Structure Determination of Ceramides and Neutral Glycosphingolipids by Collisional Activation of $[M + Li]^+$ Ions

Qinghong Ann and Jeanette Adams

Department of Chemistry, Emory University, Atlanta, Georgia, USA
(*J Am Soc Mass Spectrom* 1992, 3, 260–263)

Neutral glycosphingolipids are a general class of biological molecules that are important components of cell membranes. Their basic structure (1) comprises one or more neutral sugar residues attached to a ceramide residue. The ceramide group ($R = H$) is a long-chain sphingoid base, such as (4*E*)-sphingene [R' = CH=CH(CH₂)₁₂CH₃] or sphingane [R' = (CH₂)₁₄CH₃], that is substituted at the amino group by a fatty acyl group (R'' = alkyl chain). If the R group were a simple sugar, the glycosphingolipid would be called a cerebroside or a 1- β -glycosylceramide [1].

The structure of the glycosyl group can vary, as can the structures of both the sphingoid base and N-acyl chain. Thus, easy but complete structural elucidation of neutral glycosphingolipids has been a challenge in biochemistry, and mass spectrometry has played an important role. Derivatization and gas chromatography/mass spectrometry have been used to separately characterize the sugar, ceramide, sphingoid base, and fatty acyl components [2, 3]. Egge and Peter-Katalinić [4] reviewed the many uses of fast atom bombardment (FAB) of $[M + H]^+$, $[M + Na]^+$, and $[M - H]^-$ ions for structure elucidation of different types of glycosphingolipids. Of more recent application is the combination of collision-induced dissociation (CID) of FAB-desorbed $[M + H]^+$, $[M - H]^-$, and their fragment ions [5–7]. Costello and Vath [6] have contributed significantly in this latter area and have recently reviewed their work.

As an alternative to using CID of $[M + H]^+$ and $[M - H]^-$ ions, we have been exploring the use of alkali [8–10], and alkaline earth [11], metal ions as adducts to enhance structural determination upon CID. Here, we report our initial results of applying CID of $[M + Li]^+$ ions to the structural determination of six ceramides and 10 neutral glycosphingolipids. All spectra were acquired by using a VG Instruments (Manchester, UK) 70-S forward geometry mass spectrometer and B/E linked scans. Precursor ions were

desorbed by using FAB with either Ar or Xe at 7 kV. High-energy (8-keV) CID occurred in a collision cell in the first field-free region. The collision gas was He, and the pressure was adjusted to reduce the precursor ion abundance by ~ 50%. More experimental details can be found in refs 8–11.

Figure 1 compares structural information that can be obtained from CID of the $[M + H]^+$, $[M - H]^-$, and $[M + Li]^+$ ions obtained from N-*cis*-9-octadecenyl-(4*E*)-sphingene. The nomenclature for the cleavages labeled in our spectra is detailed in Figure 2. Although loss of H₂O is the major fragmentation route for $[M + H]^+$ ions of ceramides that contain both (4*E*)-sphingene (Figure 1a) and sphingene, there are also O' and O'' ions that presumably arise via losses of one and two H₂O molecules from O ions, respectively. The O series of fragment ions provide immediate information about the molecular weights of the sphingoid and N-acyl residues. Costello and co-workers [12] showed that derivatization of ceramides can significantly increase the amount of structural information that can be obtained from CID of $[M + H]^+$ ions.

CID of ceramide $[M - H]^-$ ions appears to provide complementary information. As an example, ceramides that contain both (4*E*)-sphingene (Figure 1b) and sphingene primarily lose 32 u (presumably as CH₃OH) and 48 u (perhaps as H₂C=O + H₂O) from the precursor ion. The product ions labeled P, S, T, U, and V all give information about the molecular weights of the N-acyl and sphingoid residues. The major effect of changing (4*E*)-sphingene (Figure 1b) to sphingene is a significantly reduced loss of 30 u. We assume loss of 30 u is loss of H₂C=O in analogy

Address reprint requests to Jeanette Adams, Department of Chemistry, Emory University, Atlanta, GA 30322.

Figure 1. CID spectra of $[M + H]^+$ (a), $[M - H]^-$ (b), and $[M + Li]^+$ (c) ions of *N*-cis-9-octadecenyl-(4*E*)-sphinganine. See Figure 2 for nomenclature for the cleavages. The analyte, dissolved in 2:1 $CHCl_3/MeOH$, was FAB-desorbed by using 8-keV Ar atoms. The protonated and deprotonated ions were desorbed from a matrix of 3-nitrobenzyl alcohol (3-NBA); the lithiated species were desorbed from a matrix of 3-NBA, saturated with LiI.

to fragmentations of $[M - H]^-$ ions of fatty alcohols [14].

CID spectra of $[M + Li]^+$ ions (Figure 1c) also reveal S, T, and U ions that are nominally analogous to those in spectra of $[M - H]^-$ ions. If the S and T ions that contained Li^+ were analogous to those proposed by Costello and co-workers [6, 13] for $[M - H]^-$ ions, they might have structures 2 and 3. The $[M + Li]^+$ ions also lose H_2O , which is analogous to the $[M + H]^+$ ions. In addition, however, there is an abundant K ion, which is more abundant than the S and T ions, that also gives information about the molecular weights of the *N*-acyl chain and sphingoid residue. More important, however, there is an abundant series of high-mass fragments formed by charge-remote cleavages [15-17] primarily of the *N*-acyl chain. These fragments give immediate information about the presence and location of the double bond in the fatty acyl chain, information that is lacking in the previous spectra. From the clear 54-u gap between peaks labeled L and M, it appears that charge-remote cleav-

Figure 2. Nomenclature for cleavages of neutral glycosphingolipids. We have as much as possible adopted the nomenclature originally proposed by Costello and co-workers [6, 12, 13]. Unfortunately, we have had to make some changes because spectra of $[M + Li]^+$ ions are more complex than spectra of $[M + H]^+$ and $[M - H]^-$ ions. Specifically, for cleavages of the ceramide, we have made the following changes: our G ion was previously [6, 12, 13] called U; our O ion was called W; our U ion was called V; and our S and T ions are the reverse of those in refs 6, 12, and 13. We have added H, J, K, L, M, P, Q, and a new designation for the V ion proposed previously. We hope that our changes will alleviate possible future confusion.

ages of the sphingoid chain are not as competitive as cleavages of the *N*-acyl chain. (Otherwise, there would be more abundant ions observed in the "gap".) We have not yet studied a ceramide that contains a double bond beyond the 4-position in the sphingoid chain, however. Thus, we do not truly know the effect of such unsaturation on the charge-remote fragment pattern. We also have not yet studied a large variety of other substituted ceramides, but we expect to be able to directly determine other substituents in the fatty acyl chain in analogy to other charge-remote fragmentations [8, 18-21]. For example, we should be able to differentiate *N*- α -hydroxyacylsphinganine from *N*-acyl-4*D*-hydroxysphinganine by an abundant radical cation that should be formed via cleavage of the C-C bond adjacent to the *N*- α -hydroxy group [8, 20] but not via cleavage of the C-C bond adjacent to the 4*D*-hydroxy group. There are no significant differences between spectra of $[M + Li]^+$ ions of ceramides that contain sphinganine instead of (4*E*)-sphinganine except that the mass of the K ion is reduced by 2 u.

The type of information that can be obtained from CID spectra of $[M + H]^+$, $[M - H]^-$, and $[M + Li]^+$ ions of neutral glycosphingolipids is demonstrated in Figure 3 for a lactosylceramide. CID spectra of $[M +$

H^+ and $[M - H]^-$ ions have been discussed in detail by Costello and co-workers [6, 13]. Our spectra of $[M + H]^+$ and $[M - H]^-$ ions (Figure 3a and b, respectively) are not identical to theirs (see CID spectra of a similar lactosylceramide in Figure 1 of ref 6). For example, we do not observe any significant charge-remote fragmentations in our spectra of $[M + H]^+$ and

$[M - H]^-$ ions, but they can be readily detected in spectra in ref 6. Another difference in our spectra of $[M - H]^-$ ions is that we do not observe abundant ions below m/z 170, which are seen in the spectrum in Figure 1 of ref 6. We believe that some of these results may be caused by differences in experimental conditions: We use 8-kV collisions with He at 50% beam reduction, but Costello and Vath [6] used 10-kV collision with He at 80% beam reduction. The increase in center-of-mass energy afforded by both the 10-kV and increased multiple collisions may provide enough extra internal energy so that the less favorable charge-remote fragmentations are accessed. This is supported by our observation that changing the FAB gas from Ar to Xe increases the detectability of the weakly abundant charge-remote fragment ions that are present above m/z 730 in Figure 3b. Fragment ions below m/z 500 in both our and previous spectra [6] of $[M + H]^+$ and $[M - H]^-$ ions, however, are too weak to be easily observed without further spectral magnification.

As shown in Figure 3c, $[M + Li]^+$ ions decompose to give a larger number of abundant ions than either the $[M + H]^+$ or $[M - H]^-$ ions. The $[M + Li]^+$ ions lose H_2O and give the typical S, T, U, and K (through M) ions. The E ion contains the intact sugar but none of the ceramide alkyl chains. The abundant C_n , B_n , Y_n , and X_n ions arise from cleavages through the sugar residue. The Y_0 and Y_1 ions in Figure 3 are presumed to arise from loss of H_2O from the Y_0 and Y_1 ions, respectively. (They could, however, be Z_0 and Z_1 ions, respectively.) Cleavage through the second sugar residue of lactosylceramides gives the $^{3,5}A_2$ ion. It is interesting that the $[M + Li]^+$ species give highly abundant B_2 and C_2 ions, but the $[M - H]^-$ species do not. Of particular importance is that the high-mass ends of spectra of $[M + Li]^+$ ions are dominated by charge-remote cleavages of the *N*-acyl chain of the ceramide. In contrast to $[M - H]^-$ ions discussed above, changing the FAB gas from Ar to Xe has no significant effect on the detectability of the charge-remote cleavages that arise from the $[M + Li]^+$ ions. For two cerebrosides that we have studied that contain monounsaturated *N*-acyl chains, the double bond and its location are determined immediately and directly from the pattern of cleavages (see Figure 1c for the fragmentation pattern). We have not yet studied neutral glycosphingolipids that contain other than saturated or monounsaturated *N*-acyl chains, but we predict that we will be able to deduce the identity and location of a variety of substituents by the characteristic charge-remote fragmentations [8, 18-21], as discussed above.

We can easily detect 3 ng of the $[M + Li]^+$ ions of ceramides in the FAB spectrum (signal-to-noise ratio of 20, FAB with Ar), which is somewhat better than our 6-ng detection limit for the $[M + H]^+$ ions. Our detection limit for obtaining interpretable CID spectra of $[M + Li]^+$ ions of ceramides is ~ 10 ng by using

Figure 3. CID spectra of $[M + H]^+$ (a), $[M - H]^-$ (b), and $[M + Li]^+$ (c) ions of lactosyl-*N*-octadecanoylsphinganine. See Figure 2 for nomenclature for the cleavages. The analyte, dissolved in 2:1 $CHCl_3$ /MeOH, was FAB-desorbed by using 8-keV Xe atoms. The protonated and deprotonated ions were desorbed from a matrix of 3-nitrobenzyl alcohol (3-NBA); the lithiated species were desorbed from a matrix of 3-NBA, saturated with LiI. Ions labeled Y_n are either $[Y_n + 2H]^+$, $(Y_n)^-$, or $[Y_n + Li + H]^+$; B_n is $[B_n + Li + H]^+$; C_n are either $[C_n]^-$ or $[C_n + Li + H]^+$; $^{3,5}A_2$ is $[^{3,5}A_2 + Li]^+$; and $^{2,4}A_2$ is $[^{2,4}A_2 - H]^-$.

FAB with Ar and ~ 5 ng with Xe. Our FAB and CID results compare quite favorably with results reported previously for 10 ng of a derivatized ceramide desorbed by FAB (see Figure 8 in ref 6). Our detection limit for CID of $[M + Li]^+$ ions of cerebroside is ~ 20 ng by using FAB with Xe. (We can obtain excellent CID spectra of both $[M + H]^+$ and $[M - H]^-$ ions of glycosphingolipids that contain one or two sugars at ≤ 100 ng.) Our detection limits are mainly hampered by chemical noise that results from poor precursor ion resolution in our first field-free region B/E linked scans. Consequently, we believe that our detection limits could be significantly lowered with true tandem techniques and the use of a photodiode array detector [6].

Further details of these results are forthcoming, and we are expanding our studies to determination of a larger variety of structurally diverse ceramides and neutral glycosphingolipids, gangliosides, and sphingomyelins. We also are applying this technique to the determination of sphingoid bases, which are in themselves biologically active and structurally diverse. We also are studying the mechanisms of formation of the diagnostic ions and are evaluating the use of other alkali and other metal ions as analytical reagents in the structure determinations.

Acknowledgments

We acknowledge funding from the Petroleum Research Fund (21755-G5), which is administered by the American Chemical Society, and the NSF (CHE-9113272). We acknowledge M. J. Contado for early technical work. Preliminary reports of this work were presented at the 1991 Sanibel Conference on Mass Spectrometry, Sanibel Island, FL, January 1991; at the 201st ACS National Meeting and Exposition, Atlanta, GA, April 1991; and at the 39th ASMS Conference on Mass Spectrometry and Allied Topics, Nashville, TN, May 1991.

References

1. IUPAC-IUB Commission on Biochemical Nomenclature. *Lipids* 1977, 12, 455-468.
2. Hansson, G. C.; Li, Y. T.; Karlsson, H. *Biochemistry* 1989, 28, 6672-6678.
3. Urdal, D. L.; Hakomori, S. *J. Biol. Chem.* 1983, 258, 6869-6874.
4. Egge, H.; Peter-Katalinic, J. *Mass Spectrom. Rev.* 1987, 6, 331-393, and references therein.
5. Ladisch, S.; Sweeley, C. C.; Becker, H.; Gage, D. *J. Biol. Chem.* 1989, 264, 12097-12105.
6. Costello, C. E.; Vath, J. E. In *Methods in Enzymology*; J. A. McCloskey, Ed.; Academic Press: San Diego, 1990; Vol. 193, pp 738-768, and references therein.
7. Hayashi, A.; Matsubara, T.; Nakamura, T.; Kinoshita, T. *Chem. Phys. Lipids* 1990, 52, 57-67.
8. Contado, M. J.; Adams, J. *Anal. Chim. Acta* 1991, 246, 187-197, and references therein.
9. Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* 1991, 113, 812-820.
10. Teesch, L. M.; Orlando, R. C.; Adams, J. *J. Am. Chem. Soc.*, 1991, in press.
11. Teesch, L. M.; Adams, J. *J. Am. Chem. Soc.* 1990, 112, 4110-4120.
12. Domon, B.; Vath, J. E.; Costello, C. E. *Anal. Biochem.* 1990, 184, 151-164.
13. Domon, B.; Costello, C. E. *Biochemistry* 1988, 27, 1534-1543.
14. Adams, J.; Gross, M. L. *J. Am. Chem. Soc.* 1986, 108, 6915-6921.
15. Jensen, N. J.; Tomer, K. B.; Gross, M. L. *J. Am. Chem. Soc.* 1985, 107, 1863-1868.
16. Adams, J. *Mass Spectrom. Rev.* 1990, 9, 141-186.
17. Contado, M. J.; Adams, J.; Jensen, N. J.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* 1991, 2, 180-183.
18. Adams, J.; Deterding, L. J.; Gross, M. L. *Spectros. Int. J.* 1987, 5, 199-228.
19. Adams, J.; Gross, M. L. *Anal. Chem.* 1987, 59, 1576-1582.
20. Adams, J.; Gross, M. L. *Org. Mass Spectrom.* 1988, 23, 307-316.
21. Contado, M. J.; Adams, J.; Gross, M. L. *Adv. Mass Spectrom.* 1989, 11b, 1034-1035.