A Conformational Study of Oligosaccharides Investigated by Tandem Mass Spectrometry and Molecular Modeling

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The purpose of this paper is to introduce the simplified linkage position determination method using tandem mass spectrometry combined with molecular modeling study. 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 of the absorbance of collision energy, leading to a statistically higher bond cleavage for sterically crowded linkages. Permethylated derivatives of the linkage-isomeric trisaccharides give useful fragmentation ratios and product-ions, including a 3-linkage specific ion. The ratios of fragment ions are related to the ability of each linkage position in the oligosaccharide to absorb collisional energy.

Keywords : Oligosaccharides, Tandem mass spectrometry, Molecular modeling.

Introduction

In addition to traditional ionic mechanism,[1,2] it was hypothesized that differences in internal, nonbonding free energy, and in entropic parameters such as degrees of freedom of motion near minimum energy conformations could allow threshold tandem mass spectrometry experiments to distinguish among the linkage-positions of the various carbohydrate isomers. The molecular modeling study was used to rationlize observed tandem mass spectrometry fragmentation ratios. 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. Linkage position among other structural parameters of carbohydrate 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.

Trisaccharides, having at least one reducing end, nonreducing end and internal monosaccharide moieties, offer minimum units to study differences of internal linkages in a linear chain of sugars. The compounds chosen for the linkage study were galactosyl(β1-3, 4, or 6)N-acetylglucosaminyl(β1-3)galactose(1-Omethyl). A variety of the glycoconjugates having galactosyl β1-4 linked to a *N*-acetylglucosaminyl(GlcNAc)β1-3galactosyl residue were related to terminal groups likely to be found on polylactosamine-like glycoproteins and glycolipids which occurred on the surface of mammalian cells. Only β1-3 linkage was related to the human cancer. The other linkages such as 1-4 or 1-6 linkages which were found from the blood, intestine and some normal tissues were not related to the cancers.[1]

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The computer program used herein, modified MM2, 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.[3,4] It includes potentials for bond stretching, bending, and stretch-bending, 3-fold torsional potentials, Van der Waals interactions, and dipole-dipole interactions.[5] The program MM2 was chosen for this work as following reasons: First, carbohydrate has a number of possible ring conformers such as $^4C_1$, $^1C_4$, $^1S_5$, etc[4]. The modified MM2 program can alter the ring geometry to a low energy form for a particular saccharide shape. This function gives advantage to carbohydrate study using MM2 program. Secondly, the modified MM2 version, automatically provides the anomeric effects that are important for sugars. Also, the lone pairs are treated as if they are atoms because MM2 requires lone pairs of electrons on all ether and hydroxyl oxygen atoms to fit the data on alcohols and ethers which are major components of carbohydrate. Finally, this 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 MM2 was further modified to give a rigid dihedral driver option that started with the same intra-residue geometry at each increment of the driven torsion angles. This avoids the propagation of residue distortions from one conformation to the next. The MM2 calculation had earlier proven to give results in good accord with the conformational properties of oligosaccharides in solution as reflected from their NMR data.[6-9]

To rationalize the results in the tandem mass spectrometry studies, MM2 and SYBYL molecular modeling programs were modified for use on an IBM3090 and were also used on DEC Microvax equipment to calculate minimum energy structures and freedom of motion volumes near the minima

but below the bond-breaking energy for the permethylated galactose($\beta 1 \rightarrow 3$, 4 or 6)GlcNAc series. 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.

Experimental Section

Materials: The linkage-isomeric trisaccharides were synthesized as described previously[10] and characterized by ^{13}C NMR. Laminaribiose, gentiobiose, UDP-galactose, α-lactoalbumin and lactose synthase were purchased from Sigma Chemical Co.. 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[12] and dissolved in chloroform. The permethylated derivatives of the trisaccharides are as follows:

MG3: Permethylated galactose(β1-3)GlcNAc(β1-3)galactose(1-O-methyl)
MG4: Permethylated galactose(β1-4)GlcNAc(β1-3)galactose(1-O-methyl)
MG6: Permethylated galactose(β1-6)GlcNAc(β1-3)galactose(1-O-methyl)

Tandem Mass Spectrometry: The FAB MS spectra were obtained on a Finnigan TSQ-70 triple quadrupole instrument using Xenon gas and an Ion Tech Saddle-Field FAB gun or an ANTEK cesium gun at 8-9 KeV.

The tandem mass spectrometry studies were performed using 0.8 mTorr of argon as collision gas and varying the collision energy offset voltage from -10 to -80 eV at -10 eV increments. The purpose of the voltage was to accelerate or decelerate ions and thus, to set the translational kinetic energy of the ions as they entered the collision cell. Each permethylated oligosaccharide (3 μg) was dissolved in 1 μL of glycerol on a copper probe tip and the spectra were scanned during 3 sec from m/z 50 to 800. For CAD MS/MS measurements, four to eight spectra from m/z 50 to 750 were averaged taken as 5 second scans at each -10 eV collision energy increment.

Molecular Modeling: The constitution of disaccharide, Galβ1-4GlcNAc(galactose(β1-4)N-acetyl glucosamine), giving atom numbering and the tortional angles, phi and psi, is shown in Figure 1. Phi and psi are defined by atoms H1-C1-O4'-C4' and H4'-C4'-O4'-C1, respectively. The definition of a torsional angle follows the IUPAC convention.[12]

Molecular calculations were performed on a DEC MicroVax 3500 or an IBM-3090 using SYBYL which was molecular modeling software and Alchemy2000 (Tripos Associates Inc., 1998) and modified MM2 software. Energy contour maps were with TOPO and SURF programs from the SURFER package (Golden Software Inc., Golden Co.) which was a graphic software for visualization of minimized energy levels from modified MM2. The first step in the modified

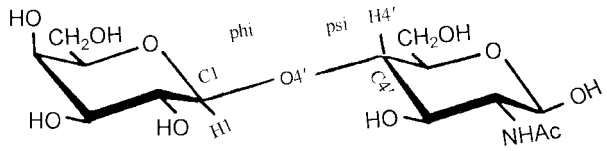

Figure 1. The constitution of disaccharide, Galβ1-4GlcNAc, giving atom numbering and the torsional angles phi and psi. and psi are defined by atoms H1-C1-O4'-C4' and H4'-C4'-O4'-C1, respectively.

MM2 calculations was determination of the interatomic distances, bond angles and torsional angles in the starting geometry made by SYBYL and Alchemy 2000 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 MM2 pro-

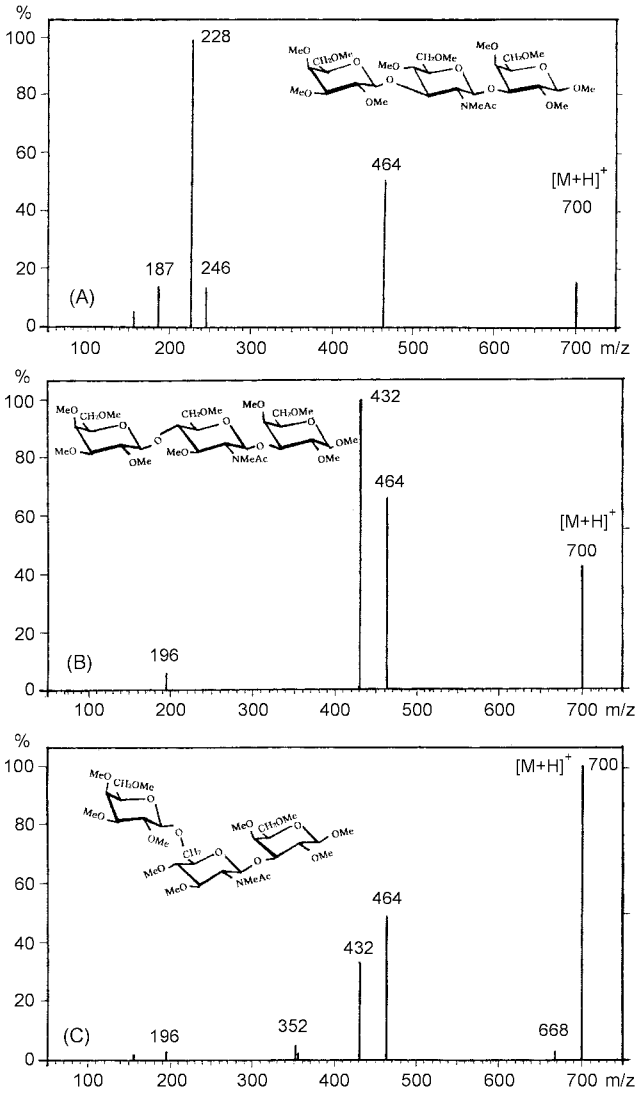

Figure 2. Tandem mass spectra of m/z 700 for permethylated galactose (β1-X)GlcNAc(β1-3)galactose(β1-O-methyl) at -30 eV where X=3(A), 4(B) or 6(C).

gram used 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 was minimized, providing a value for a point on the energy map.

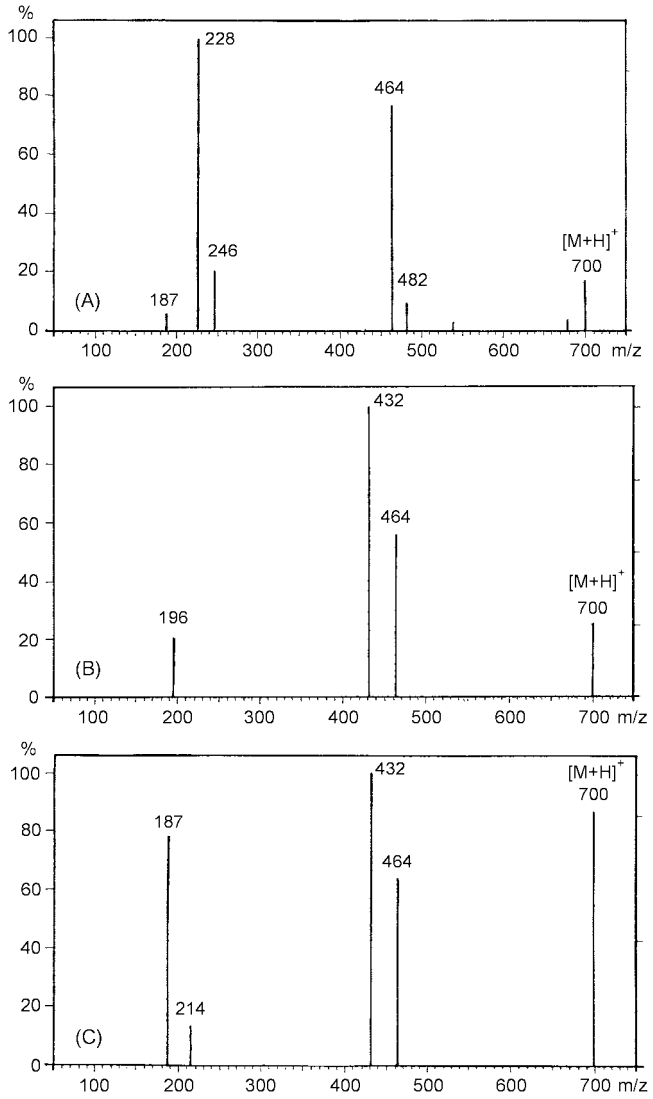

Figure 3. Tandem mass spectra of m/z 700 for permethylated galactose(β1-X)GlcNAc(β1-3)galactose(β1-O-methyl) at -70 eV where X=3(A), 4(B) or 6(C).

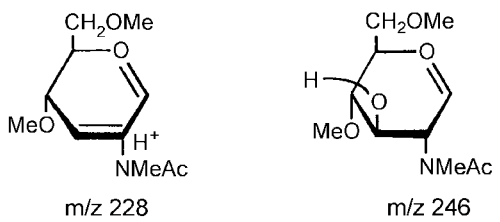

Figure 4. Structures of an oxonium ions form of the permethylated GlcNAc (m/z 246 and m/z 228).

Results and Discussion

The tandem mass spectrometry spectra of permethylated isomeric trisaccharides, MG3 (permethylated galactose(β1-3)GlcNAc(β1-3)galactose(1-O-methyl)), MG4 (permethylated galactose(β1-4)GlcNAc(β1-3)galactose(1-O-methyl)) and MG6 (permethylated galactose(β1-6)GlcNAc(β1-3) galactose(1-O-methyl)) at -30 eV were shown in Figure 2. Figure 3 showed the tandem spectra of the same compounds at -70 eV collision energy offset. The spectra all showed the same $[M+H]^+$ ion at m/z 700 as expected, as well as common fragment ions at m/z 464. The survival rate (relative intensity of collided ion) of the molecular ion (m/z 700) in compounds decreased differently according to linkage as the collision offset increases, and permethylated MG6 had the highest survival m/z 700 ion at -70 eV among the set of three permethylated trisaccharides (Figure 3). The relative intensity of the molecular ions with respect to the daughter ions in the MG3, MG4 and MG6 at -70 eV collision offset and 0.8 mTorr argon was as follows: MG6 (80%) > MG4 (21%) > MG3 (15%).

The major fragment ion at m/z 464 was formed by loss of permethylated galactose with cleavage of the glycosidic bond between GlcNAc and reducing end galactose according to the a-type pathway,[13] which was characterized by a hydrogen transfer from the amino-containing GlcNAc to the permethylated galactose, generating an oxonium ion on the permethylated GlcNAc (m/z 246, Figure 4). Compared with the spectra of the other permethylated saccharides, those of MG3 (which contains the β1-3 linkage) at each collision energy level exhibited a relatively intense peak at m/z 228 (Figure 4) that was diagnostic for the remnant penultimate aminosugar moiety from the nonreducing terminal loss of 3-linked permethylated sugars. Similar observation for a 3-specific ion had been made by Domon *et al.* and Egge *et al.*.[14,15] The a-type fragment ion at m/z 432 was diagnostic for β1-4 or β1-6 linkages in the same class of molecules, and was due to the loss of reducing end permethylated galactose with cleavage of the glycosidic bond between galactose and GlcNAc moieties and consecutive losses of methanol. Fur-

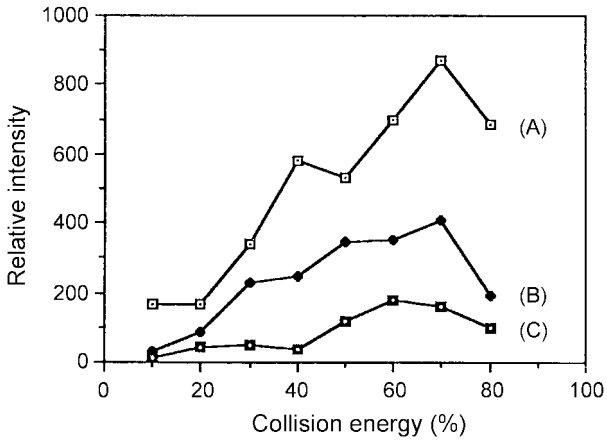

Figure 5. Plot of collision offset *vs.* ion ratio in MG3(3-linkage), MG4(4-linkage) and MG6(6-linkage): m/z (228+246+464)/700.

ther loss of the permethylated nonreducing galactose moiety yielded an unsaturated, partially methylated GlcNAc ion at m/z 196.

A particularly interesting relationship was found between physical parameters of the analysis and the linkage position in the MG3, MG4 and MG6. A plot of collision offset energy *vs*. parent/daughter ratios (Figure 5) gave a unique linkage-related slope across a gradient of collision energy, another way to depict the relative stability among the three linkage positions. This relationship broke down above collision offset energies of -70 eV. Parent ion survival *vs*. daughter ion ratios in the fragmentations of the isomeric trisaccharides in each collision energy set was a strong indicator of linkage position in tandem mass spectra in which the order of bond stability was 1-6 > 1-4 > 1-3. This was especially clear in the plot of collision offset vs. relative intensity of ion ratio, m/z (228 + 246 + 464)/700. In the linkage-isomeric oligosaccharides, the compound containing the 1-3 linkage was always more labile because of the propensity for charge retention on the nearby amino group on GlcNAc. In this comparison, the 1-4 linkage compound was always intermediate in stability and the 1-6 linkage-containing compound the most stable. The intensity ratio of major fragments reflects variations in the internal energy of the precursor ions with fragmentation of the permethylated 1-6 linkage-containing compound requiring higher energy.

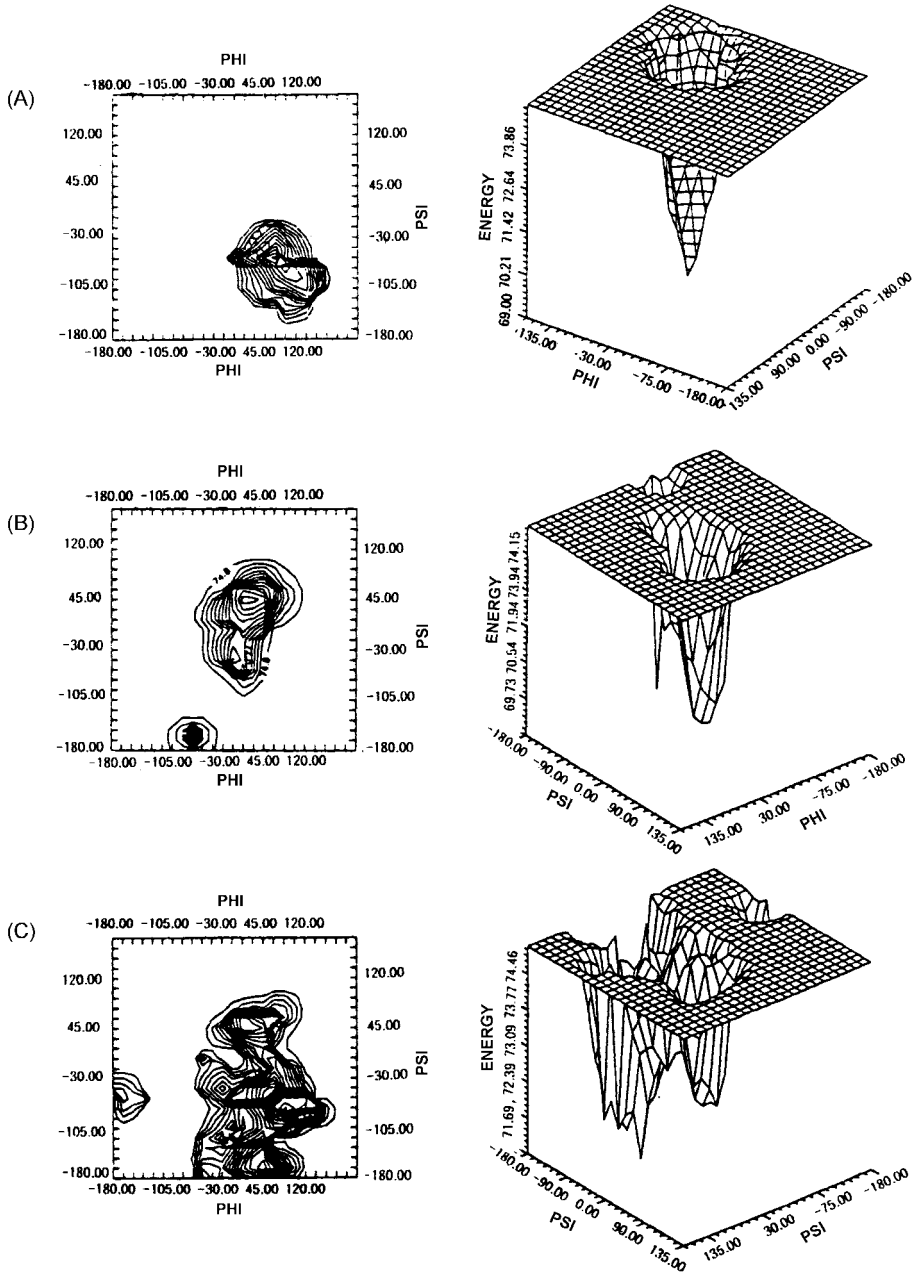

Figure 6. The phi and psi plots of the total energies and energy wells derived from the modified MM2 calculation. (The drawings were made with the SURF program of SURFER from Golden software.)

Molecular modeling of the permethylated derivatives supported the rationale for the above-described order of stability by examining the degree of rotational freedom (number of available vibronic states) around the isomeric linkage.[16] Figure 6 showed energy wells derived from the modified MM2 calculations on neutral, uncharged molecules which depicted degrees of phi-psi rotational freedom. MG3, the most rigid isomer, generates a volume which I would depict as 1.0, while MG4 generated an intermediate volume of 1.2. MG6, being the most flexible with its three rotational bonds generated a much larger well (1.8). Using low energy tandem mass spectrometric experiments and molecular modeling, it had been suggested that significant differences in glycosidic bond cleavage occurred due not only to ionic considerations but also had contributions from steric hindrance of the absorbance of collision energy, leading to a statistically higher bond cleavage for sterically crowded linkages.

Tandem mass spectrometry in combination with molecular modeling may lead to useful procedures to recognize linkage position in oligosaccharide structures with much less effort than conventional methylation linkage analysis. Although the link between the tandem mass spectrometry and molecular modeling was a worthwhile approach to distinguish linkage position of carbohydrates, it needed further works to apply this study to biologically active glycoconjugates. Some specific linkages such as 1-3 linkage between hexNAc and hexose gave dignostic signals which could be used directly to identify these linkage positions. While other linkages could be determined using survival rates of molecular ions or major fragment ions at each collision energy level. In order to apply this method to the biologically active components, general criteria for linkage determination are required at optimized conditions of derivatization, ionization and collision with development of chemical and enzymatic fragmentation method of biological components to small loigosaccharides. It will be possible by creating a database with a number of set of synthetic isomeric oligosaccharides.

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