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Hydration of T-antigen Galβ(1-3)GalNAc and the isomer Galβ(1-3)GlcNAc by molecular dynamics simulations

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We present a 250 ps molecular dynamics simulation of the T-antigen Galβ(1-3)GalNAc and its isomer Galβ(1-3)GlcNAc in the classic Gibbs Ensemble, Number of particles, Pressure and Temperature (NPT) with explicit representation of 432 water molecules. We computed the radial distribution function, equilibrium conformation, intramolecular and intermolecular hydrogen bonds, and water residence time to characterize the hydration pattern of these sugars, which are not very different and exhibit hydrophilic behavior. Based on hydration dynamics, it was concluded that these sugars should be classified as negative hydrated. Formation of an intramolecular hydrogen bond between the ring oxygen atom O5 of the first unit and the OH4′ group of glycoside of the second unit might influence interaction with the antigenic receptor and could explain the main difference of affinities between them. © 2000 by Elsevier Science Inc.

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INTRODUCTION

Carbohydrates, which often are conjugated to proteins and lipids, play a fundamental role in essentially all living organisms. Many carbohydrates are involved in signaling and recognition processes and are agglutinated by a family of proteins called lectins.[1] Their specificity and ability to be bounded by the different lectins depend not only on the characteristics of the binding site but also on the interaction of these molecules with water. It has been suggested that sugars interacting with proteins can form water bridges at the binding site of the protein. In this respect, the water molecules become structural and have play a fundamental role in stabilizing the carbohydrate within the interaction site.[2]

Thompsen-Friedenreich antigen, generally known as T-antigen [Galβ(1-3)GalNAc], is a well-defined tumor-associated antigen that causes severe malignancy in humans. It is expressed in cells as O-linked glycans [Galβ(1-3)GalNAc-αSer/Thr]. It is expressed prominently in more than 85% of human carcinomas, such as those located in the colon, breast, bladder, bucal cavity, and prostate, as well as on poorly differentiated cells.[3]

Structural studies of monosaccharides suggest that the positions of some OH groups in the pyranosic ring, mainly the third and fourth positions, are important for the selectivity of the sugar in relation with the lectin.[4]

Among the proteins that bind T-antigen, peanut agglutinin (PNA) is the most widely used in recognition processes. T-antigen is distinguished by the presence of an axial 4-hydroxyl group and equatorial 3-hydroxyl group in both pyranosic units. It binds to the PNA with high specificity.

Galβ(1-3)GlcNAc, when complexed with fucose and N-acetyl neuraminic acid, is recognized by a different family of lectins (selectins and sialoadesinas).[2,5] Figure 1 shows schematic diagrams of the T-antigen and its isomer. Both disaccharide molecules are in β anomeric form.

In this paper we present molecular dynamics simulations of the T-antigen Galβ(1-3)GalNAc and its isomer Galβ(1-3)GlcNAc in the Gibbs ensemble, Number of particles, Pressure and Temperature (NPT) with explicit representation of Single point charge/Extended (SPC/E) water molecules. To characterize the hydration of these molecules, we studied structural parameters, such as radial distribution functions and glycosidic torsional angles, and dynamic parameters, such as the mean number of intermolecular H-bonds, the intramolecular

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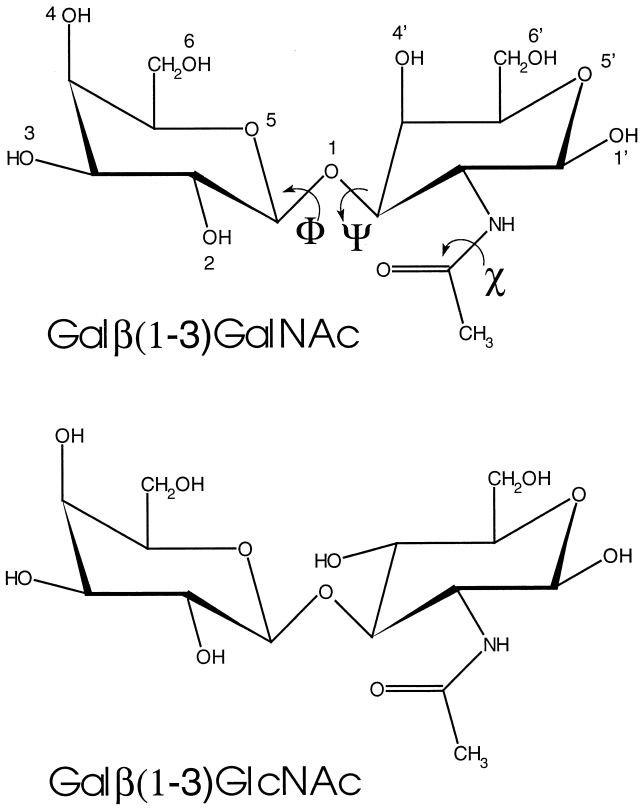

Figure 1. Schematic diagrams of disaccharide molecules. (a) T-antigen molecule Galβ(1–3)GalNAc. (b) Isomer Galβ(1–3)GlcNAc.

and intermolecular H-bond network, and the relative residence times of water molecules near the solute.

COMPUTATIONAL METHOD

We performed molecular dynamics simulations using the THOR package,[6] which is a simulation program initially developed to work with water as an effective medium. The program was updated to consider explicit solvent molecules[7] and to accomplish pressure control.[8] In this package, the mechanical evolution of the system is computed by solving the classic equations of motion using the leap frog algorithm.

An isothermal-isobaric ensemble is obtained by coupling the system to a thermal bath (T = 300 K). The system was allowed to equilibrate at atmospheric pressure (101,325 Pa). The constancy of density was used as a criterion for equilibrium, because average fluctuations do not exceed 1% (Figure 2).

After equilibrium was attained (50 ps), trajectories were collected each 20 fs for ulterior analysis. The simulation was carried out at 250 ps using an integration time step of 1 fs.

Simulations were performed using a Silicon Graphics Origin 2000 and a Pentium II 300. Portions of the analysis and graphics were performed using a personal computer.

The Model

The THOR program uses the GROMOS[9] force field for simulations, which have been highly reliable for simulation of carbohydrates in solution if a good choice of atomic partial

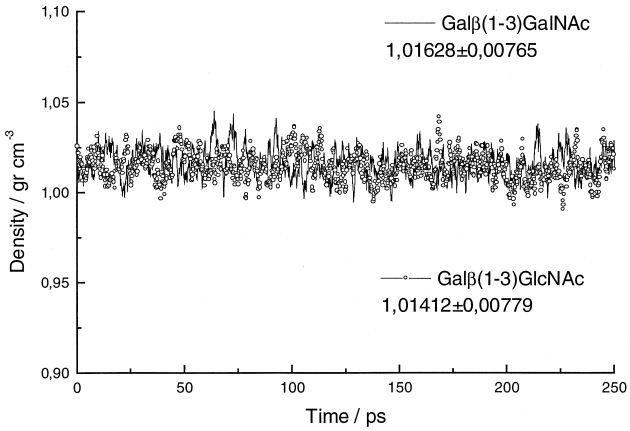

Figure 2. Mean density at T = 300 K.

charges is made. Care must be taken, especially when studying interactions with water. Oxygen atoms with small atomic partial charges would not be expected to hydrogen bond to solvent, and the molecule may behave essentially as a hydrophobic species.[10] In contrast, oxygen atoms with very high values could overestimate the hydration pattern. Atomic partial charges were calculated using the semiempirical AM1 method of the SPARTAN PRO program (Wavefunction Inc., Irving, CA) and are listed in Table 1.

Interactions between nonbonded atoms were represented with a 6-12 Lennard Jones potential, and the electrostatic interaction between the atomic partial charges was taken into account by adding a Coulombic term to the expression for effective potential.

Both isomers initially were kept in the $^4C_1$ molecular conformation, and improper torsion angles were applied to avoid conformational transitions to less probable states during the simulation. The $^4C_1$ conformation is highly probable because of the large number of equatorial hydroxyl groups in the molecules. No torsional restriction was applied to the glycosidic linkage when studying the equilibrium conformation of the disaccharides in solution. A proper torsional angle was applied to the C2′-N-C-O atoms to account for the resonance effect between the nitrogen atom and the carbonyl group (C═O). Two other torsional proper angles were applied to keep the planarity of the acetamido group.

The SHAKE procedure was used to keep bond lengths rigid, and solvent molecules were simulated using the SPC/E[11] model. Periodic boundary conditions were applied in all directions.

The tetrahedral geometry of carbons was maintained using improper torsion potentials. CH and CH2 groups of atoms were treated as united atoms, and only one center of interaction was considered. The interactions between bonded atoms in the solute were represented with bond and valence angle harmonic potentials. The nonbonded and long-range electrostatic interactions were truncated using a cut-off radius of 9 Å and a switching function that goes smoothly to zero when atom pair distance approaches the cut-off radius, thus avoiding discontinuity in the Coulombic potential term and any significant distortion in the structural properties of water.

The T-antigen molecule Galβ(1-3)GalNAc was simulated in a cubic box of initial size of 25 Å surrounded by 432 molecules of water (0.12 molal). For T-antigen, this should be considered

Table 1. Partial atomic charges for the disaccharide atoms obtained by the SPARTAN PRO program

Atom	Charge (e)
C1	0.260
C2	0.160
C3	0.160
C4	0.160
C5	0.140
C6	0.160
O5	−0.380
O2	−0.480
O3	−0.480
O4	−0.480
O6	−0.480
HO2	0.320
HO3	0.320
HO4	0.320
HO6	0.320
C1′	0.310
C2′	0.160
C3′	0.140
C4′	0.160
C5′	0.140
C6′	0.160
C	0.800
CH3	0.180
O5′	−0.380
O1′	−0.480
N	−0.600
O	−0.550
O1	−0.380
O4′	−0.480
O6′	−0.480
HO1′	0.320
HN	0.320
HO4′	0.320
HO6′	0.320

an infinite dilution because there are no solute–solute interactions. The same was done for the isomer Galβ(1-3)GlcNAc.

RESULTS

Radial Distribution Functions

Radial distribution functions were calculated for nitrogen and all oxygen atoms in both isomers. We will analyze separately those atoms belonging to the first and second units of monosaccharide.

Figure 3 shows the radial distribution functions of water oxygen atoms centered in the oxygen atoms of the sugar.

For the first pyranosic ring (Figure 3a), the radial distribution functions show the hydrophilic behavior characteristic of carbohydrates. The oxygen atom of the ring O5 and the glycosidic oxygen atom O1 present some structure probably due to the presence of the neighboring hydroxyl groups. The correlation function for the O5 atom displays a small-intensity peak, indicating the formation of H-bonds between water molecules and this atom in the case of the T-antigen. The radial distribution functions for O2, O3, O4, and the side group O6 show sharp peaks at an average distance of 2.7 Å, corresponding to the first hydration shell and typical for hydrogen bonds.

The intensity of the first hydration layer in the distribution function for O6 is higher than for the other oxygen atoms in this ring, suggesting a greater presence of water molecules near this atom. The hydration structure appears more well-defined and stronger for T-antigen than for its isomer.

Some appreciable differences appear in the second ring (Figure 3b). The most hydrophobic atom is the nitrogen of the acetamido group, although some structured water seems to be present for the T-antigen molecule.

In the side-chain oxygen atom O6′, the first peaks are of the same intensity but the isomer also has a well-defined second hydration layer.

The oxygen atom of the acetic group (carbonyl oxygen) reveals the presence of a well-structured hydration shell, but the intensity of the peak is not as high as the other oxygen atoms. The ring oxygen atom presents some degree of structure but, as in the case of the first unit, the peaks are mainly due to the hydration layers of the neighboring hydroxyl groups.

For the O4′ oxygen atom, the main difference between radial distribution functions in both isomers is in the first hydration shell. The T-antigen has a more defined and stronger hydration, which could be explained by the existence of an important hydrogen bond established between the O5 atom and the OH4′ hydroxyl group.

Conformation

T-antigen and the isomer molecules initially were in $^4C_1$ conformation, i.e., the values of the angles that define the chair conformation were approximately 130°. As stated previously, transitions to other conformational states are improbable because of the high number of hydroxyl groups in the equatorial conformation in both molecules.

The conformation of these two disaccharides can be characterized by two angles: ϕ (H1-C1-O1-C3′) and ψ (C1-O1-C3′-H3′). In our topological model for the disaccharide molecule, we worked with united atoms for CH groups, so the positions of hydrogen atoms were estimated, taking into account the tetrahedral character of the carbon atom. Figure 4a shows the values of these angles represented as a function of time. They also are given in a (ϕ/ψ) plot in Figure 4b.

Gilleron and coworkers[12] calculated the most probable conformations for these molecules, representing water as an effective medium with a dielectric constant $\epsilon = 80$. They found five possible conformations for ϕ/ψ angles. Our results, obtained using explicit water molecules, agree with the 30°/15° value. We also can see that the glycosidic linkage has greater mobility and flexibility for the antigen than for the isomer.

For Galβ(1-3)GlcNAc, we did not observe any transition to the conformation that is established when glycosidic C-H bonds of both pyranosic units form an angle of approximately 180°, with an H-bond between the OH4′ hydroxyl group and the OH2 hydroxyl group[13,14] (anti-conformation). No appearance of this H-bond could be explained because of the formation of a more stable bond between the O5 oxygen atom and the OH4′ hydroxyl group of the glucosidic unit.

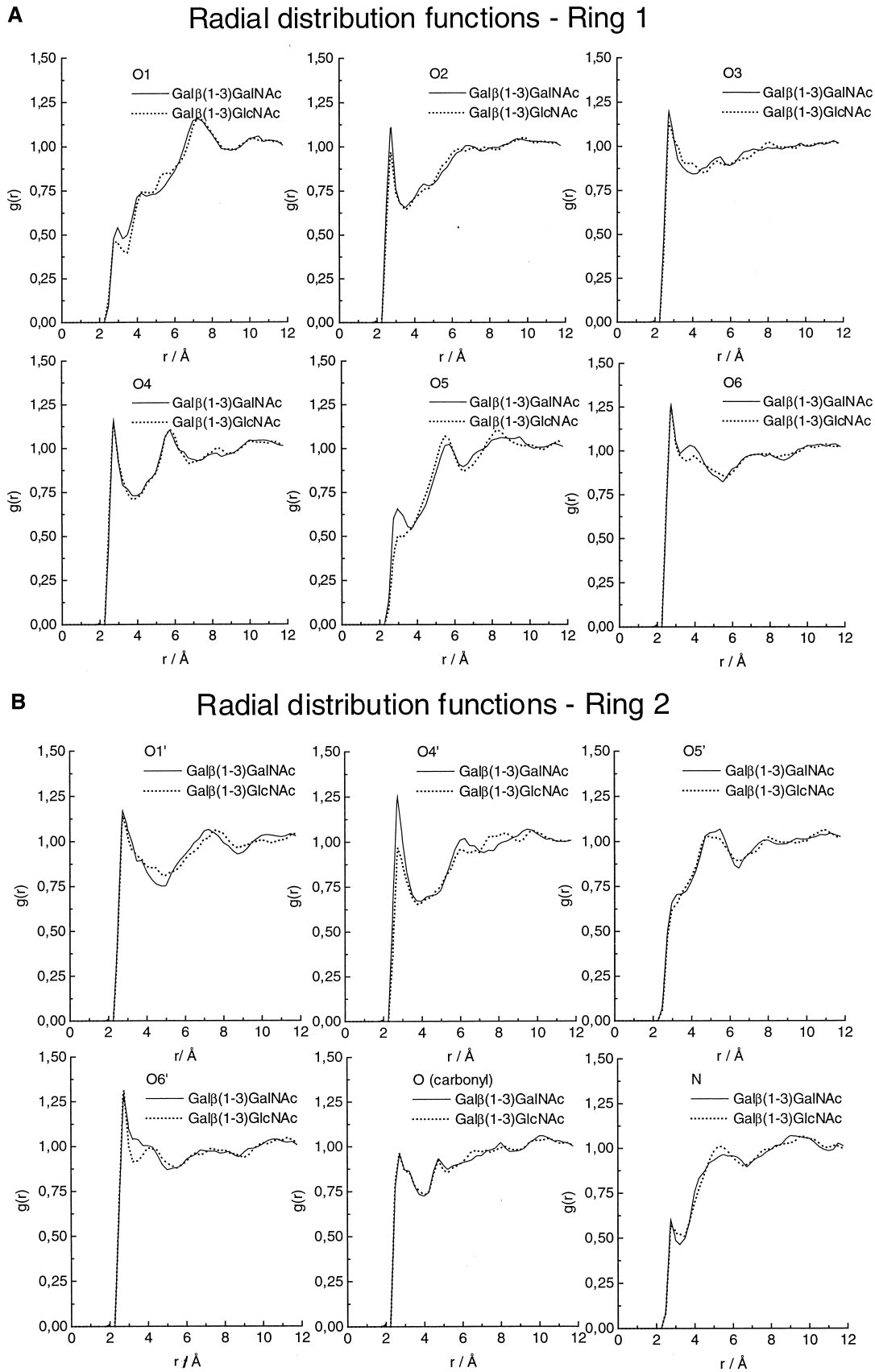

Figure 3. Radial distribution functions of water for the different oxygen atoms (and nitrogen atom) of the sugar molecules. (a) Pyranosic ring 1. (b) Pyranosic ring 2.

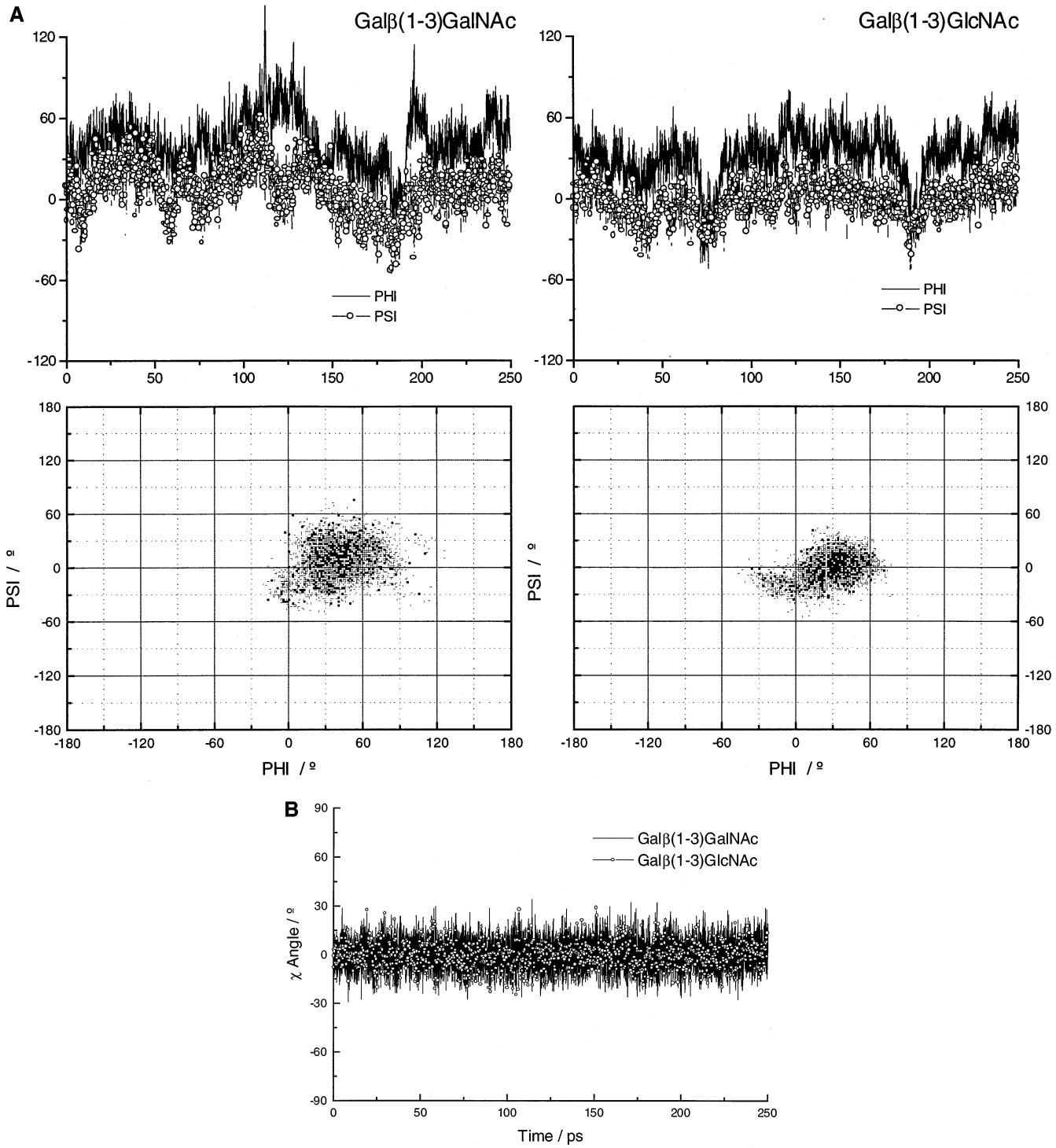

Figure 4. Schematic representation of glycosidic torsion angles ϕ and ψ. (a) Temporal evolution of dihedral angles. (b) ψ vs ϕ. (c) Temporal evolution of χ torsional angle.

The other dihedral angle that was monitored is the angle χ, which is defined by the atoms C2′-N-C-O of the acetamido group. A proper torsional potential was applied to these atoms to mimic the resonance effect of the N-C═O group. Two other improper angles were applied to maintain the planarity of this group of atoms. The first proper potential function has two minima, one at 0° and the other at 180°. During the 250 ps of simulation, both molecules remained in the same conformation, and no transitions from *trans* to *cis* were observed. Temporal evolution of χ is shown in Figure 4c.

Hydrogen Bonds

Sugars are polyhydroxylated compounds and have some properties similar to high polymers, probably due to the capacity to form multiple hydrogen bonds. To characterize the formation

of H-bonds, we used a geometrical criterion in which it is considered that a donor or acceptor oxygen atom forms a hydrogen bond when the distance between the acceptor and the hydrogen atom is less than 2.4 Å, and the H-O-H angle lies between 145° and 215°.[15,16]

The mean lifetime of H-bonds could be overestimated due to the discrete character of time in simulation processes. To minimize this error, we saved the configurations of the system at each 20 fs, which is a short compared with the mean lifetime of hydrogen bonds between water molecules of 150 fs.

The most significant intramolecular H-bonds are listed in Table 2. The lifetimes of these H-bonds are on order of tens of femtoseconds, possibly due to the interaction with water molecules and thermal agitation. We also observed the formation of H-bonds between the atoms of the side chain (CH_2OH) group and the OH4 group of the second monosaccharide unit with very short lifetimes.

Column "P" in Table 2 indicates the ratio (as a percentage) between the time the H-bond was found connected and the total simulation time. We use this number as a measure of the probability of occurrence of H-bonds.

The main difference between isomers is the formation of the more important H-bond between the OH4′ hydroxyl group and the ring oxygen atom O5 of the first moiety of Gal(1-3)GlcNAc. This hydrogen bond is well defined for the isomer of T-antigen, and it has a highly significant probability of occurrence (P) value of 44.64%.

Mean lifetimes for intermolecular H-bonds for both disaccharides are shown in Table 3. Their values are higher than for internal H-bonds, indicating a stronger interaction with solute than the molecule itself.

The histogram in Figure 5 shows the mean number of H-bond linkages formed with water by the T-antigen and by its isomer during the simulation. The maximum of the histogram corresponds to 11 water molecules forming H-bonds with the solute simultaneously in case of the isomer and 12 for the T-antigen. We also can see that the T-antigen establishes more H-bonds simultaneously than its isomer, indicating more hydrophilic-like behavior.

Residence Times

Residence time was calculated for all the oxygen atoms of the disaccharide molecules. It was computed as the time that a water molecule remains within a sphere of radius 3.5 Å centered in the position of the oxygen atom. That distance was chosen considering the average of the minima of radial distribution functions.

Table 2. Mean lifetimes of intramolecular H-bonds of the disaccharides

H-bond		Galβ(1-3)GalNAc		Galβ(1-3)GlcNAc	
Donor	Acceptor	Mean lifetime (ps)	P (%)	Mean lifetime (ps)	P (%)
O2	O	0.0543	0.304	0.0509	1.93
O6′	O4′	0.0422	0.152	0.0286	1.09
O4′	O6′	0.0300	0.432	0.0267	0.16
O4′	O5	—	—	0.1092	44.64

Table 3. Mean lifetimes (ps) of H-bonds of the disaccharides and water molecules

Atom	Galβ(1-3)GalNAc	Galβ(1-3)GlcNAc
O1	0.068	0.067
O2	0.103	0.095
O3	0.085	0.084
O4	0.087	0.083
O5	0.068	0.054
O6	0.100	0.096
O1′	0.081	0.077
O4′	0.086	0.075
O5′	0.059	0.055
O6′	0.099	0.091
N	0.098	0.104
O	0.093	0.091
Ow-Ow	0.150	

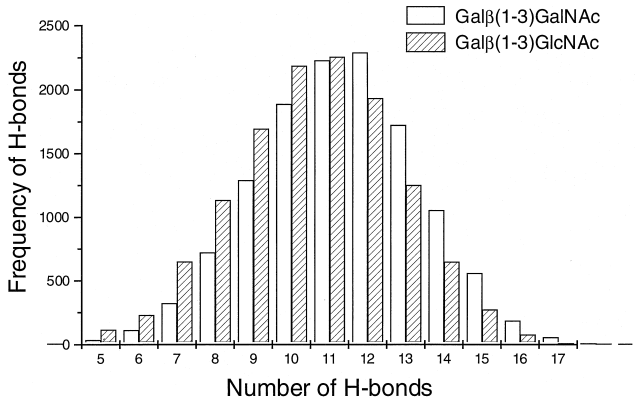

Figure 5. Histograms for intermolecular hydrogen bond distribution.

Table 4 shows the ratio between the residence time of water near a T-antigen oxygen atom (or isomer) and the residence time of water near another water molecule in the bulk. The factor is equivalent to the Samoilov[17] factor used to define positive ($R > 1$) or negative ($R < 1$) hydration. In our case, there was negative hydration for all atoms.

CONCLUSIONS

Carbohydrates can always make hydrogen bonds, but they can have either positive or negative hydration. The hydration properties of T-antigen and its isomer, as described by the simulations, exhibit hydrophilic behavior with negative hydration.

From analysis of the spatial correlation functions, it can be noted that a slight variation of the position of one hydroxyl group does not cause a dramatic change in the hydration pattern of these disaccharides, but this conclusion cannot be extended to other carbohydrates.[18] Further, we can conclude that the straight conformation adopted by these sugar molecules is mainly due to the interaction with water, as observed in simulations performed on other sugars.[19]

X-ray crystallography data[3] of PNA lectin complexed with

Table 4. Relative residence time ratio R of water near the sugar oxygen atom

Atom	Galβ(1-3)GalNAc	Galβ(1-3)GlcNAc
O1	0.5562	0.6431
O2	0.7395	0.6826
O3	0.6641	0.6276
O4	0.6968	0.7211
O5	0.5935	0.4847
O6	0.6313	0.6417
O1′	0.6375	0.6247
O4′	0.7692	0.6530
O5′	0.4700	0.4630
O6′	0.6191	0.6545
O	0.6584	0.6627
Water residence time (ps)	0.587	

R = $(\tau_{s\text{-}w})/\tau_{w\text{-}w}$). Residence time was calculated for SPC/E model in a sphere of radius 3.5 Å.

T-antigen reveal that the OH4′ group of T-antigen is involved in the formation of two H-bonds with Ser-211 and Gly-213, both residues belonging to the loop that determines specificity. The ring oxygen atom of galactose is involved in another H-bond, also with Ser-211. In the case of Galβ(1-3)GlcNAc, the internal H-bond established between the OH4′ group of glucose and the pyranosic ring oxygen of galactose might explain the difference of affinity between the two isomers, because some other interactions could be avoided.

The high flexibility of glycosidic linkage, as observed in our simulations, produces conformational heterogeneity, including the conformation adopted by the sugar inside the binding site. T-antigen dissacharide has an average conformation that is more flexible in water than within the binding site, where it adopts a specific conformation involving structural water H-bonds.[3]

Water-mediated hydrogen bonds between sugar and protein can be as strong as direct protein–sugar hydrogen bonds. In that case, the water molecules act as fixed structural elements, with long mean residence lifetimes. Because we obtained very short lifetimes for intramolecular and intermolecular H-bonds in solution (shorter than the water–water H-bond lifetime), we can conclude that water-mediated H-bonds are part of the binding site structure and are not a hydration property of these sugars.

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