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Invited Paper

Comparison of Triple Quadrupole and Double Focusing Mass Spectrometers to Investigate Collision-Induced Dissociation and Metastable Ions of Glycoconjugates

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Glycoconjugates, such as chromophore-labeled disaccharides and permethylated glycosphingolipids (GSL) were used for comparison of triple quadrupole and double focusing mass spectrometers in analysis of product ions. A profound effect of collision energy was observed in the product ion spectra of ceramide ions (fragment ions of permethylated GSL): more product ions were observed from a double focusing mass spectrometer. Besides collision energy, the structure of the analyte had a significant effect on the formation of product ions. Despite the fact that masses of protonated molecular ions (MH^+) of permethylated GSL are significantly larger than their ceramide fragments, the low-energy and high-energy product ion spectra of MH^+ are, in general, similar. In a double focusing mass spectrometer of reversed geometry, more metastable ions were observed in the first field free region (FFR) than in the second FFR. The metastable ions observed in the second FFR were similar to those observed in low-energy collision-induced dissociation (CID). Although a double focusing mass spectrometer is superior to triple quadrupole instrument for detection of product ions, the poor resolution in either the selection of precursor ion or in the product ion spectra can be a serious problem in analysis of a mixture with similar masses.

INTRODUCTION

Collision-induced dissociation (CID) and metastable ions are widely used in mass spectrometric analysis of organic and biological molecules.[1-3] Major applications of these techniques include establishment of precursor-product ion relationships in structural elucidation, elimination of chemical noise, and enhancement of fragmentation efficiencies in conjunction with soft ionization.[4-7]

Among many types of mass spectrometers, triple quadrupole and double focusing mass spectrometers are the two most popular instruments for analysis of product ions. Although the resolution of precursor ion selection of a triple quadrupole mass spectrometer is superior to that of a double focusing mass spectrometer, the collision energy of the former (1-250 eV) is considerable smaller than the later (3-10 keV). The much smaller collision energy is reported to be a drawback in the low-energy CID study of peptides with molecular weight above 1000 Da.[6]

Glycoconjugates are molecules including a carbohydrate residue.[8] Increasing interest in these molecules for diverse biological functions has stimulated the search for new analytical techniques for their structural analysis.[5,8-10] In our laboratory, triple quadrupole and double focusing mass spectrometers are used to seek new mass spectrometric techniques for structural analysis of glycosphingolipids (GSL) and oligosaccharides. The potentials and problems of these techniques in the study of glycoconjugates are reported here.

EXPERIMENTAL SECTION

Materials and Methods

p-Aminobenzoic acid butyl ester (ABBE) (Aldrich Chemical Co., U.S.A.), isomaltose and GSL, such as N-palmitoyl-, N-stearoyl-, N-lignoceroyl-dihydrolactocerebroside, N-palmitoyl-, N-oleoyl-, N-stearoyl-, and N-nervonoyl- cerebrosides, Gangliosides GM1, Glucocerebrosides from human (Gaucher's) spleen (Sigma Inc., U.S.A.), helium, argon, and xenon (San-Fu Co., Taiwan) were purchased from the indicated suppliers. The procedure of Her et al.[4] was adopted to prepare of ABBE-labeled disaccharides. GSL were permethylated as described by Ciucanu and Kerek[11] and adapted for glycolipids according to Larson et al.[12] Briefly, a sample was placed in a screw-top glass tube and subsequently dissolved in anhydrous dimethyl sulfoxide (DMSO, 0.2 mL) which contained finely

powdered sodium hydroxide. The solution was vortexed for 4 min at room temperature (25 °C). Iodomethane (10 μL) was added and the solution was treated with ultrasound at room temperature for an additional hour. Dichloromethane (1 mL) and water (1 mL) were added to the reaction mixture. After vortex and centrifugation, the dichloromethane phase was washed with water (3 × 2 mL).

Mass Spectrometry

Low-energy CID spectra were acquired with a triple quadrupole mass spectrometer (VG-Biotech Quattro, Fisons/VG, England), schematically shown in Fig. 1(a). The collisional energy used for MS/MS analysis was 50 eV because the qualities of the CID spectra were not improved with increased collisional energy. Argon was used as the collision gas; the pressure of argon was adjusted to reduce the ion beam to half its initial value. In the ionization process, we used liquid secondary-ion mass spectrometry (LSIMS); the primary beam consisted of Cs^+ ions (11 keV).

Mass spectra of constant B/E (product) linked scans and mass analyzed ion kinetic energy mass spectra (MIKES) were recorded with a double focusing mass spectrometer (JEOL SX-102A, JEOL, Japan, Fig. 1b). This instrument is a two-sector machine with reversed geometry (magnetic sector before the electrostatic sector). The acceleration voltage was 10 kV. Helium was used as the collision gas and the pressure of helium was adjusted to reduce the ion beam to half its initial value. The mass scale in the linked scan mode was calibrated with a mixture of alkali halides.[13] Fast atom bombardment (FAB) was used as the ionization method, and the FAB gun was operated at 6 kV using xenon as ionizing gas. Both in LSIMS and FAB/MS, a sample solution (1 μL) was mixed with matrix (3-nitrobenzyl alcohol, 1 μL) on the probe tip for product ion analysis.

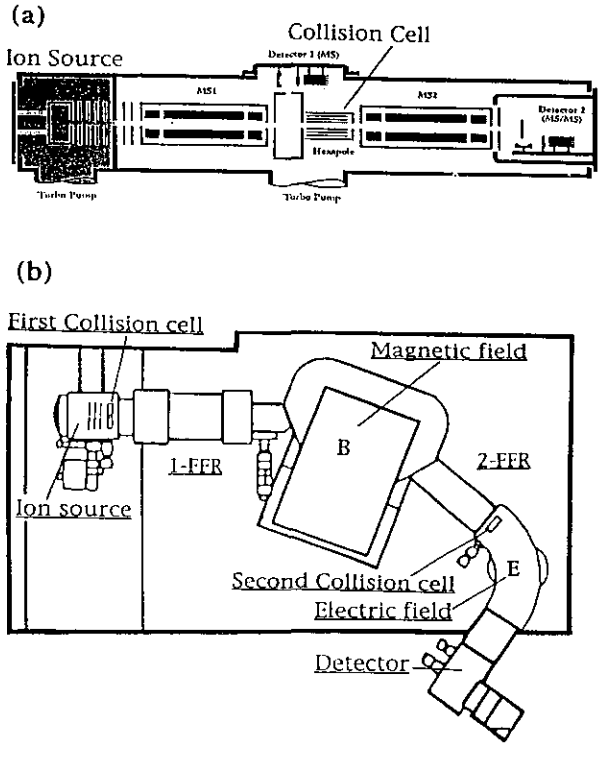

Fig. 1. Schematic diagram illustrating the different field free regions and collision cells in (a) a triple quadrupole mass spectrometer and (b) a double focusing mass spectrometer of reversed geometry.

RESULTS AND DISCUSSION

The fragmentation of a gas-phase ion is closely associated with the internal energy of the ion. Translational energy (energy in the laboratory frame, E_{LAB}) of the precursor ions plays an important role in the CID of gas-phase ions. The maximum energy E_{CM} that can be converted from translational energy to internal energy is:[14]

$$E_{CM} = E_{LAB} * Mg/(M_p+M_g)$$

E_{CM} is the energy of collision in a coordinate system moving with the center of mass of the collision partners. M_p is the mass of the precursor ion and M_g is the mass of the target gas. Triple quadrupole mass spectrometers are normally operated in the range 1-250 eV as the low-energy CID case. In order to obtain a large value of collision energy, massive target gases such as argon are preferred in CID operation.[15] The acceleration voltage of sector instruments is generally in the range 3-10 kV. A light gas such as helium is preferable in CID with sector instruments because its use minimizes major processes competing with CID, i.e., scattering beyond the acceptance angle of the mass spectrometer.[16] The effect of collision energy is the subject of numerous papers.[16-18] It is generally believed that the higher is the collision energy, the better is the quality of the product ion spectra. For example, a upper mass limit approximately 1000 Da is proposed to obtain useful sequence ion data in the analysis of peptides with low-energy CID.[6]

When the chromophore-labeled disaccharide-isomaltose (M - H = 516) was subjected to negative ion CID (Fig. 2), similar product spectra were observed although the E_{CM} of the precursor ion in a triple quadrupole mass spectrometer was much smaller than that of a sector instrument (3.6 eV

versus 76.9 eV). These values were calculated with E_{LAB} of 50 eV in the triple quadrupole (**Mg** = 40) and 10 keV in the sector instrument (**Mg** = 4). Considering the relatively poor reproducibility of ion intensity in CID, the two spectra are similar because the product ions in Fig. 2(a) have the same m/z values as in Fig. 2(b). In all spectra, the masses of the precursor and product ions are shown as nominal masses (e.g., m/z 516 is actually 516.2). In CID analysis of peptides, the greater is the mass of the precursor ion, generally the larger is the variation between high- and low-energy CID spectra. To ascertain whether similar phenomena were observed for glycoconjugates, permethylated GSL with molecular weight significantly larger than that of chromophore-labeled disaccharides were examined with high- and low-energy CID. The spectra obtained with the triple quadrupole mass spectrometer varied little from those obtained with the sector instrument. For example, the product ion spectra of the protonated molecular ion of permethylated GM1 m/z 1828 were similar in high- and low-energy CID (Fig. 3). The similarities between high- and low-energy CID indicated that, unlike peptides,[6] collision energy had little effect on the CID of glycoconjugates.

There are two different structural moieties in GSL: a ceramide and a carbohydrate residue. Ions resulting from fragmentation of the ceramide moiety were generally not observed in the MH^+ product ion mass spectra of GSL (e.g., Fig. 3). Therefore, in order to elucidate the detailed structure of the ceramide, ceramide fragments instead of MH^+ ions were selected as precursor ions. The results show that, unlike CID of MH^+ ions (e.g., Fig. 3), the product ion spectra of ceramide ions from low-energy CID were distinct from high-energy CID (e.g., Fig. 4a, 4b); more fragment ions (F2, F3′, K)[19] including those of charge-remote fragmentation[20] (a cluster of ions with 14 u interval in the high mass region) were observed in the high-energy CID product ion spectra. The collision energy, which was uncritical in CID of MH^+ apparently played an important role in CID of ceramide ions. The similarity in the product ion spectra of molecular ions and the variations in the spectra of ceramide fragments indicated that both collision energy and structure of the precursor ion were important in the CID of glycoconjugates. Collision energy was important in the CID of deglycosylated residue (e.g., ceramide), whereas structure (sugar) is important in the CID of glycoconjugates (e.g., GSL).

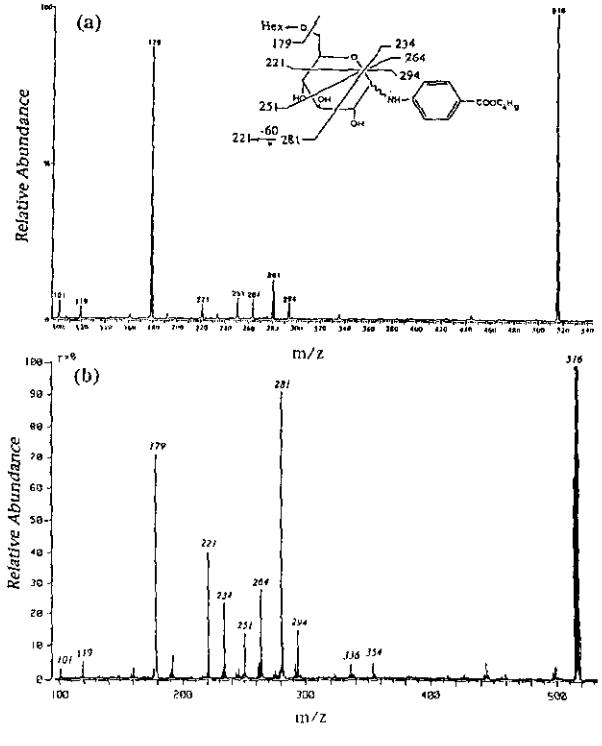

Fig. 2. Negative product ion spectra of ABBE labeled isomaltose (M-H$^-$, m/z = 516), (a) obtained with a triple quadrupole mass spectrometer as low-energy CID. (b) obtained with a double focusing mass spectrometer with linked scanning at constant B/E as high-energy CID.

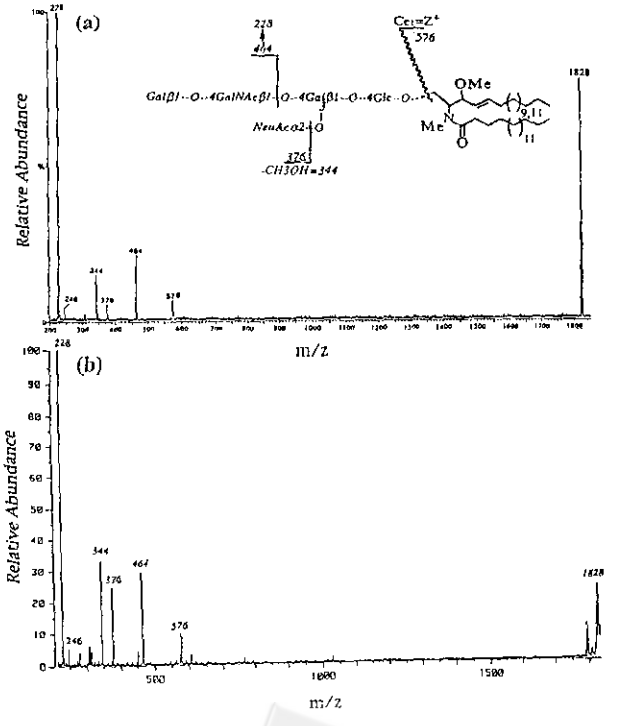

Fig. 3. Product ion spectra of permethylated GM1, (MH^+, m/z = 1828), (a) obtained with a triple quadrupole mass spectrometer as low-energy CID. (b) obtained with a double focusing mass spectrometer with linked scanning at constant B/E as high-energy CID.

After ionization, ions with a range of internal energy such that they are stable in the ionization source but fragment before reaching the detector are called metastable ions. Since the observation and explanation of metastable ions in 1945,[2] they are widely used by organic chemist to interpret electron impact (EI) mass spectra. In a double focusing mass spectrometer, ions are separated by momentum and energy. Because ions fragmented in the field free regions have momenta and kinetic energies different from those formed in the ionization source, these ions are differentiated with double focusing instruments. For a double focusing instrument of reversed geometry (BE instrument), fragment ions formed in the first field free region (before the B field) can be detected with a constant B/E scan whereas ions produced in the second field free region (before the E field) can be detected with an E scan (MIKES; mass analyzed ion kinetic energy spectroscopy). For the same precursor ion in Figs. 4a and 4b, the metastable ion mass spectra obtained in the first and second FFR are shown in Figs. 4c and 4d. The metastable spectrum from the first FFR (Fig. 4c) is similar to its CID spectrum (Fig. 4b). Despite its intrinsic property of poor resolution, the metastable ion spectrum from the second FFR region (Fig. 4d) is similar to the CID spectrum from the triple quadrupole instrument (Fig. 4a).

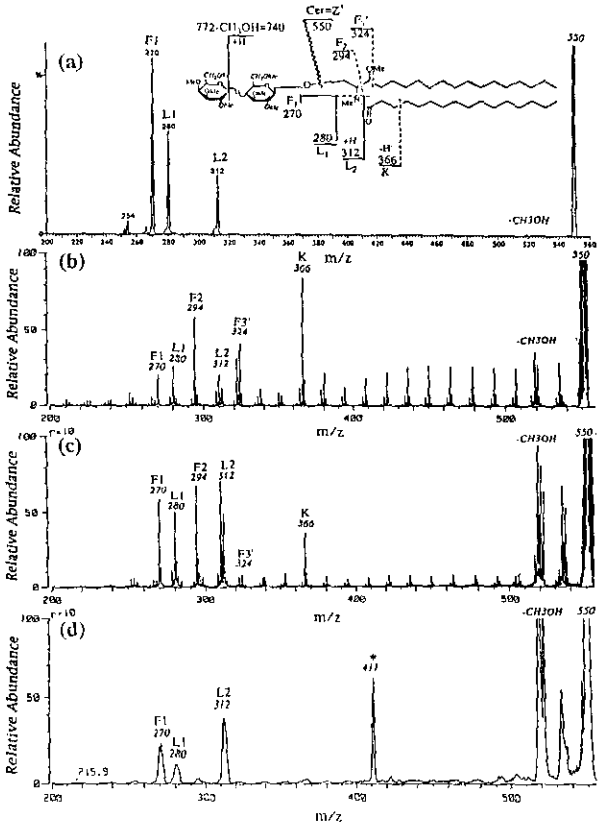

Fig. 4. Product ion spectra of a ceramide fragment from permethylated N-palmitoyl-dihydrolactocerebroside, (Z^+, m/z = 550), (a) obtained with a triple quadrupole mass spectrometer as low-energy CID, (b) obtained with a double focusing mass spectrometer with linked scanning at constant B/E as high-energy CID, (c) obtained with the same scanning mode as (b) but without collision gas (metastable ion), (d) obtained with the MIKES mode but without collision gas (metastable ion).

The narrow peak in Fig. 4d (m/z 411) is an artifact peak and corresponds to an ion with m/z 740 (loss of sugar and methanol from MH^+, m/z 990) formed in the first FFR. In a double focusing mass spectrometer, the magnetic field is considered a momentum analyzer. As the ion (m/z 740) has similar momentum to the selected precursor ion (m/z 550) {momentum = $(2*m*K.E.)^{1/2}$ = $(2*550*10\ keV)^{1/2} \cong (2*740*10\ keV*740/990)^{1/2}$}, it passed through the magnet with the ceramide precursor ion (m/z 550). In MIKES experiment, the electrostatic field functions as an energy analyzer. The energy of the ion with m/z 740 (10 keV*740/990 = 7.6 keV) is less than the ion of m/e 550 (10 keV); therefore, it appears at the position 7.6/10 in the MIKES spectrum (an energy spectrum). The regular peaks in MIKES are much broader than one u because of the release of internal energy into translational energy during fragmentation. The artifact peak is narrower than those regular peaks because this ion was formed before the magnetic field, rather than in the electrostatic field as for regular peaks, and only a limited range of momentum was transmitted through the magnet at a fixed field strength.

Ions having greater internal energy generally have a greater rate of reaction. Therefore, ions with a greater life time possess smaller internal energy and thus fewer fragmentation pathways. In comparison with fragments observed in the first FFR, fewer fragments were detected in the second FFR. The reason is likely that precursor ions fragmented in the second FFR have a greater life time and therefore possess smaller internal energy than the ions fragmented in the first FFR. The times taken for ions of mass 500 u to arrive at the first and second FFR are calculated to be 0,48 μs and 24 μs respectively at an acceleration voltage 10 kV.

Based on spectra obtained from collision and metastable ions, internal energies of the precursor ion in the double focusing mass spectrometer were in the order: CID in first or second FFR, metastable ion in first FFR, and metastable ion in second FFR. The differences between CID and metastable ions provide the possibility to correlate internal energy with the formation of product ions. For the ceramide

fragment in Fig. 4, the formation of ions at m/z 322, 324, 366 required greater internal energy because these ions were observed to have large intensity under high-energy CID (Fig. 4b), a smaller intensity as metastable ions of first FFR (Fig. 4c) and to have vanished entirely in the metastable spectrum of second FFR (Fig. 4d). The utility of this information in the explanation of fragmentation mechanism is currently under investigation.

For a triple quadrupole mass spectrometer, metastable ions may, in principle, be detected if precursor ions were stable enough to pass the first quadrupole but to fragment before entering the third quadrupole. When the metastable ion experiments were performed with a triple quadrupole mass spectrometer, the product ion spectra observed were similar to those observed in low-energy CID and for metastable ions in the second FFR. There are two possibilities for observation of these ions. Because the duration of the precursor ions traveling to the second quadrupole was comparable with that of the second FFR (58 μs, 24 μs respectively), similar product ions were observed. Another possibility for the observation is the collision with residual gas in the second quadrupole. Both explanations were consistent with the observation that the ions recorded were in much smaller abundance (approximately 1%) than in CID.

The primary beams used differed in both energy (11 keV versus 6 keV) and charge (positive ion versus neutral atom); therefore this condition may be a possible cause for the variation of high- and low-energy CID. Growing evidence indicates that the fundamental mechanisms of ionization by FAB and LSIMS are quite similar.[21-22] The major difference between a Cs^+ beam and a Xe beam is the sensitivity for massive analytes, not in fragmentation; the more expensive and more energetic Cs^+ gun has the advantage of increased sensitivity for massive analytes.

In the analysis of permethylated GSL by FAB, many ceramide product ions observed in high-energy CID but not in low-energy CID were also detected in the regular FAB mass spectrum (Fig. 5). The observation of these ceramide fragments in FAB but not in low-energy CID indicates that the maximum energy provided by the sputtering process of FAB exceeded the maximum amount of translational energy converted during collision at a small energy. Furthermore, the failure to detect the fragment ions in regular FAB spectrum by low-energy CID indicates another problem: the relationship between fragment ions that is important in structural elucidation may not be able to be established by low-energy CID.

Although a double focusing mass spectrometer might be superior to triple quadrupole mass spectrometer in producing structurally characteristic product ions, the lack of resolution in the selection of precursor ion in a B/E linked scan (first FFR) and the poor resolution in MIKES (second FFR) may be a serious problem in MS/MS analysis. For example, in the analysis of a monolithiated precursor ion by linked scan, product ions with two lithium atoms were also observed.[23] The detection of these dilithiated product ions was due to poor resolution in the B/E linked scan; thus the dilithiated molecular ion was selected with the monolithiated molecular ion. A similar problem was encountered in the analysis of ceramides. For example, in the analysis of permethylated glucocerebrosides, most likely because of a double bond in the long chain base or in the fatty acid, the difference between two of four major ceramides was only two mass units (m/z 658, 660), which made it impossible to select only one ceramide ion in the B/E linked scan at one time.

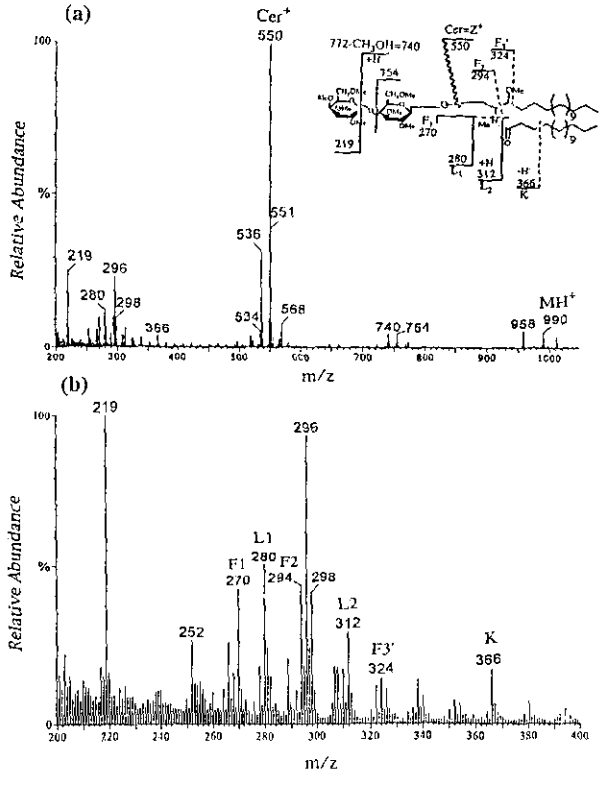

Fig. 5. Positive ion FAB spectra of permethylated N-palmitoyldihydrolactocerebroside (MH^+, m/z = 990), (a) full mass range, (b) partial range covering the mass range of the ceramide fragment ions.

CONCLUSION

For molecules with a sugar residue, collision energy has little effect on the fragmentation, and the product ion

spectra obtained from a triple quadrupole instrument have quality similar to those from a double focusing instrument. Collision energy is important if deglycosylated fragments (e.g., ceramide) are chosen as precursor ions. The differences between CID and metastable ions facilitate explanation of product ions and therefore the structure of the analyte.

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Key Words

Triple quadrupole; Double focusing mass spectrometer; Glycoconjugates; Collision-induced dissociation (CID); Metastable ion; Mass analyzed ion kinetic energy spectroscopy (MIKES).

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