Evidences that β-Lactose Forms Hydrogen Bonds in DMSO

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Glycoproteins and glycolipids play key roles in intracellular reactions between cells and their environments at the membrane surface. For better understanding of the nature of these events, it is necessary to know three-dimensional structures of those carbohydrates, involved in them. Since carbohydrates contain many hydroxyl groups which can serve both as hydrogen bond donors and acceptors, hydrogen bond is an important factor stabilizing the structure of carbohydrate. DMSO is an aprotic solvent frequently used for the study of carbohydrates because it gives detailed insight into the intramolecular hydrogen bond network. In this study, conformational properties and the hydrogen bonds in β-lactose in DMSO are investigated by NMR spectroscopy and molecular dynamics simulations. NOEs, temperature coefficients, deuterium isotope effect, and molecular dynamics simulations proved that there is a strong intramolecular hydrogen bond between O3 and HO2' in β-lactose and also OH3 in β-lactose may form an intermolecular hydrogen bond with DMSO.

Key Words : β-Lactose, DMSO, NMR spectroscopy, Molecular dynamics simulation, Hydrogen bond

Introduction

Control of important biological processes such as growth and defence against invading organisms, requires that a cell interacts with its environment at the level of its external membrane surface.[1-3] The interactions between cell and its environment include biological processes such as cell-cell recognition, intercellular adhesion, growth, and the defence against invading organisms.[1-3] For better understanding of the nature of these events, it is necessary to know three-dimensional structures of the carbohydrates, involved in them. Even small difference in glycoprotein or glycolipid residues or their primary sequence can cause significant changes in the rate, the way or the products of the biochemical reaction.

Lactose (4-o-β-D-galactopyranosyl-β-D-glucopyranoside) contains β-1-4 linkage and consequently possesses two variable torsion angles, φ and ψ, about the glycosidic linkage. β-lactose is a recognition signal for lectins such as ricin which recognizes galactose-terminated oligosaccharides, specifically at the membrane surface.[4,5] Also, β-lactose unit can be found in many kinds of ganglioside and glycolipids as a recognition signals.[6,7] Therefore, it is important to investigate the structure and the hydrogen bond of β-lactose.

Hydrogen bonds are often quite important in determining the conformations of carbohydrates. These interactions are 3-6 kcal/mol in magnitude, are much weaker than typical chemical bonds; however, they are at least an order of magnitude larger than the strongest van der Waals interactions, and substantially larger than kT at room temperature.[8,9] Furthermore, these interactions are both short range and angle-dependent. Since carbohydrates contain many hydroxyl groups which can serve both as hydrogen bond donors and acceptors, hydrogen bond is an important factor stabilizing the structure of carbohydrate.[8,9]

Since intramolecular hydrogen bond plays a fundamental role in the determination of the active conformations of carbohydrate, and thus the finding of convenient structuring solvents that favor intramolecular hydrogen bond s allows us to study more easily these active forms. In this regard, a lot of works have been done for carbohydrates in DMSO.[6,7] Because spectra of carbohydrate in H_2O suffer from peak overlapping with the huge water signal, carbohydrates are usually dissolved in D_2O for NMR studies. Since hydroxyl protons in carbohydrates are exchanged rapidly with D_2O, it is impossible to observe the NMR signals of hydroxyl protons. Therefore, water is not a good solvent to study the intramolecular hydrogen bond of carbohydrates. In order to study intramolecular hydrogen bonds in carbohydrates, DMSO is an aprotic solvent frequently used for the study of carbohydrates because it gives detailed insight into the intramolecular hydrogen bond network.

Functions of cabohydrates often involve the binding of a protein to an oligosaccharide receptor anchored to the membrane as a part of an integral glycoprotein or glycolipid. They also take part in some intracellular reactions for these interactions at the membrane surface. Since many kinds of carbohydrates such as ganglioside and glycolipids are not water-soluble, DMSO which has high polarity, high dielectric constant, small size and can dissolve those carbohydrates, is most popular solvent to study of those carbohydrates.[6,7]

NMR is a good method to obtain structural data of carbohydrate in solution where motional variations are less restricted than in crystals.[10-14] Also, in many cases, the crystal structures of carbohydrates are not available. Because of severe spectral overlaps, NMR experiments do not provide enough NOE distance constraints to allow a complete structure determination of carbohydrates.[10-14] Sometimes,

theoretical modeling of carbohydrates can provide more informations.[15-25] However, NMR techniques such as chemical shift variation, temperature coefficients, nuclear Overhauser effects, and coupling constant have been used to investigate the hydrogen bonds in carbohydrate structures.[26-30]

Previously, we reported the flexibilities in the structure of β-lactose which can be a receptor for protein at the membrane surface are studied using the adiabatic potential map and molecular dynamics simulations.[15] Adiabatic energy map shows that lactose can exist among the various conformations. While the lowest energy structure in vacuum adiabatic energy map has Φ=165° and Ψ=8° (L1), the lowest energy structure in the adiabatic energy map in $\varepsilon = 50$ has Φ=61°, $\Psi = -3°$ (L2). L2 agrees with NMR solution structure in water and crystal structure. In these calculations, no intramolecular hydrogen bond has been found for β-lactose in water. Water molecule is well known to make strong hydrogen bond with carbohydrate in the aqueous solution because it is very polar and small enough to be inserted deep into the disaccharide and weaken the intramolecular hydrogen bonds in carbohydrates.

Here, we studied the hydrogen bonds existed in the structure of β-lactose in DMSO. DMSO is known to increases the ability of hydroxyl groups in carbohydrates to participate in intramolecular hydrogen bonding. In this study, we have utilized molecular dynamics simulations in DMSO box in conjunction with NMR spectroscopy to examine intramolecular hydrogen bonds in β-lactose in DMSO.

Experimental Section

Nomenclature. Figure 1 shows the naming scheme of the β-lactose molecule. Flexibilities around the glycosidic linkages are described by torsion angles: ϕ and ψ defined by H1'-C1'-O4-C4 and C1'-O4-C4-H4, respectively. χ1 and χ2 is O5'-C5'-C6'-O6' and O5-C5-C6-O6.

NMR Experiments. β-lactose was purchased from Sigma. The NMR samples for the resonance assignment were dissolved in 100% DMSO-d_6 and 12.5 mM sample was made in 0.45 mL. NMR experiments were performed at 30 °C on a Bruker AVANCE 400 MHz spectrometer at Konkuk University and 500 MHz spectrometer at KBSI. All the data were processed off-line using FELIX software on SGI workstation in Department of Chemistry at Konkuk University.[31] For spectral assignments a double quantum filtered COSY spectrum was obtained using time proportional phase incrementation (TPPI) with spectral width of 3205.128 Hz, 2048 t_2 point, and 600 t_1 point.[32] We collected a ^{1}H-^{13}C heteronuclear multiple quantum coherence (HMQC) spectrum and heteronuclear multiple bond correlation (HMBC) to aid the spectral assignments.[33-35] 2D ^{1}H-^{1}H phase sensitive NOESY and ROESY experiment was performed with mixing times of 600 and 800 msec.[36,37] In order to calculate temperature coefficients, chemical shifts were measured every 5 °C from 298 K to 318 K.

In order to calculate temperature coefficients, chemical shifts were measured every 5 from 298 K to 318 K. Sample for the measurement of the deuterium isotope effect on ^{1}H and ^{13}C chemical shifts was deuterated and dried by lyophilizing from D_2O/acetone solutions and then dissolved in dry DMSO-d_6. Deuterium isotope effects were measured by the magnitudes of the ^{13}C chemical shift variation from the isotope effects.

Molecular Dynamics Simulations of β-Lactose. In order to investigate the dynamic behavior of the β-lactose, molecular dynamics simulation on β-lactose in DMSO was proceeded. All calculations were performed with CHARMM (Chemistry at HARvard Macromolecular Mechanics). The potential function of CHARMM is as follows[38]

$$V(q) = \sum k_{bi}(r_i - r_{0i})^2 + \sum k_{\theta i}(\theta_i - \theta_{0i})^2 + \sum k_{UBi}(s_i - s_{0i})^2 + \sum k'_{UBi}(s_i - s_{0i})^2 + \sum k_{\phi_i}[1 + \cos(n_i\phi_i - \delta_i)] + \sum k_{\omega i}(\omega_i - \omega_{0i})^2 + \sum \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right) + \sum \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}$$

This equation contains the terms such as bond energy, angle energy, dihedral energy, Urey-Bradley 1-3 interaction energy, improper energy, electrostatic energy, and van der Waals energy. Hydrogen bond energy term is not handled separately, but treated as nonbonding interactions in the CHARMM potential. Parameters used here are available through QUANTA.[31]

The conformational behavior of β-lactose was examined through the dynamics simulation with explicit DMSO molecules. For the simulations a cubic DMSO box (density 1.1) consisting of 125 DMSO molecules with a length of 24.52 Å in each dimension was set-up. Parameters for DMSO molecules were adopted from the reference.[37] They were equilibrated for 40 ps and 1 ns molecular dynamics simulations were performed before carbohydrate molecule was positioned in the center of the DMSO box.[22,38-40] Adiabatic energy map of β-lactose in vacuum was calculated

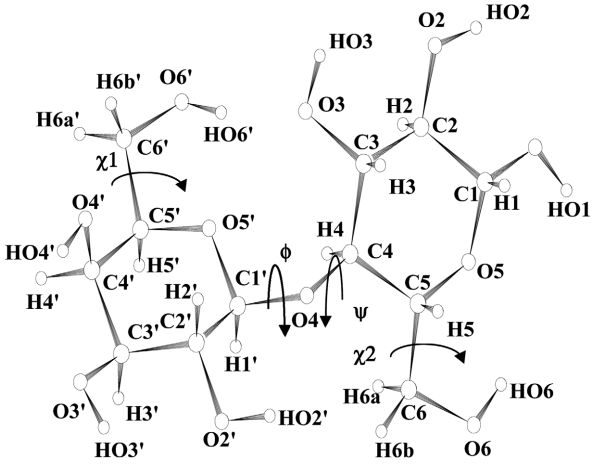

Figure 1. Naming schemes of β-lactose in the vacuum map. The dihedral angles, ϕ, ψ, are defined as H1'-C1'-O4-C4, C1'-O4-C4-H4.

Table 1. Conformational features and relative energy of the low energy conformations of β-lactose found on the vacuum energy map

	L1	L2	NMR data In DMSO
ϕ (°)	164.8	64.6	
ψ (°)	1.8	−17.4	
χ_1 (°)	45.6	41.6	
χ_2 (°)	−50.9	45.1	
H1'-H4 (Å)	3.5	2.4	[a]
HO6'-H4 (Å)	2.5	3.2	2.6
HO2'-H4 (Å)	3.2	4.2	3.4
HO2'-HO3 (Å)	2.6	6.1	3.0
HO6'-H6a (Å)	2.8	5.5	2.7
HO6'-H6b (Å)	3.7	6.0	4.0
HBond (Å)	HO2'-O3 (1.9) HO6'-O6 (2.0)	HO6'-O3 (2.0)	HO2'-O3 (2.6)
Relative energy (kcal/mol)	0.0	4.93	

[a]Distance can not be measured because of the peak overlapping.

and it has five low energy minima as reported previously.[13] The lowest energy structure L1 as listed in Table 1 satisfies well the NMR data of β-lactose in DMSO. L2 was the lowest energy structure in adiabatic energy map calculated with a dielectric constant of 50 and it represents the NMR data of β-lactose in water. Therefore, L1 was utilized in generating initial geometries of β-lactose in DMSO. All solvent molecules closer than 2.8 Å to any heavy atom of the carbohydrate molecule were deleted. 5000 cycles of ABNR energy minimization were carried out, keeping the carbohydrate harmonically constrained to its original structure. During the whole simulations, minimum-image periodic boundary conditions were used to eliminate edge effects. The simulation involved an equilibration period of 40 ps and the production run was performed for 1 ns, from which the dynamics trajectory was obtained for the conformational analysis. Simulations and analysis were performed on a SGI O2 workstation and Cray-C90 supercomputer at SERI.

Results and Discussion

Resonance Assignment and NOE Measurement. The proton resonance assignment in β-lactose in DMSO was proceeded on the basis of DQF-COSY and HMQC spectrum shown in Figure 2. Heteronuclear correlated experiments such as HMQC and HMBC were used to complete assignment. Table 2 lists ^{1}H chemical shifts and coupling constant data of β-lactose. On the top of the Figure 2 assignment of the ^{1}H and ^{13}C spectrum is shown. As shown in this spectrum chemical shifts of most of the ring protons are crowded between 3.0 and 3.5 ppm except the anomeric protons.

Distance information derived from NOE data should lead to definition of the solution structure. The distance information obtained from NOESY and ROESY experiments are

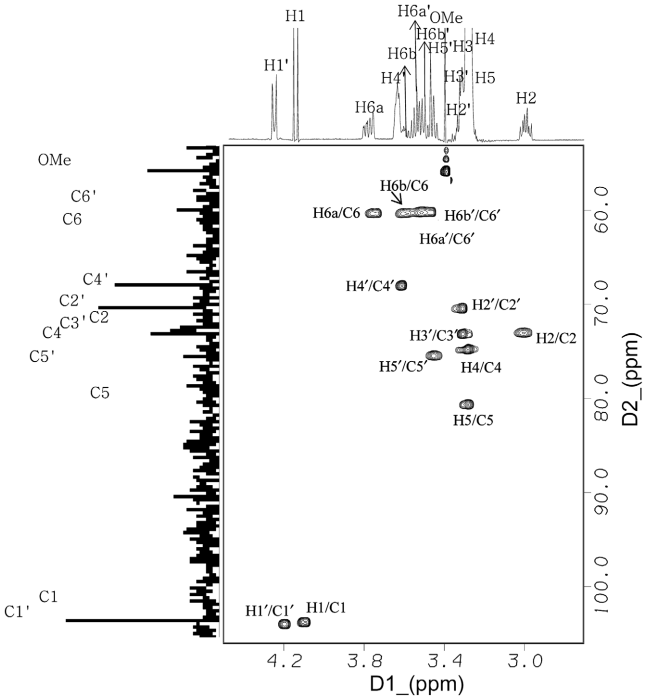

Figure 2. Phase sensitive, ^{13}C-decoupled, ^{1}H detected multiple quantum correlation (HMQC) spectrum of 12.5 mM β-lactose in DMSO-d_6 at 303 K. Assignment of ^{1}H NMR spectrum of β-lactose is shown on the top and assignment of ^{13}C NMR spectrum is shown on the left of the HMQC spectrum.

Table 2. Chemical shifts and scalar couplings of β-lactose in DMSO-d_6

proton	δ(ppm)[a]	proton pair	^3J (Hz)[b]
H1'	4.20	H1'-H2'	7.8
H2'	3.33	H2'-H3'	
H3'	3.31	H3'-H4'	3.1
H4'	3.61	H4'-H5'	
H5'	3.45	H5'-H6a'	
H6a'	3.53	H5'-H6b'	
H6b'	3.49	H6a'-H6b'	
HO2'	5.06		
HO3'	4.75		
HO4'	4.48		
HO6'	4.62		
H1	4.10	H1-H2	9.3
H2	3.00	H2-H3	9.3
H3	3.31	H3-H4	
H4	3.29	H4-H5	
H5	3.28	H5-H6a	3.1
H6a	3.75	H5-H6b	3.2
H6b	3.61	H6a-H6b	12.5
HO2	5.14		
HO3	4.65		
HO6	4.54		
OMe	3.39		

[a]Chemical shift of the DMSO peak was set at 2.50 ppm. [b]Digital Resolution is 0.0061 Hz/point (1D) and 0.195 Hz/point (2D).

Table 3. Distances obtained from nOe of β-lactose in DMSO-d_6

proton pair	distance (Å)
H1-H2[a]	2.9
H1-H5	2.9
H1-H6a	3.7
H1-H6b	3.8
H1'-H3'	2.3
H1'-H4'	3.2
H1'-H4	a
H3-HO2	2.8
H3'-HO3'	2.4
H3'-HO4'	2.9
H4-HO2'	3.5
H4-HO6	2.6
H4-HO6'	2.6
HO6'-H6a	2.7
HO6'-H6b	4.0
HO3-HO2'	3.0
HO3-HO2	3.9
HO4'-HO3'	2.8

[a]The H1-H2 distance was set to 2.89 Å. [b]Distance can not be measured because of the peak overlapping.

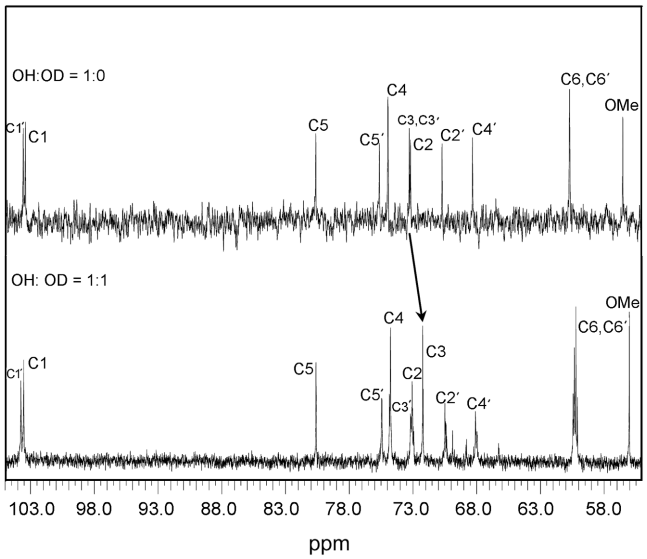

Figure 3. 500 MHz ^{13}C NMR spectrum of the β-lactose in DMSO-d_6 at OH:OD ratios of 1 : 0 and 1 : 1. Hydroxyl protons in β-lactose were deuterated and dried by lyophilizing from D_2O/acetone solutions. Then, β-lactose was dissolved in dry DMSO-d_6. Arrow shows the deuterium isotope effect on ^{13}C NMR spectrum of C3 by measurement of the magnitudes of the ^{13}C chemical shift variation from the isotope effects.

listed in Table 3. For the calibration, distance between H1-H2 was set to 2.89 Å and this distance is the value in charmm-minimized structures. Particular interest should be imposed on the protons around the glycosidic linkage. Distance between HO2'-HO3 was 3.0 Å and this result means that there may be a hydrogen bond between HO3 and HO2' hydroxyl groups. NOEs between H1' and H4 were observed but there were severe peak overlapping. Therefore, accurate distance between H1'-H4 were not measurable. Distance between HO6'-H4 were 2.6 Å and that between HO6' and H6a were 2.7 Å. These results obtained from NMR experiments satisfied well the distances measured from L1 conformation as listed in Table 1.

Table 4 also shows the temperature coefficients and coupling constant of hydroxyl groups of β-lactose in DMSO. A reduction in temperature susceptibility (ppb/K) has been commonly accepted as an indicator of reduced interaction with solvent, due to intramolecular hydrogen bonding. A very small temperature coefficient and coupling constant are observed for HO3 proton compared to other hydroxyl protons. This may correspond to a transfer of electron density from the OH bond as a result of hydrogen bonding with the other atoms as electron donors.

According to the our previous work on GlcNAc(β1,3)-Gal(β)OMe, it was found that the hydroxyl group acting as donor exhibits an isotope shift in ^{1}H NMR spectrum, when the hydrogen atom in hydroxyl group as an acceptor is replaced by deuterium.[24] The ^{13}C NMR spectrum of β-lactose with fully deuterated hydroxyl groups in DMSO-d_6 exhibits shifted resonance signals for C3 compared to that of β-lactose with fully protonated hydroxyl groups in DMSO-d_6 as shown in Figure 3. Isotope effect observed for C3 resonance is transmitted through hydrogen bondings between OH3 hydroxyl group and other atoms. This isotope effect observed for C3 is caused by blocking formation of hydrogen bond between OH3 hydroxyl group and other atoms by deuterium substitution of OH3. There were no isotope effects found for the other carbons. NMR data can prove that there are strong hydrogen bondings including OH3 hydroxyl group, but it is not enough to determine the exact location and the stability of hydrogen bond. Since deuterium in DMSO-d_6 is not labile, hydroxyl protons in β-lactose can not be exchanged with them. However, deuterium in D_2O can be exchanged with protons in β-lactose. Therefore, compared to NMR experiments of β-lactose in water, those in DMSO can give useful information about hydrogen bonds in β-lactose as shown in the present study.

Molecular Dynamics Simulations in DMSO box. In order to investigate the hydrogen bond existed in DMSO,

Table 4. Temperature coefficients and coupling constants of hydroxyl group for β-lactose in DMSO-d_6

	HO2'	HO3'	HO4'	HO6'	HO2	HO3	HO6
temperature coefficient (ppb/K)	−8.97	−11.21	−9.43	−7.84	−11.53	−3.55	−9.53
coupling constant (Hz)	4.19	5.24	4.71	6.28*	5.24	0.91	5.76[a]

[a]Pseudo-triplet for HO6 signals due to coupling to the methylene protons.

Table 5. β-lactose's conformational features obtained from averaged dynamics trajectories generated in DMSO box

	L1	L2	NMR data In DMSO
ϕ^b (°)	166.8(± 5.7)[a]	63.9(± 8.2)	
ψ (°)	2.7(± 6.9)	−9.7(± 8.3)	
χ_1 (°)	19.0(± 13.5)	24.8(± 11.5)	
χ_2 (°)	−51.0(± 7.5)	−7.9(± 16.3)	
H1'-H3[c] (Å)	4.6(± 0.1)	4.5(± 0.1)	
H1'-H4 (Å)	3.5(± 0.1)	2.5(± 0.1)	
H1'-H5 (Å)	4.5(± 0.1)	4.1(± 0.2)	
O3-HO2'--O2'	1.9 Å (± 0.1) 135.5° (± 12.7)[b] 0.52[c] (1.9 Å, 144.9)[d]	5.6 Å (± 0.3)	2.6 Å (HO2'-HO3)
O6-HO6'--O6'	2.0 Å (± 0.2) 143.9° (± 12.6) 0.73 (2.0 Å, 149.1°)	6.4 Å (± 0.4)	2.7 Å (HO6'-H6a)
O3-HO6'--O6'	5.9 Å (± 0.2)	2.0 Å (± 0.3) 156.6° (± 9.1) 0.95 (2.0 Å, 156.9°)	

[a]Numbers in the parenthesis are the rms deviations from the averaged values. [b]Angles are in degree. [c]Occurance probability that bond distance is less than or equal to 2.5 Å and angle is greater than or equal to 135°. [d]Average distance (Å) and angle (degree) during bond distance is less than or equal to 2.5 Å and angle is greater than or equal to 135°.

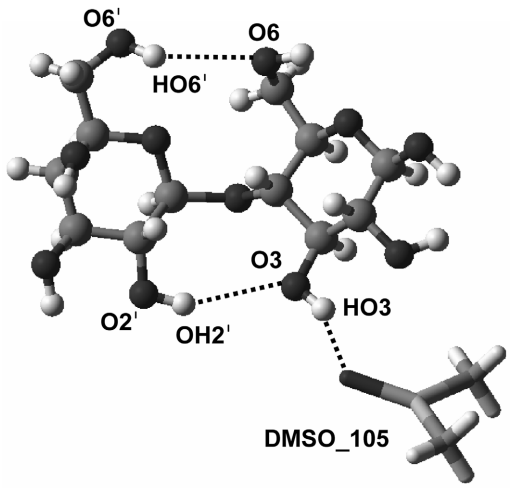

Figure 4. Hydrogen bond network of β-lactose in DMSO box. Stable intramolecular hydrogen bonds and the intermolecular hydrogen bond with DMSO are shown with dotted lines.

molecular dynamics simulations with explicit DMSO molecules were performed. Table 5 lists a set of the selected torsion angles and distances of the low energy conformers of β-lactose located on the energy map generated in vacuum. Table 5 shows the conformational features of β-lactose, L1 and L2, obtained from the average dynamical trajectory generated in DMSO box. As listed in Table 5, L1 at ϕ = 166.8° and ψ = 2.7° has two hydrogen bonds of O3-HO2' with a distance of 1.9 Å and O6-HO6' with a distance of 2.0 Å. And L2 at ϕ = 63.9° and ψ = −9.7° has only one hydrogen bond O3-HO6' with a distance of 2.0 Å. L1 reproduces the structural constraints determined by NMR experiments such as HO2'-HO3 distance of 2.6 Å and HO6'-H6a distance of 2.7 Å. Also temperature coefficient and coupling constant of HO3 was very small in Table 4. Temperature coefficients of HO2' and HO6' are also smaller than the other hydroxyl proton. As a result, MD trajectory in DMSO fluctuates near L1 state in the adiabatic energy map and satisfies the experimental NMR data well. The intramolecular hydrogen bond HO2'-O3 in the final shot of MD simulation of β-lactose is shown in Figure 4. In order to understand the details about the hydrogen bonds observed during the simulation, we looked at the occurrence of the hydrogen bonds as listed in Table 5. We calculated the occurrence of the hydrogen bond by counting how frequently the distance between the hydrogen atom and the acceptor was r_{ha} < 2.5 Å and the angle between the donor, the hydrogen atom and the acceptor is greater than 135°, simultaneously. Hydrogen bond between O3-HO2'--O2' is frequently observed with an occurrence of 0.52 and is also strong as measured by the distance of 1.9 Å and the angle of 144.9°. Interestingly, HO3 forms an intermolecular hydrogen boding with oxygen in DMSO_105 molecule with an occurrence of 0.40 within the distance of 2.5 Å. Therefore, deuterium isotope effects observed in C3 resulted from these stable hydrogen bonding network including intramolecular hydrogen bond between O3 and HO2' or intermolecular hydrogen bond between HO3 and DMSO.

Conclusion

Hydrogen bond is an important factor in the structures of carbohydrates. Because of great strength, short range, and strong angular dependence, hydrogen bonding is an impor-

tant factor stabilizing the structure of carbohydrate. Structures of carbohydrates such as gangliosides and many different kinds of disaccharides or trisaccharides have been determined in water or DMSO by NMR spectroscopy.[8-12,23-27] Water molecule is well known to make strong hydrogen bond with carbohydrate in the aqueous solution because it is very polar and small enough to be inserted deep into the disaccharide and weaken the intramolecular hydrogen bonds in carbohydrates. Hydroxyl protons are exchanged rapidly with water and hydrophobic carbohydrates such as glycolipids and ganliosides are not soluble in water but soluble in DMSO. Therefore, DMSO is often used to study intramolecular hydrogen bonds in carbohydrate. MD trajectory in DMSO fluctuates near L1 state in the adiabatic energy map and satisfies the experimental NMR data well. Molecular dynamics simulation in conjunction with NMR experiments proves to be efficient ways to investigate the intramolecular hydrogen bonding existed in carbohydrate in DMSO. Results obtained from NMR experiments proved the presence of hydrogen bonds in β-lactose. However, they did not give information to discriminate between the donor and acceptor hydroxyl groups or to provide a basis for comparison of the relative strengths of hydrogen bonds in β-lactose. Molecular dynamics simulations in explicit DMSO molecules provided this information. By using the data obtained from the NOEs, temperature coefficients, coupling constant, deuterium isotope effect, and molecular dynamics simulations, we can conclude that there are stable intramolecular hydrogen bonding between O3 and HO2' in β-lactose and intermolecular hydrogen bonding between HO3 and DMSO. Molecular dynamics simulation in conjunction with NMR experiments proves to be efficient way to investigate the intramolecular hydrogen bonding existed in carbohydrate.

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