Molecular Simulations and Conformational Studies of Fucose(α1-3)Gal(β1-X)GlcNAc where X=3, 4, or 6 Oligosaccharides

Eunsun Yoo[*] and Inmo Yoon[†]

Dept. of Oriental Medicine Industry, [†]Dept. of Game Animation, Honam University, Gwangju 506-714, Korea
[*]E-mail: yooeun@honam.ac.kr
Received March 2, 2008

Energy minimization and conformational studies of molecular ions generated by ESI (electrospray ionization) tandem mass spectrometry (MS/MS) can be used for the discrimination of stereoisomeric permethylated and sodium cationized trisaccharides. Sets of fucose-containing trisaccharides having different internal and terminal linkages have been synthesized to analyze the reducing terminal linkage positions using BT and IT fusion approaches. A detailed investigation has been undertaken on the conformational behaviors of four trisaccharide fragments from human milk and blood group determinants of Type 1 and Type 2, namely Fuc(α1-3)Gal(β1-3)GalNAc and Fuc(α1-3)Gal(β1-X)GlcNAc where X = 3, 4 and 6 using molecular modeling methods. Three dimensional rigid and adiabatic phi-psi-energy maps (Surfer program) describing the energy as a function of rotation around corresponding glycosidic linkages were calculated by SYBYL molecular modeling and MM4 force field programs conjunction with cleavage energies of ESI MS/MS for the side group orientations. This approach predicted conformational behaviors exhibited by isomer saccharides for future applications on biologically active glycoconjugates and to exploit a faster method of synthesizing a series of structural isomeric oligosaccharides.

Key Words : Molecular modeling, Fucose, ESI tandem mass spectrometry

Introduction

L-Fucose residues in complex carbohydrates are widely distributed in mammalian glycolipids and glycoproteins and play important roles in a variety of biological processes. Fucoses are usually found in terminal positions linked through an α-glycosidic linkage.[1,2] Given their terminal location, it is easy to understand why fucoses have been implicated in many cell-cell signaling and cell-cell adhesion events and in mediating leukocyte trafficking and recruitment to inflammatory sites.[1,2] Enzymatic synthesis using fucosyltransferase is an alternative and efficient method of preparing fucose-containing oligosaccharides. The syntheses of oligosaccharides which are modified at a specific position are gaining interest. The availability of such molecules can provide further insights into their biological functions and might lead to the discovery of novel carbohydrate-based therapeutics.[3,4] Biosynthetically fucosyltransferases act on very large and complex glycoproteins and glycolipids, therefore small molecule acceptors, such as linkage isomeric oligosaccharides might be viewed as non-natural acceptors. In this study, however, we considered a change of linkage position between sugar units as creating several non-natural acceptors.

The forces stabilizing the conformations of complex carbohydrates including small and oligosaccharides have been the subject of newly introduced study by both theoretical techniques of computer modeling and by various experimental methods such as NMR (Nuclear Magnetic Resonance), X-ray crystallography, CD (Circular Dichroism) and MS studies.[5-8] Several important forces have been identified and each force is represented in the terms of the various force fields that are used in computer modeling studies. The interplay of forces stabilizing the conformations of complex carbohydrates of glycoproteins and glycolipids has been less studied than have the interactions stabilizing peptide and/or nucleic acid conformations.[9] The dominant forces in oligosaccharides seem to be somewhat different from those in proteins and nucleic acids. Although hydrophobic effects are thought to play a substantial role in stabilizing many peptide conformations, their clear dependence on the solvent structure makes it difficult to include them in a force field representing the peptide alone. In the case of nucleic acids, the effects of polarizability of the aromatic heterocyclic bases are also added to the electrostatic dipole effect.

The NMR and CD experimental studies[5-9] indicate that van der waals effects are dominant in carbohydrates and that electrostatics, hydrogen bonding and hydrophobic effects are less important. NOE (Nuclear Overhauser Effect) data on human milk and blood group oligosaccharides having four to six sugar residues can be harmonized with single low-energy conformations determined mainly by van der waals interactions rather than by hydrogen bonding and electrostatic effects.[1] In this study, the molecular modeling combined with MS/MS experiments was used to rationalize relative energy states and also degrees of bond strength of isomer oligosaccharides obtained from tandem mass spectrometry (MS/MS) fragmentation ratios. The compounds chosen this study were fucose(α1-3)Gal(β1-3, 4, or 6)GlcNAc. A variety of the glycoconjugates having galactosyl β1-3 and β1-4 linked to a GalNAc or GlcNAc residue were

related to terminal groups likely to be found on human milk and blood group glycoproteins and glycolipids. Trisaccharides, having at least one reducing end, non-reducing end and internal monosaccharide moieties, offer minimum units to study differences of internal linkages in a linear chain of sugars. Threshold energies imparted to oligosaccharide ions in collision cells of tandem mass spectrometers may give different patterns of fragmentation based on differential ability of isomers to absorb and dissipate vibrational energy due to aminosugar of reducing end. Linkage position among other structural parameters of saccharide may be the most sensitive to variance in vibrational freedom, particularly rotation, due to the different steric hindrance and degrees of freedom of motion between sugars.

The computer program used herein, modified MM4,[10] is one of the molecular mechanics programs that optimize the atomic coordinates of a molecule to produce a structure at a local minimum on a multidimensional hypersurface of potential energy. It includes potentials for bond stretching, bending, and stretch-bending, 3-fold torsional potentials, van der waals interactions, and dipole-dipole interactions. This modified version has "dihedral driver" facility which accepts the initial, final, and increment size values of two tortsional angles and energy-minimization at each increment of these torsion angles. It permits conformational analysis of a disaccharide by rotating of glycosidic bond. The MM4 calculation has earlier prove to give results in good accord with the conformational properties of oligosaccharides in solution as reflected from their NMR data.[5-9] The primary structures of the fragments of the human milk and blood group oligosaccharides used in present study are given in Table 1.

There were four trisaccharides serving as models for the nonreducing or reducing terminal fragments of human milk or blood group structures (Table 1). These structures were chosen because of the availability of NMR data including NOE and T1 data.[11,12] In human milk and blood group related oligosaccharides, all the sugar residues existed in the pyranose form and belong to the D series, except for fucose, which is an L sugar. Our previous reports using FAB (Fast Atom Bombardment) CID MS/MS[13-16] and sialic acid containing oligosaccharide using ESI CID MS/MS[17] suggested that the rationale for most of the fragmentation (daughter ion) assignment was due to steric factors. To rationalize the results in the ESI CID MS/MS studies, SYBYL and modified MM4 molecular modeling programs were also used to calculate minimum energy structures of the permethylated MFX series. Earlier, the modified MM4 calculation was proven to provide results consistent with the conformational properties of oligosaccharides in solution as reflected from their NMR data.[18,19]

Table 1. Oligosaccharide fragments used in the molecular modeling study

No.	primary structures of oligosaccharide[a]
MF2	permethylated Fuc(α1-3)Gal(β1-3)GalNAc
MF3	permethylated Fuc(α1-3)Gal(β1-3)GlcNAc
MF4	permethylated Fuc(α1-3)Gal(β1-4)GlcNAc
MF6	permethylated Fuc(α1-3)Gal(β1-6)GlcNAc

[a]all monosaccharide units are D sugars except for fucose which is an L sugar.

Experimental Section

Materials. The linkage-isomeric trisaccharides were synthesized as described previously[13,15] and characterized by ^{13}C NMR. The saccharides, Gal(β1-3)GalNAc, Gal(β1-3)-GlcNAc, Gal(β1-4)GlcNAc, Gal(β1-6)GlcNAc, GDP-fucose and fucosyl(α1-3)transferase were purchased from Sigma Chemical Co. and CalBiochem. All other chemicals were reagent grade quality. The synthetic trisaccharides have the three possible different linkage positions of terminal galactose to *N*-acetylglucosamine (GlcNAc). The trisaccharides were permethylated by the method of Ciucanu and Kerek[20] and dissolved in chloroform. The permethylated derivatives of the trisaccharides are in Table 1.

Synthesis of trisaccharides. The reaction mixture contained 20 mM disaccharide (Gal(β1-3)GalNAc, Gal(β1-3)GlcNAc, Gal(β1-4)GlcNAc, Gal(β1-6)GlcNAc) as substrate, 0.50 mM GDP-fucose, 0.2 mg/mL α-lactoalbumin and $MnCl_2$ with 1 unit of EC 2.4.1.65. 50 mM sodium cacodylate in a total volume of 500 μL. Assay mixtures were prepared in ice and the reaction started by the addition of disaccharide. After incubation (3 hrs, at 37 ^{o}C), the reaction was stopped by cooling to 0 ^{o}C.

Purification. The incubated sample was applied to a 1 × 100 cm Bio-Gel P2 (100-200 mesh) and eluted with water containing 10% acetic acid. Fractions of 1 mL were collected and the saccharide content in 6 μL aliquots was determined by the phenol-sulfuric acid method.

Permethylation of synthetic trisaccharides. The permethylation technique best suited for this approach was essentially that of Ciucanu and Kerek[20] as modified by Gunnarsson.[21] The method was rapid and gave high yields (98+/–2%) without the formation of the non-sugar products. The trisaccharide samples (1 mg) was dissolved in methylsulphoxide (0.1 ml). Then, finely powdered NaOH (4 mg) and methyl iodide (0.25 ml) were added to the sample solution. Each mixture was stirred (100 r.p.m.) for 6 minutes in a closed vial at 25 ^{o}C, respectively. Water (0.5 mL) and chloroform (0.5 mL) were then added and the chloroform layer was washed with water (3 × 5 mL) and dried under nitrogen gas.

Instrumentation. All experiments were performed on the ThermoFinnigan LCQ ion trap mass spectrometer (ThermoFinnigan Co., San Jose, CA, USA) using electrospray ionization. Ultra high purity helium was introduced as the buffer and collision gas and dry NF grade nitrogen was used for sample nebulization. Samples were infused by syringe into the source at 5 μL/min. The electrospray source was operated at a voltage of 4.5-5 kV and the capillary heater was set to 200 ^{o}C. For the generation of most MS^{n} spectra, collision energies were set to 40-45% of maximum, though

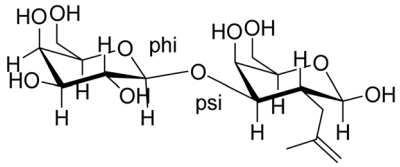

Figure 1. The constitution of disaccharide, Galβ1-3GalNAc and the torsional angles, ϕ (phi) and ψ (psi).

values much lower (20-25%) were sometimes used for optimal transmission of certain ions into the next stage of analysis. The maximum ion collection time was set at 2000 ms, and 2-3 microscans were performed per each individual spectrum. Between 25 and 100 spectra were summed to yield the MS^n spectra reported here. All experiments were performed in the positive-ion mode.

Energy minimization and molecular modeling. The constitution of disaccharide, Galβ1-3GalNAc, giving atom numbering and the tortional angles, ϕ (phi) and ψ (psi), are shown in Figure 1. The definition of a torsional angle follows the IUPAC convention.[22]

Molecular calculations were performed using SYBYL (Tripos Associates Inc., 2006) and modified MM4 software. Energy contour maps were with TOPO and SURF programs from the SURFER package (Golden Software Inc., Golden Co.). The first step in the modified MM4 calculations was determination of the interatomic distances, bond angles and torsional angles in the starting geometry made by SYBYL and SURFER programs. The values obtained were used in the different potential function expressions to calculate an initial steric energy, which was simply the sum of various potential energies calculated for all bonds, bond angles, torsional angles, nonbonded pairs of atoms and so forth in the molecule. The modified MM4 program uses a block diagonal Newton-Raphson optimization. Once the optimization had converged, the program printed the final steric energy and optimized geometry. Calculation of minimized energies and optimization of geometries were repeated at each 20° increment of phi and psi torsional angles from −180° to 160°. At each 20° increment of torsion angles, the energy is minimized, providing a value for a point on the energy map.

Results and Discussion

The tandem mass spectrometry spectra of permethylated isomeric trisaccharides, MF3 (permethylated Fuc(α1-3)-Gal(β1-3)GlcNAc), MF4 (permethylated Fuc(α1-3)Gal(β1-4)GlcNAc) and MF6 (permethylated Fuc(α1-3)Gal(β1-6)GlcNAc) at −40 eV are shown in Figure 2. After synthesis using fucosyl transferase, MF2 trisaccharide almost could not obtained at above various reaction conditions. Later, different glycosyl donors may be needed for the effective formation of a variety of reactivities and optimizing conditions, necessary to obtain consistently high yields for each new linkage to create linkage isomeric oligosaccharides for important molecular tools for medical applications.

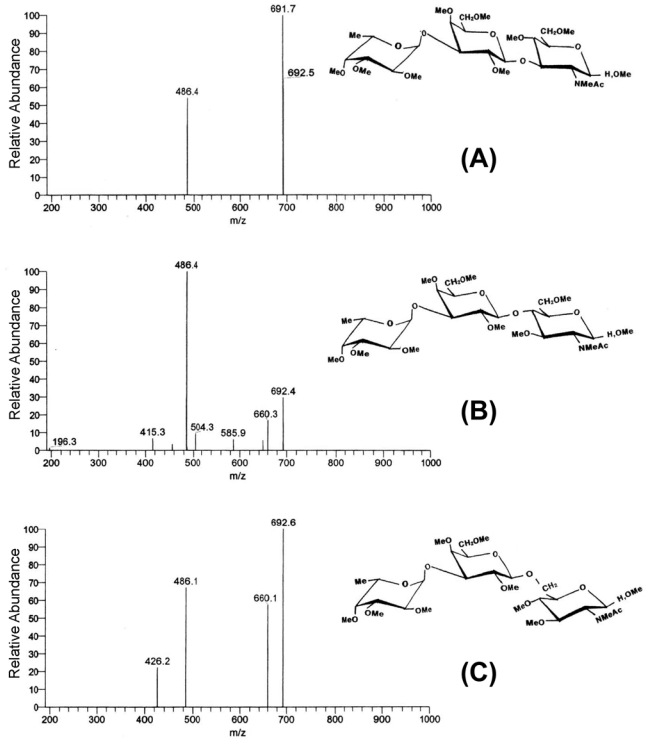

Figure 2. Tandem mass spectra of m/z 692 for permethylated Fuc(α1-3)Gal(β1-X)GlcNAc at −40 eV where X = 3(A), 4(B) or 6(C).

The spectra all show the same $[M+Na]^+$ ion at m/z 692 as expected, as well as common fragment ions at m/z 486. Under ESI conditions, permthylated neutral oligosaccharides show only weak molecular ion signals.[12] By contrast, carbohydrates containing HexNAc units (basic oligosaccharides) give rise to abundant $[M+Na]^+$ ions because of increased proton affinity. The $[M+Na]^+$ species were selected as precursor ions of the MS/MS experiments. The survival rate (relative intensity of collided ion) of the molecular ion (m/z 692) in compounds decreases differently according to linkage as the collision offset increases, and permethylated MF6 trisaccharide has the highest survival m/z 692 ion at −40 eV among the set of three permethylated trisaccharides (Figure 2). The relative intensity of the molecular ions with respect to the daughter ions in the MF3, MF4 and MF6 trisaccharides at −40 eV collision offset and 0.8 mTorr argon was as follows: MF6 (98%)> MF3 (65%)> MF4 (35%).

The major fragment ion at m/z 486 is formed by loss of permethylated fucose with cleavage of the glycosidic bond between galactose and reducing end GlcNAc according to the α-type pathway,[22] which is characterized by a hydrogen transfer from the fucose to the permethylated galactose. Compared with the spectra of the other permethylated saccharides those of MF3 trisaccharide (which contains the β1-3 linkage) at each collision energy level does not exhibit a relatively intense peak at m/z 660 (Figure 2) that is diagnostic for 3-linked permethylated sugars. Similar observation for a 3-specific ion has been made by Domon *et al.* and Egge *et al.*[23,24] The a-type fragment ion at m/z 660 is dia-

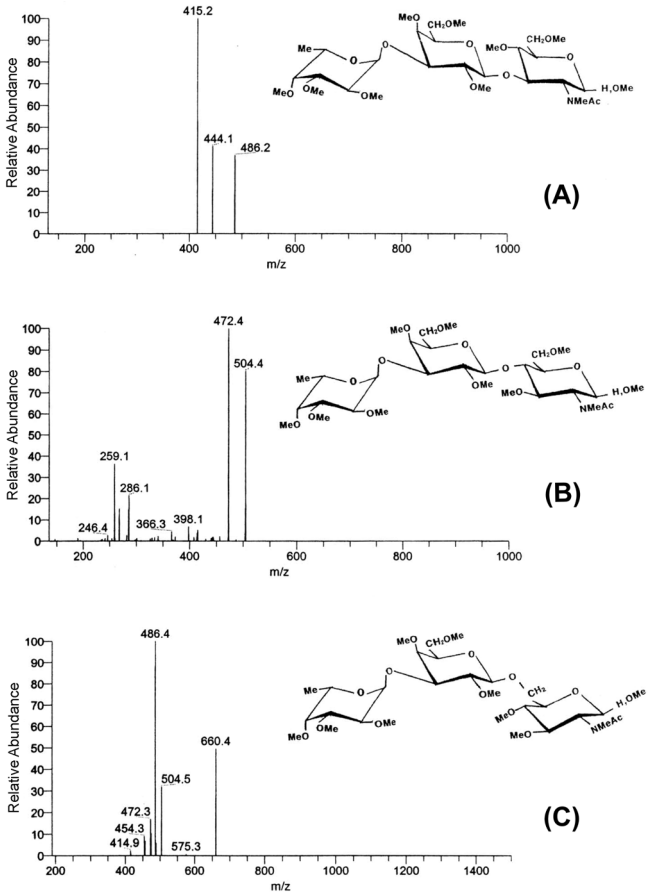

Figure 3. Tandem mass spectra of m/z 486 for MF3(A), m/z 504 for MF4(B) and M/z 660 for MF6(C) at −50 eV.

gnostic for β1-6 linkages in the same class of molecules (Figure 3) due to the loss of methanol and a fragment ion at m/z504 is the diagnostic for β1-4 linkages (Figure 3). In the linkage-isomeric oligosaccharides, the compound containing the β1-4 linkage was always more labile because of the propensity for charge retention on the nearby amino group on reducing end GlcNAc. In this comparison, the β1-3 linkage compound was always intermediate in stability and the β1-6 linkage-containing compound the most stable.

Molecular modeling and energy minimization methods were carried out without explicit consideration of solvent to explore the conformational mobility of blood group oligosaccharides and also human milk oligosaccharides (Figure 4).

To rationalize the results we observed in the ESI CID MS/MS studies, SYBYL and MM4 molecular modeling programs were used to calculate minimum energy structures of the permethylated MF2, MF3, MF4 and MF6 trisaccharides (Figure 4). Minimum energy structures were calculated on the basis of thermodynamic considerations that the most stable equilibrium state of a system was the one with the lowest free energy. The above relative intensity results correlated the freedom of motions around glycosidic bonds of molecular modeling study for these isomeric saccharides (Figure 4). In the linkage-isomeric oligosaccharides, the compounds containing the β1-6 linkage (MF6) were always

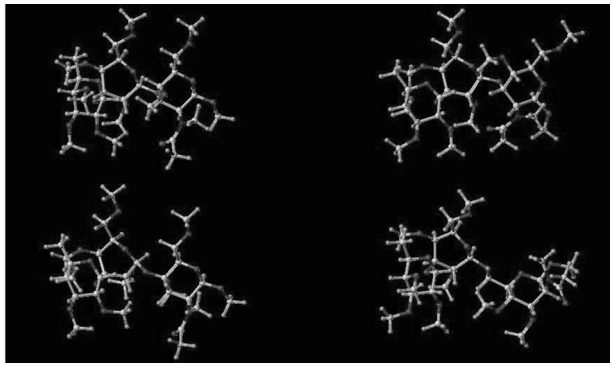

Figure 4. Optimized molecular modeling structures of MF2, MF3, MF4 and MF6 (clockwise). White circles with four connected atoms denote carbon atoms, red circles with two short bonds indicate oxygen atoms with lone pairs electrons, blue circles are nitrogens, and light blue circles are hydrogen atoms.

more labile because of the propensity for charge retention on the nearby amino group on GlcNAc. The exceptional glycosidic cleavage could probably be related to bond energies. In ESI CID MS/MS, vibronic energy absorbed from collision kinetic energy imparted to molecules and distributed among several modes included normal, bending, and rotation. Also, hydroxyl group blocking by permethylation could stabilize the ring and restrict the rotation of sugar rings. Vibronic normal and bend modes were strongly connected to rotation, thereby providing spectra with strong dependence on freedom of motion at the glycosidic bonds. Structures with weaker charge centers and more freedom of motion such as methylated MF4 trisaccharide would more readily dissipate energy absorbed from collision events due to lowered probability of populating the reaction coordinate for glycosidic bond cleavage. The methylated MF2, MF3, MF4 and MF6 trisaccharides which had reducing end amino group had strong intramolecular hydrogen bonds between the methyl proton in fucose or galactose and were stabilized energetically (Figure 4). They did not have linear structures but had bended structures. Methylated aminosugar-containing trisaccharides were good examples for suspecting an ionic effect to the cleavage events due to charges on the *N*-acetyl groups of reducing end amino sugar. Amino sugar containing trisaccharides (MF2, MF3, MF4 and MF6), free from a dominant charge center, could provide two or three charge centers and credence for the effect of ionic effects and steric hindrance to saccharide cleavage events. Structures with more freedom of motion would more readily dissipate energy absorbed from collision events due to lowered probability of populating the reaction coordinate for glycosidic bond cleavage.

Figures 5-7 were trajectories mapped onto the energy surface of the disaccharides, represented by the 2-D and 3-D contour plots of energy as function of ϕ and ψ calculated at 20° intervals, with all other degrees of freedom fixed.

Energy minimization and conformational studies of the permethylated derivatives supported the rationale for the above-described order of stability by examining the degree

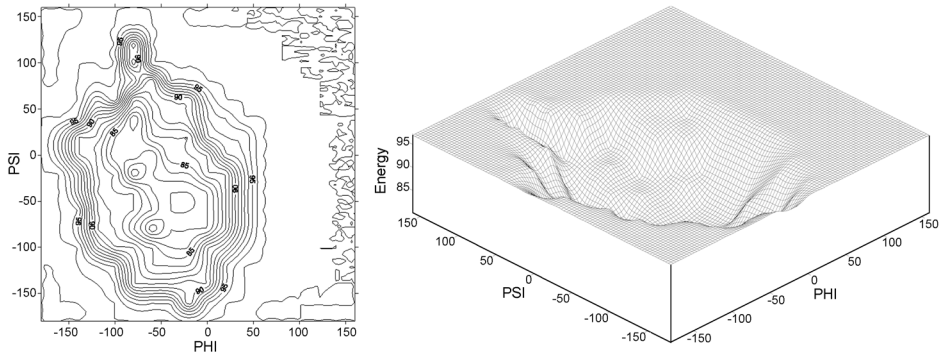

Figure 5. 2-D and 3-D Energy contour maps (not relaxed) of Gal(β1-3)GlcNAc.

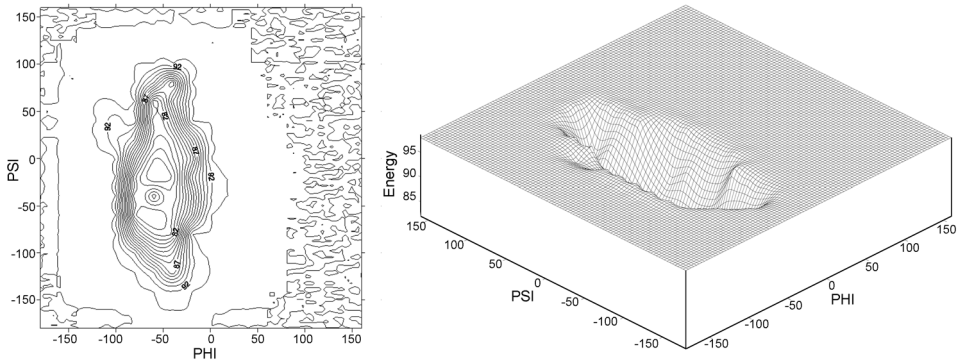

Figure 6. 2-D and 3-D Energy contour maps (not relaxed) of Gal(β1-4)GlcNAc.

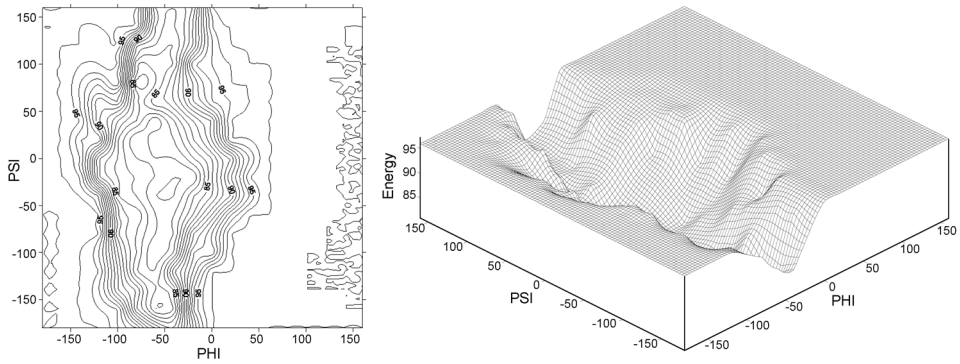

Figure 7. 2-D and 3-D Energy contour maps (not relaxed) of Gal(β1-6)GlcNAc.

of rotational freedom (number of available vibronic states) around the isomeric linkage. Figure 5-7 shows 3-D ϕ-ψ maps derived from the modified MM4 calculations on amino group containing molecules which depict degrees of phi-psi rotational freedom. MF4, the most rigid isomer, generates a volume which I will depict as 1.0, while MF3 generates an intermediate volume of 1.4. MF6, being the most flexible with its three rotational bonds generates a much larger well (1.7). Using low energy tandem mass spectrometric experiments and molecular modeling, it has been suggested that significant differences in glycosidic bond cleavage may occur due not only to ionic considerations but also may have contributions from steric hindrance (steric energy; 82.5 kcal/mol (MF3), 79.2 kcal/mol (MF4), 82.9 kcal/mol (MF6)), of the absorbance of collision energy, leading to a statistically higher bond cleavage for sterically crowded linkages. ESI MS/MS in combination with conformational study may lead to useful procedures to recognize linkage position in oligosaccharide structures with much less effort than conventional methylation linkage analysis. Conformational energy calculations and proton NOE on a series of synthetic oligosaccharides closely related to the human milk and blood group oligosaccharides. The results of molecular modeling studies concluded that the conformations about the 1-3 and 1-4 glycosidic linkages showed well-defined conformations. The Gal(β1-6)GlcNAc linkage of MF6, which can occurred as a branch point in complex carbohydrates, is a special case as a result of the rotation of three single bonds in the intersaccharide linkage leading to great flexibility. The energy map of Gal(β1-3)GlcNAc of MF3, showing that the disaccharide is more flexible than Gal(β1-4)GlcNAc of MF4 . The flexibility of

the disaccharide can be explained by multiminimum potential surface for van der waals interactions. The disaccharide oscillates in each potential well and exhibited numerous transitions among three minima that were identified by minimizations again all degrees of freedom on the energy surface. Addition of the amide group to C2 of GlcNAc yields the Gal(β1-3)GlcNAc of MF3 found in many blood group glycoproteins. The decreased flexibility of this (β1-3) linkage on addition of the Fuc(α1-3) substituent has also been pointed out in modeling studies by previous our lab, although present results differ in the detail from the conformation. The pronounced decrease in the conformational space available to the Gal(β1-3)GlcNAc linkage of MF3 caused by the fucosyl substituent results mainly from the van der waals repulsive interactions between fucose and GlcNAc that keep the trisaccharide oscillating in a single potential well in a tightly folded conformation.[15] The major conformation found for this trisaccharide in these simulation is essentially the same as that deduced from NOE data and modeling studies of French *et al.*[25,26]

Consistent with this interpretation is the observation of a correlation between the dynamic behavior of the angles ϕ and ψ. Some of the glycosidic linkages of human milk and blood group oligosaccharides show numerous transitions among distinct minimum energy conformations, as was observed by French *et al* for maltose.[25,26] But other oligosaccharides that have only a single minimum energy conformation show essentially no conformational transitions at all. We have found two especially interesting cases in which the addition of a third residue to a disaccharide greatly restricts the motion of the glycosidic linkage. The flexibility of the (β1-3) linkage is greatly diminished by the addition of fuc(α1-3) to Gal(β1-3)GalNAc of MF2 to make the human milk and blood group trisaccharide. The disaccharide (β1-3) or (β1-6) is very flexible but (β1-4) linkages in the trisaccharide is extremely rigid, and neither of the three glycosidic linkages shows any conformational transitions. It suggests that the oligosaccharide moves as a single unit. It shows also a marked difference in flexibility according to linkage positions.

Conclusion

Carbohydrate composition and structure strongly influenced the abundance of fragment ions observed in the ESI CID tandem mass spectra. Chemically derived biomolecules also might be beneficial for increasing fragmentation during electrospray ionization. Linkage-specific fragments had been observed for oligosaccharides with N-acetyl residues on the reducing end sugars; a structural feature that appeared to enhance cleavage at the adjacent glycosidic bond. In the fucose containing oligosaccharides, MFX series, the compound containing β1-6 linkage was always more labile because of the propensity for charge retention on the nearby amino group on GlcNAc. In the molecular modeling comparison, the β1-3 linkage compound was always intermediate in stability and the β1-4 linkage-containing compound the most stable. Using ESI CID tandem mass spectrometric experiments and molecular modeling, it had been suggested that significant differences in glycosidic bond cleavage occurred due to ionic considerations from *N*-acetyl groups and also from contributions from steric hindrance of the absorbance of collision energy, leading to a higher bond cleavage for sterically crowded linkages. Complementary information for linkage positions of oligosaccharides will be obtained using several sample derivatizations such as peracetylation or cation adducts of intact samples. This will be possible by creating a database with a number of sets of synthetic isomeric oligosaccharides and using frame structures for medical therapeutics.

Acknowledgments. This work was supported by the Honam University Research Fund (2007).

References

1. Schachter, H. *PNAS* **2005**, *102*, 15721.
2. Werz, D. B.; Castagner, B.; Seeberger, P. H. *JACS* **2007**, *129*, 2770.
3. Seeberger, P. H.; Werz, D. B. *Nature* **2007**, *446*, 1046.
4. Scanlan, C. N.; Offer, J.; Zitzmann, N.; Dwek, R. A. *Nature* **2007**, *446*, 1038.
5. Bosques, C. J.; Tschampel, S. M.; Woods, R. J.; Imperiali, B. *JACS* **2004**, *126*, 8421.
6. Chol, H.; Choe, E.; Yang, E.; Jang, S.; Park, C. *Bull. Korean Chem. Soc.* **2007**, *28*, 2354.
7. Yu, S.; Wu, S.; Khoo, K. *Glycoconj. J.* **2006**, *23*, 355.
8. Kang, D.; Jung, K.; Kim, S.; Lee, S.; Jhon, G.; Kim, Y. *Bull. Korean Chem. Soc.* **2007**, *28*, 2209.
9. Kelienberger, E.; Rodrigo, J.; Muller, P.; Rognan, D. *Proteins* **2004**, *57*, 225.
10. French, A. D.; Johnson, G. P. *Cellulose* **2004**, *11*, 5.
11. Rao, V. S. R.; Qasba, P. K.; Balaji, P. V.; Chandrasekaran, R. *Conformation of Carbohydrates*; Harwood Academic Publishers: 1998; pp 49-190.
12. Boons, G.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539.
13. Yoo, E.; Yoon, I. *Key Engineering Materials* **2005**, *277*, 33.
14. Yoo, E.; Laine, R. A. *Biol. Mass Spectrom.* **1992**, *21*, 479.
15. Yoo, E. *Bull. Korean Chem. Soc.* **2003**, *24*, 339.
16. Yoo, E. *Bull. Korean Chem. Soc.* **2001**, *22*, 293.
17. Yoo, E. *Bull. Korean Chem. Soc.* **2005**, *26*, 339.
18. Duus, J.; Gotfredsen, C. H.; Bock, K. *Chem. Rev.* **2000**, *100*, 4589.
19. Lee, K.; Kim, Y. *Bull. Korean Chem. Soc.* **1996**, *17*, 18.
20. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209.
21. Gunnarsson, A. *Glycoconjugate J.* **1987**, *4*, 239.
22. IUPAC Tenetative Rules for the Nomenclature of Organic Chemistry *Eur. J. Biochem.* **1971**, *18*, 151.
23. Domon, B.; Costello, C. E. *Glycoconjugate J.* **1988**, *5*, 397.
24. Egge, H.; Peter-Katalinic, J. *Mass Spectrometry Review* **1987**, *6*, 331.
25. French, A. D.; Tran, V. H.; Perez, S. *Computer Modeling Carbohydrate Molecules*; American Chemical Society: Washington D. C., 1989; pp 191-212.
26. French, A. D. *Carbohydr. Res.* **1989**, *188*, 206.