a collection of shifts in the spectrum. In previous publications,[6-8] it was demonstrated that the hydroxyl groups of sugars are usually forming cooperative hydrogen bond networks that hamper (but do not completely block) their interaction with other molecules.[9, 10]

The interpretation of the spectra in Figure 2 requires the guidance of predictions from quantum mechanics. Thus, we explored the conformational landscapes for the interaction between the two molecules using a combination of molecular mechanics and DFT methods. As demonstrated elsewhere,[11, 12] the interaction between sugars is a balance of changes in enthalpy and entropy and therefore both quantities have to be taken into account when analyzing the energetic order of the calculated structures (see Supporting Information, Theoretical Methodology Section). The

final assignment of β-PhGlc···α-MeGlc dimer is shown in the upper panel of Figure 2. No single isomer was able to reproduce all the features present in the spectrum and a combination of two species was required. In order to observe additional conformers of this system, we performed UV/UV hole burning experiments but, due to the broad nature of the spectrum, this attempt was unsuccessful. Nevertheless, the obtained IR/UV traces, probing different wavenumbers of the REMPI spectrum (see Figure S2), point out the existence of at least two isomers contributing to the spectrum, even if the complete separation of their spectra was not possible.

The structures of the two assigned isomers, reported in Figure 3, demonstrate that they correspond to very different interacting geometries: while in isomer 1 the hydroxymethyl group of α-MeGlc is incorporated into the hydrogen bond network of β-PhGlc (O4-H···O2′-H··O3; the upper marker after the number distinguishes one molecule from the other) and at the same time there is a direct interaction with the aromatic ring of the chromophore; in isomer 2, the interaction between the two molecules takes place away from the aromatic ring through O3-H···O6‘ + O2-H···O4′-H···O1 interactions. Moreover, in this second isomer several intermolecular hydrogen bond networks are formed. The Gibbs free energy difference between the two structures is less than 3 kJ/mol in the interval of temperatures achieved in the expansion (around 100 K of vibrational temperature; see Figure S3, S4 and S5 in the Supporting Information), supporting the existence of both species in the beam.

Regarding the mass-resolved IR/UV spectrum of β-PhGlc···β-MeGlc, the OH stretches also form two groups, but the bands to the blue are closer together, pointing to a very similar environment for those OH, while the three bands around 3500 cm^{-1} are better resolved than in the case of β-PhGlc···α-MeGlc. The comparison with the DFT predictions shows that the spectrum

of the global minimum reproduces very accurately the experimental observation (Figure S11) and therefore one can conclude that in this case a single species is formed in the beam (Figure S6). This is further supported by the energies of the calculated structures (see Table S2, Figures S7 and S8): the ΔG of the second most stable structure is >10 kJ/mol greater than that of the global minimum, during the whole interval from room temperature to 0 K. The existence of a single isomer of β-PhGlc···β-MeGlc dimer, despite detecting three isomers of β-PhGlc in the expansion, is due to a significant reduction of the isomerization barrier that connects the three bare conformers upon formation of the aggregate. Actually, the interaction with the β-MeGlc reduces the barrier from 23 kJ/mol in the bare molecule to 12 kJ/mol when complexed (Figure S10).

Thus, the assigned isomer presents a structure in which the hydroxymethyl substituent of each molecule is included in the hydrogen bond network of the partner molecule through O2-H···O6′-H···O1 interactions, embracing one each other and putting the hydrophobic CH groups in contact. The interaction between the substituents of the anomeric carbons add extra stabilization to the complex, thanks to C-H···π interactions. The elegancy of this symmetric stacked structure results in a significantly high binding energy of 67 kJ/mol, according to the calculations run at the M06-2x/6-311++G(d,p) level. This value is substantially bigger than the binding energy found for the isomers of β-PhGlc···α-MeGlc.

It is well known that the small structural difference between α and β-anomers, just the equatorial/axial position of the hydroxyl group, results in a small extra stabilization of the α-anomers. However, our results indicate that the intermolecular interactions dramatically change the conformational landscape. Clearly, the symmetric and energetically favorable structure

adopted by the most stable isomer β-PhGlc···β-MeGlc cannot be reproduced by the β-PhGlc···α-MeGlc complex. It could be still possible for the α-PhGlc···α-MeGlc dimer to reach structures of similar or even higher relative stability than those observed for the β-β complex, but, unfortunately, we were not able to find α-PhGlc to run such experiment. Nevertheless, we run calculations on the α-PhGlc···α-MeGlc dimer, which shows a great abundance of conformations interacting with the chromophore (Supporting Information, Section VI and Table S5). For this reason, we extended the theoretical analysis to the α-MeGlc···α-MeGlc dimer, investigating their interaction by hydrogen bond networks (Supporting Information, Section VII). Finally, we completed the general view of the interaction landscapes calculating the α-MeGlc···β-MeGlc and the β-MeGlc···β-MeGlc, using the same calculation level used to reproduce the experimental results shown in Figures 1 and 2. The results are summarized in Figure 4, where the relative binding Gibbs free energies of the most stable structures of each system are plotted between 0 and 400 K. It is clear that the β-β complexes (blue lines) always present the highest interaction energy, well separated from the α-β (green) and the α-α (red) systems.

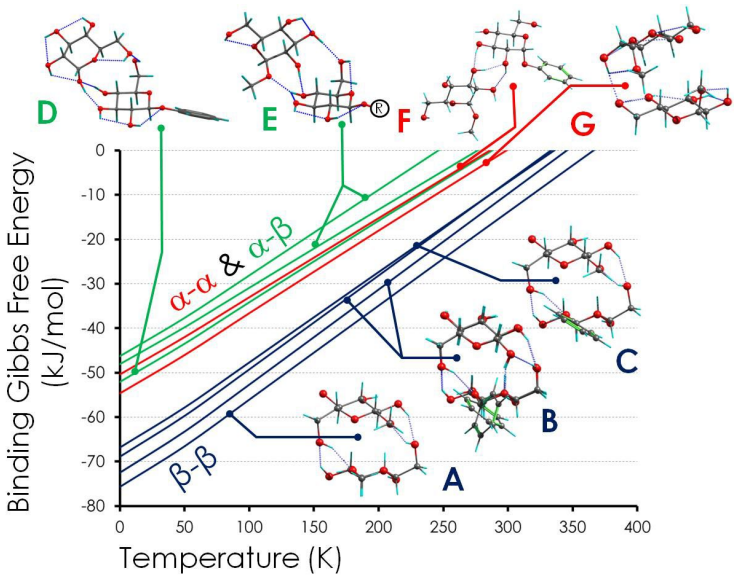


Figure 4. Relative binding Gibbs free energy of the most stable structures of: A) β-MeGlc···β-MeGlc B) β-PhGlc···β-Glc C) β-PhGlc···β-MeGlc D) β-PhGlc···α-Glc (β-PhGlc·α-Glc4) E) β-Ph/MeGlc···α-MeGlc (β-Ph/MeGlc·α-MeGlc-08) F) α-PhGlc···α-MeGlc G) α-MeGlc···α-MeGlc. The most stable isomer without interaction with the chromophore was considered (for more details see Supplemental Information, Table S6 and Table S7).

As a final test, we introduced in the expansion a mixture of β-PhGlc and D-glucose, in order to form the corresponding aggregates. In this case, the absence of a substituent in the anomeric carbon of D-glucose resulted in a mixture of β and α-anomers, as they can interconvert through the linear form of the sugar. The resulting REMPI and IR/UV spectra can be found in the Supporting Information (Figures S1 and S16) together with the quantum mechanical predictions (Tables S3, S4 and Figures S12, S14). The existence of both anomers in the expansion reach the formation of complexes of β-PhGlc with both species, largely complicating the spectroscopy of the system. Actually, the comparison between the experimental spectrum and the predicted traces showed that at least three species, one with the α and two with the β-anomers (see Figure S13 and S15), may be contributing to the experimental spectrum. The structures of these species are

very close to those presented in Figure 3, and they also are enclosure in Figure 4 since they follow the same trend in stability: the complexes with the β-anomer are significantly more stable than those with the α-anomer.

Finally, all these results point out the high degree of selectivity in the intermolecular interactions of sugars. The already known small energy difference between glucose's anomers, appears reversed and seems to be even amplified by the intermolecular interactions, which lead to specific molecular aggregates with a very different stability. The complexes with a β-β interaction result to be the most stabilized ones, pointing out as the intrinsic properties of the anomeric interactions are fundamental in influencing the intermolecular binding preferences. The outcomes of this work were obtained thanks to the synergy between accurate experiments and theoretical calculations. Indeed, if there are many theoretical studies showing the extra stability of the β-anomer in water solution,[13] in this work the stabilization preferences between anomers are proved using an experimentally validated theoretical method.

Starting from these findings, a comparison between the isolate phase and the condensed phase can be done, in order to get insight of which interactions represent the driving forces in the molecular aggregation process. This analysis was carried out comparing crystallographic structures (see Supporting Information, Section VIII and references within) with the ones calculated for the investigated dimers (see Tables from S1 to S7). It is worth noting that the crystal matrix produces a high influence on the structural arrangements, evidenced by the hydrogen bond interactions. Searching for the same kind of interactions in our dimers, it can be noted that the corresponding conformations are significantly higher in energy with respect to the assigned minima in the gas phase. This can be ascribed to the stabilizing interactions that occur

and compete in the condensed phase, like in foldamers or crystal matrices. Furthermore, comparing β-β dimer with the cellulose crystal, it can be point out that both present the general trend to assemble with planar and stacked geometries, although the hydrogen bonds change a bit their distribution passing from the reduced glucose's dimer to the structured cellulose, as depicted in Figure S23. This feature arises from the O1 of the β-anomer that lies in the equatorial plane of the molecule. This property does not means that each β-anomer achieves a similar stacked arrangement (see Figure S23), but it represents the key factor to get access to this type of further stabilized structures. Actually, many factors are involved in defining the macroscopic behavior of molecular systems, like the difference in solubility between the cellulose and the starch; however the intermolecular anomeric preference can be consider one of the main forces. In conclusion, the difference in the equatorial-axial orientation of hydroxyls groups between sugars epimers represents a determinant role in the molecular aggregation process.

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The authors declare no competing financial interests.

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REFERENCES

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(2) Bi, D.; Yang, L.; Boschloo, G.; Hagfeldt, A.; Johansson, E. M. J. Effect of Different Hole Transport Materials on Recombination in $CH_3NH_3PbI_3$ Perovskite Sensitized Mesoscopic Solar Cells. *J. Phys. Chem. Lett.* **2013**, *4*, 1532-1536, DOI:10.1021/jz400638x.

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