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Comparison between Positive and Negative Ion Mode FAB CAD MS/MS Spectra of Linkage-Isomeric Oligosaccharides

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Abstract : Negative ion fast atom bombardment (FAB) mass spectra were found to allow the determination of the linkage positions in a series of underivatized linkage-isomeric oligosaccharides. A previous work (Laine *et al.*, 1988) reported that ion patterns of linkage-isomeric trisaccharides could be distinguished by a positive ion. Negative ion FAB collison-activated dissociation (CAD) mass spectrometry (MS) spectra of trisaccharides exhibited better sensitivity than the positive ion mode and provided specific fragmentation patterns according to the linkage positions. Especially, the fragmentations, m/z 205 in F6 and m/z 221 in G6, not occuring in 1-3 or 1-4 linkage, were an indication of 1-6 linkage, by changing collision energies from +10 eV to +60 eV. The survival ratios of molecular ions in each collision energy set gave support to previous results in which the order of bond stability was 1-6>1-4>1-3 linkage.

Key words : fast atom bombardment collision-activated dissociation mass spectrometry/mass spectrometry (FAB CAD MS/MS), linkage-isomeric oligosaccharides, negative ion

Carbohydrates had been understood to play passive roles as energy sources, space fillers or stabilizers of protein structure. One of the most significant recent discoveries (reviews; Rudd, 1993; Dwek, 1996; Kim, 1996) is that oligosaccharides play important roles in cellular events as modulators of cell-cell recognition and mediators of cell adhesion.

The fact that glycoconjugates frequently found on cell surfaces and oligosaccharides are often large in comparison to the proteins or lipids to which they are attached make them well suited to mediate recognition events. Eight monosaccharides (mannose, galactose GlcNAc;N-acetylglucosamine, GalNAc;N-acetylgalactosamine, fucose, NeuAc;N-acetyl neuraminic acid, NeuGc;N-glycolyl neuraminic acid and xylose) found in the oligosaccharide chains of the mammalian cell surface may be assembled into many different structures by means of different linkages, by chain branching and the inclusion of substituents such as sulfate, phosphate or acetyl groups (Dwek, 1996). It is important to understand the structure of oligosaccharides as a major factor which has affected their active biological functions.

The structural elucidation of carbohydrates is very difficult because of their complex structures. There are a very large number of possible structures for simple saccharides which even have the same mass. The number of possible structures for the simple saccharides which have the same mass is as follows: $S=N!\times 2^{Na}\times 4^{N-1}\times 2^{Nr}$, where N is the number of different hexoses, N! is the sugar epimer order, 2^{Na} is an anomeric configuration, 4^{N-1} is position of linkage, 2^{Nr} is the ring size (such as 5- or 6-membered)(Laine *et al.*, 1989). For a linear string of 3 hexoses, $S=3!\times 2^3\times 4^2\times 2^3=768$, we can obtained 768 structural isomeric trisaccharides.

Although carbohydrates have been investigated for many decades, there is no comprehensive or sensitive methodology to determine all the structural parameters of carbohydrates (Reinhold *et al.*, 1995). As of yet, structural studies of oligosaccharides have relied mainly on enzymatic analysis, mass spectrometry and NMR spectroscopy to elucidate one or some parameters (Dell, 1987; Cole *et al.*, 1989; Peter-Katalinic, 1990; Burlingame *et al.*, 1996). Enzymatic analysis is clearly the method of choice in biological systems for which often only very small amounts of samples (picomoles level) are available. There are still many linkages for which specific enzymes are not available. The NMR method requires a relatively large amount of samples (mg level) for structural analysis. It is the weak point of this analytical method for biological samples. Mass spectrometry requires small amounts of samples (μg level) and pro-

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vides a unique method for investigation of the type, number of sugar constituents, sequence and sugar composition by molecular ions as well as key fragments. FAB ionization, one of the major ion forming modes, is the most useful technique for nonvolatile and polar compounds such as oligosaccharides and their derivatives.

In the FAB experiment, an accelerated beam of atoms is fired from the gun towards the target, which has been preloaded with a liquid matrix containing the sample to be analyzed. When the atom beam collides with the matrix, kinetic energy is transferred to the surface molecules, many of which are sputtered out of the liquid into the high vacuum of the ion source. A significant number of these molecules are ionized during the sputtering process. Thus, gas-phase ions are generated without prior volatilization of the sample. FAB MS gives molecular ions as well as major fragments and thus generates useful structural information (Dell, 1987).

A FAB CAD MS/MS using a triple quadrupole mass spectrometer (Fig. 1; TSQ70 Operator's mannual, 1986; Thomson *et al.*, 1995) has the two stages of analyses. In the first stage, the ions (*parent ions*) formed in the ion source are analyzed by the first analyzer (Q1). In the second stage, after choosing ions (*mass-selected ions*) which have an interesting mass from parent ions, the ions are activated by collision with a neutral gas (Q2). Finally, the resultant ionic fragments (*daughter ions*) are mass analyzed by the second analyzer (Q3).

The rationale of linkage study using FAB CAD MS/MS is that structures with more freedom of motion will more readily dissipate energy absorbed from collision events. Also, negative and positive FAB CAD MS/MS will show different fragment ions due to the influence of the charge in functional groups.

Materials and Methods

The samples, linkage-isomeric trisaccharides, were synthesized as described previously (Jain *et al.*, 1988) and characterized by ^{13}C-nuclear magnetic resonance (NMR) spectroscopy. Trisaccharides have the three different linkage positions of either terminal fucose or galactose to N-acetyl glucosamine (GlcNAc) as follows:

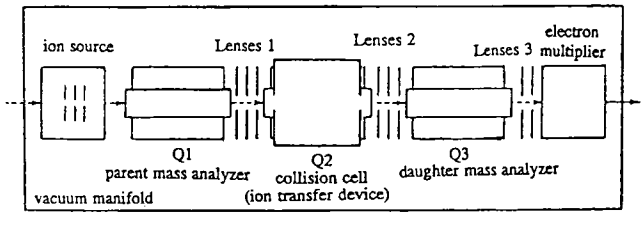


Fig. 1. Schematic representation of three quadrupole rod assembles in the TSQ70.

FX series
F3: Fuc(α1-3)GlcNAc(β1-4)Gal(1-OCH_3)
F4: Fuc(α1-4)GlcNAc(β1-4)Gal(1-OCH_3)
F6: Fuc(α1-6)GlcNAc(β1-4)Gal(1-OCH_3)

GX series
G3: Gal(β1-3)GlcNAc(β1-4)Gal(1-OCH_3)
G4: Gal(β1-4)GlcNAc(β1-4)Gal(1-OCH_3) and
G6: Gal(β1-6)GlcNAc(β1-4)Gal(1-OCH_3).

All other reagents were purchased from Sigma Chemical Co. (St. Louis, USA) and used without further purification. The FAB MS spectra were obtained on a Finnigan TSQ-70 using Xenon gas and an Ion Tech saddle-Field FAB gun at 8-9 KeV. Each oligosaccharide (10 μg) was dissolved in 1 μl of glycerol on a copper probe tip and the spectra were scanned at 3 seconds from m/z 50 to m/z 600 for a positive mode and from m/z 50 to 700 for a negative mode. The positive and negative FAB CAD MS/MS studies were performed using 0.8 mTorr of argon gas and varying the collision energy from -10 to -60 eV and +10 to +60 eV at 10 eV increments, respectively. For FAB MS/MS measurements, four to eight spectra from m/z 50 to 600 or 700 were averaged taken as 5 second scans at each collision energy increment.

Results and Discussion

Negative mode

Fig. 2 shows the negative FAB CAD MS/MS spectra of F3 (Fuc(α1-3)GlcNAc(β1-4)Gal(1-OCH_3)), F4 (Fuc(α1-4)GlcNAc(β1-4)Gal(1-OCH_3)), and F6 (Fuc(α1-6)GlcNAc(β1-4)Gal(1-OCH_3)) compounds collided at +40 eV collision energy offset. The spectra all show the same molecular ion ($[M-H]^-$) at m/z 542 as expected, as well as common fragment ions at m/z 348 and m/z 193 (Scheme 1). The survival rates (relative intensity of the collided ion of the molecular ion) in compounds decrease differently according to linkage positions as the collision energy offset increases. F6 (1-6 linkage containing compound) has the highest survival rate of m/z 542 ion, F4 (1-4 linkage containing compound) has a middle level, and F3 has the lowest survival molecular ion level at each collision energy level (Fig. 3). At +40 eV collision offset and 0.8 mTorr argon, the relative intensity of the molecular ion peaks with respect to the daughter ions in the FX series, as follows: F6(90%) > F4(50%) > F3(20%).

Fragmentation patterns and ratios could be correlated with structural features. The dominant glycosidic cleavage can probably be related to bond energies. There are two primary fragment ions at m/z 348 and m/z 378 in

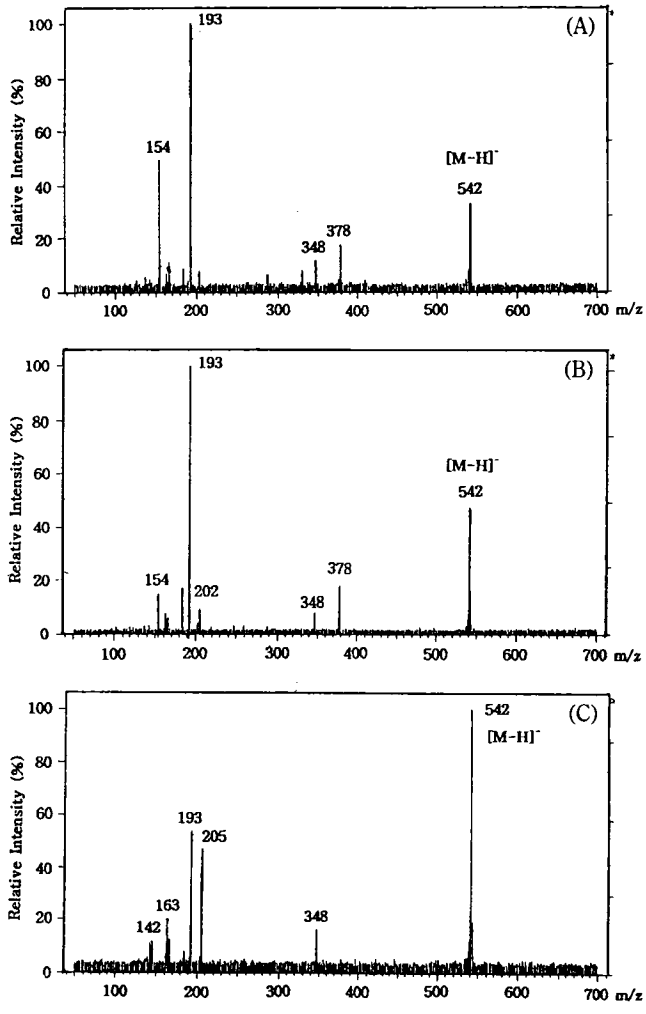
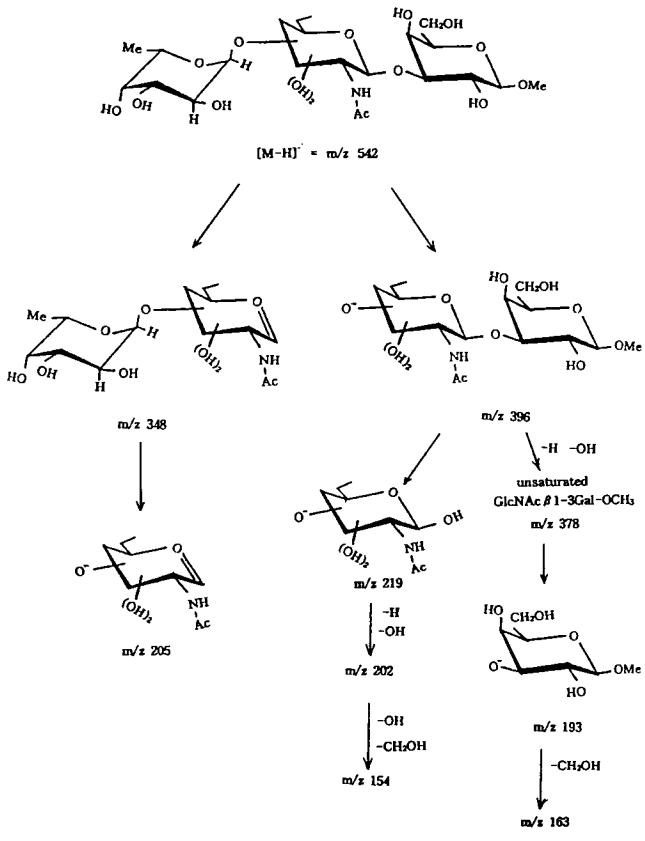


Fig. 2. Negative FAB CAD MS/MS spectra of m/z 542 for F3 (A), F4(B), and F6 (C) at +40 eV.

Fig. 2. The major fragment ion at m/z 348 is formed by loss of galactose with cleavage of the glycosidic bond between GlcNAc and galactose according to the Bn ion series (Paulter *et al.*, 1990) which is characterized by a hydrogen transfer from the amino-containing sugar to the galactose, generating an oxyanion on the GlcNAc (Scheme 1). It is a common fragment ion from the cleavage of glycosidic bonds between GlcNAc and galactose in F3, F4, and F6 compounds.

Another primary fragment ion, m/z 378 is obtained from m/z 396 by loss of a hydrogen and a hydroxyl group. Loss of the fucose moiety from the molecular ion (m/z 542) by cleavage of the glycosidic bond between fucose and GlcNAc with a negative charge transfer to GlcNAc and consecutive loss of a hydrogen and hydroxyl group results in an ion at m/z 378 (Yn ion series). The m/z 378 ion contains the linkage in question. It is interesting to note that F6 does not exhibit m/z 378 peak at each collision energy level. It suggests that the 1-6 linkage is more difficult to break



Scheme 1. Negative FAB CAD MS/MS fragment pathway of F3, F4, and F6.

than the 1-3 or 1-4 linkage-containing compounds due to the greastest rotational freedom around the glycosidic bond. A similar observation for a 1-6 linkage specific ion has been made by Dallinga *et al.* (1991). Continuous loss of galactose and several functional groups (hydrogen, hydroxyl group, or methanol group) give secondary fragment ions such as m/z 163 and m/z 154.

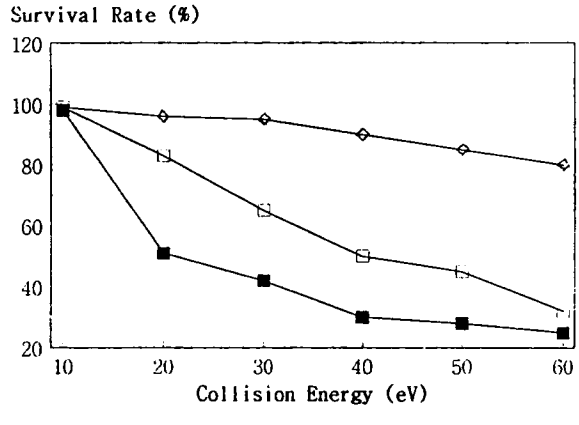


Fig. 3. Plot of collision energy offset versus survival rate of m/z 542 peak in F3 (1-3 linkage), F4 (1-4 linkage), and F6 (1-6 linkage). ■—■: 1-3 linkage, □—□: 1-4 linkage, ◇—◇: 1-6 linkage.

which are another diagnostic peak of 1-3 and 1-4 linkages.

The m/z 205 peak is formed from m/z 348 by cleavage of a glycosidic bond between fucose and GlcNAc and consecutive loss of fucose moiety. The m/z 205 peak is the another diagnostic peak of 1-6 linkage containing amino sugars. It shows that cleavage of a 1-6 linkage occurs after a 1-3 linkage breakdown. In 1-3 or 1-4 linkage-containing compounds, the cleavage of a glycosidic bond occurs after cleavage of a 1-3 linkage between GlcNAc and galactose. The major fragment pathway of these compounds is m/z 219 → m/z 202 → m/z 154. The major cleavage pathway of a 1-6 linkage-containing compound is m/z 348 → m/z 205 by selective cleavage between GlcNAc and galactose.

The negative FAB CAD MS/MS spectra of G3 (Gal(β1-3)GlcNAc(β1-4)Gal(1-OCH_3)), G4 (Gal(β1-4)GlcNAc(β1-4)Gal(1-OCH_3)), and G6 (Gal(β1-6)GlcNAc(β1-4)Gal(1-OCH_3)) at +40 eV are shown in Fig. 4. FX and GX sets exhibited the same fragmention patterns according to the position of linkages. A significant diagnostic ion for the 1-3 linkage and 1-4 linkage at m/z 348 in FX appeared at m/z 364 in the FAB CAD MS/MS spectra of GX series at +40 eV.

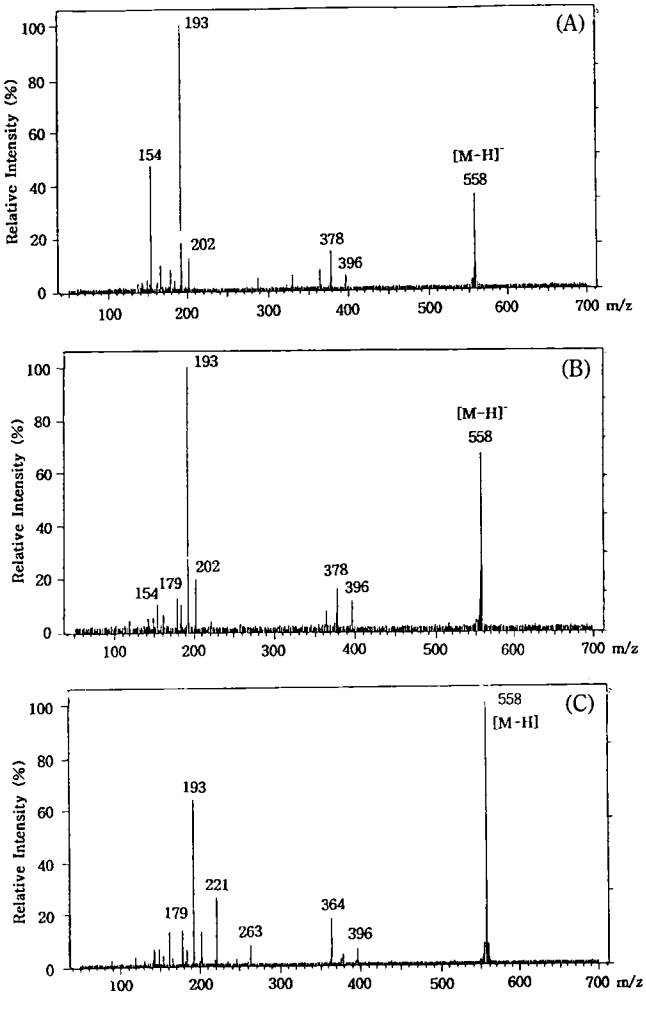
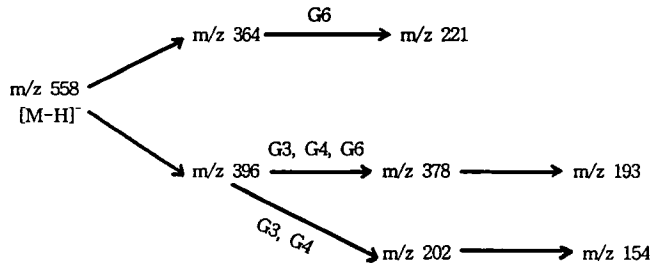


Fig. 4. Negative FAB CAD MS/MS spectra of m/z 558 for G3 (A), G4 (B), and G6 (C) at +40 eV.



Scheme 2. Negative FAB CAD MS/MS fragment pathway of G3, G4, and G6.

F3 and G3 have identical cleavage pathways as do those of the 1-4 linkage containing F4 and G4, respectively (Scheme 2). Significant differences in the fragment ratios contribute to discernment between the 1-3 and 1-4 linkages. In both the FX and GX series, the compound containing the 1-3 linkage was always more labile because of the crowding of atoms around the glycosidic bond. In this comparison, the 1-4 linkage compound was always intermediate in stability and the 1-6 linkage-containing compound was the most stable. The intensity ratios of major fragments reflect variations in the internal energy of the precursor ions, with fragmentation of the 1-6 linkage containing a compound requiring higher energy.

Positive ion mode

The positive FAB CAD MS/MS spectra of F3, F4, and F6 are shown in Fig. 5 at a -40 eV collision energy offset. The spectra all show the same [M+H]+ ion at m/z 544. The significant fragmentation is apparent corresponding to the loss of reducing end galactose with cleavage of the glycosidic bond between GlcNAc and galactose, accompanied by a hydrogen transfer from the GlcNAc to the galactose, giving a nonreducing disaccharide ion at m/z 350.

A loss of fucose moiety from m/z 544 by cleavage of the glycosidic bond between fucose and GlcNAc with a hydrogen transfer to GlcNAc results in an ion at m/z 398. The loss of both neutral sugars in a like manner with charge retention on the GlcNAc results in a major fragment at m/z 204. Compared to the negative mode, the positive FAB CAD MS/MS spectra of FX compounds have no significant fragment ions according to linkage positions. It shows the only different fragment ratios according to the linkage positions.

Observed FAB CAD MS/MS stability levels of molecular ions and observed intensity differences in product

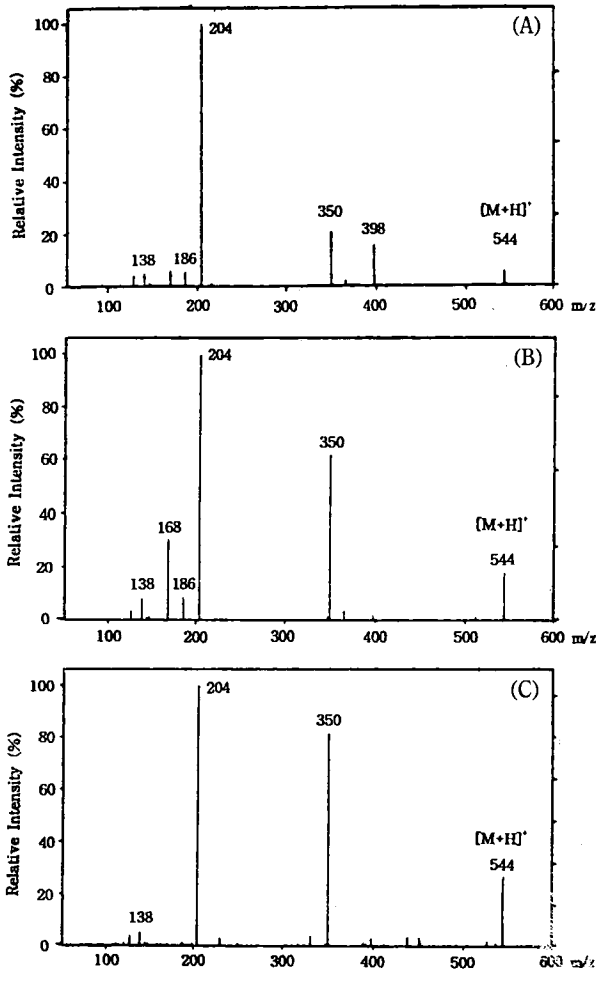


Fig. 5. Positive FAB CAD MS/MS spectra of m/z 544 for F3 (A), F4 (B), and F6 (C) at -40 eV.

ions from collisionally-activated glycosidic cleavages of fucosyl-GlcNAc bonds gave strong indications that steric factors in the positions of fucose linkage to GlcNAc in the three trisaccharides contribute to bond stability during FAB CAD MS/MS experiments in the relationship 1-6>1-4>1-3. Negative FAB CAD MS/MS molecular ion ([M-H]$^-$) gave linkage-dependent fragment patterns superior to previous observations with protonated molecular ions (Laine et al., 1988). The negative ion FAB CAD MS/MS spectra of trisaccharides exhibited better sensitivity than the positive ion mode and provided specific fragmentation patterns according to linkage positions (Fig. 2 and Fig. 5). Complementary information for linkage positions of oligosaccharides will be obtained using a different ionization mode, such as a positive or negative mode, and several sample derivatizations such as permethylation, peracetylation, or cation adducts of intact samples.

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