ORGANIC MASS SPECTROMETRY, VOL. 29, 700–702 (1994)

OMS Letters

Dear Sir,

Linkage Position Determination of Lithium-cationized Disaccharides by Surface-induced Dissociation Tandem Mass Spectrometry

Surface-induced dissociation (SID) has proved to be an effective means of ion activation for both small polyatomics[1–6] and macromolecules[7–13] and serves as an alternative to gas-phase collision-induced dissociation (CID). When a projectile ion collides with a surface, several different processes can occur.[14] SID involves the partial conversion of the kinetic energy of the projectile into ion internal energy, inducing fragmentation of the projectile ion. Surface-induced dissociation is known to deposit high average internal energies, the distribution of which is narrow; the average energy transfer can be easily controlled by varying the kinetic energy of the precursor ion.[15,16]

We illustrate here the use of SID as a possible ion activation technique for the determination of the linkage position of hexose sugar units in disaccharides. Linkage position determination in oligosaccharides has been studied extensively using different ionization techniques including chemical ionization (CI),[17] laser desorption ionization[17–19] and fast atom bombardment (FAB).[21–27] Earlier results for FAB ionization combined with CID tandem mass spectrometry (MS/MS) of lithiated disaccharides showed that different patterns of fragment ions are obtained depending on the position of the glycosidic linkage of the disaccharides.[21–26] A similar trend has been observed for fragment ions obtained by FAB negative ion CID-MS.[27] Leary *et al.*[25] performed detailed studies on lithium-cationized disaccharides to determine linkage positions and semi-empirical calculations to define the role of lithium in fragmentation of the disaccharides.

We report here the SID spectra of four isomers of mannosylmannose and compare them with fragmentation patterns observed in the CID spectra of disaccharides. The instrument used was a tandem quadrupole mass spectrometer constructed with Extrel 4000 u quadrupoles. The instrument design and set-up have been described elsewhere.[28] The precursor ion, selected by the first quadrupole, collides with the surface and the fragment ions are analyzed by the second quadrupole. The surface used in this investigation was prepared by self assembly of 2-(perfluorooctyl)ethanethiol on plasma-cleaned vapor-deposited gold.[4]

The disaccharides studied were four isomers of mannosyl-α(1 → Y)-mannose, where Y is the linkage position, $C_6H_{11}O_5$-α(1 → Y)-$C_6H_{11}O_6$. The four disaccharide isomers of mannosyl-α(1 → Y)-mannose (where Y = 2, 3, 4, 6) were provided by Dr Eric Jacobson, Department of Internal Medicine, MCV, Virginia Commonwealth University, and have been characterized by NMR spectroscopy.[29] A stock solution was prepared by dissolving 0.5 mg of the disaccharide in 100 μl of water. The sample was prepared by adding 1–3 μl of this stock solution and 0.5–1 μl of 0.05 M $LiCO_3$ to 3 μl of 3:1 dithiothreitol–dithioerythritol (DTT–DTE) matrix. Approximately 15–45 nmol of disaccharide were used for each analysis. The lithium-cationized disaccharide ions were produced by liquid secondary ionization, by bombarding the probe tip with a 6 keV Cs^+ ion beam (cesium ion gun from Antek, Palo Alto, CA, USA).

Figure 1(a)–(d) show the high-mass region of the SID spectra of the four isomers of mannosyl-α(1 → Y)-mannose, obtained on the 2-(perfluorooctyl)ethanethiolate surface at 30 eV laboratory collision energy. Although the spectra of all the four isomers look very similar at $m/z < 150$, there are significant differences in the SID spectra above m/z 150. All of the main fragment ion peaks observed are listed in Table 1. Table 1 clearly shows that the four different linkage positions lead to four distinct fragmentation patterns. All the isomers gave two predominant fragment ion peaks at m/z 169 and 187 which result from the cleavage of the glycosidic linkage and retention of Li^+ by each of the hexose sugar units. Each of the isomers analyzed gives a certain unique combination of ions at m/z 229, 259, 289 and 331 in addition to the strong peaks at m/z 169 and 187. The dependence of the fragmentation patterns obtained for the four isomers on collision energy was also investigated for the 2-(perfluorooctyl)ethanethiolate self-assembled monolayer surface, a surface known to deposit higher internal energy into projectiles. No new fragmentation peaks were observed, but the breakdown curves for the four isomers show that at collision energies beyond 45–50 eV, the fragment ions at m/z 229, 259, 289 and 331 further fragment to low-mass ions and are not present in the spectra for linkage position determination of disaccharides.

The characteristic fragment ion peaks observed by SID above m/z 150 are similar to those observed in low-energy CID-MS/MS experiments on other hexose disaccharides by Leary *et al.*[25] The coordination of lithium to the disaccharide is presumed to help in ring fragmentation, thereby leading to two, three and four carbon losses which gives rise to the unique fragments necessary in linkage position determination. Semi-empirical calculations[25] show that it is plausible that lithium cation coordinates with the maximum number of oxygens permitting certain fragmentation pathways, yielding a unique set of fragment ions for different linkage positions.

Comparing our results [Fig. 1(a)–(d)] with those obtained by CID for disaccharides with different linkage positions, the linkage positions for the mannosylmannose isomers could be assigned.[25] There are differences, however, in SID *vs.* CID fragment ion abundances and also, in the case of the 1 → 3 linkage isomer, the combination of fragment ion peaks which help assign the linkage position. The SID spectra of the disaccharide with a 1 → 3 linkage contain peaks at m/z 331, 187 and 169 as expected, but not the m/z 259 peak which was reported in the CID spectrum. This could be due to the higher internal energy deposited by SID, which is manifested as enhanced fragmentation of the fragment ion at m/z 259. This means the fragment ion at m/z 259 has enough energy to fragment further and might lose $C_3H_6O_2$ to yield an ion at m/z 187, which is the base peak in the 30 eV SID spectrum [Fig. 1(b)] for the 1 → 3 isomer. In general, the relative abundances of fragment ions with respect to the parent ion are also different in SID and CID spectra. The parent ion is the most intense peak in low-energy CID, unlike in SID, where m/z 187 is the most intense peak. Comparison of the relative ratios of the peaks at m/z 229, 259 and 289 to that at m/z 187 in SID and CID spectra support the conclusion that SID deposits

Table 1. Fragment ions observed in 30 eV SID spectra for different isomers of mannosyl-α(1 → Y)-mannose[a]

Isomer	Product ions (m/z)					
	169	187	229	259	289	331
Mannosyl-α(1 → 2)-mannose	×	×	×			
Mannosyl-α(1 → 3)-mannose	×	×				×
Mannosyl-α(1 → 4)-mannose	×	×			×	×
Mannosyl-α(1 → 6)-mannose	×	×	×	×	×	×

[a] All spectra were obtained on a 2-(perfluorooctyl)ethanethiolate monolayer surface. Y denotes the linkage position (Y = 2, 3, 4, 6).

CCC 0030–493X/94/110700–03

Received 28 July 1994
Accepted 28 July 1994

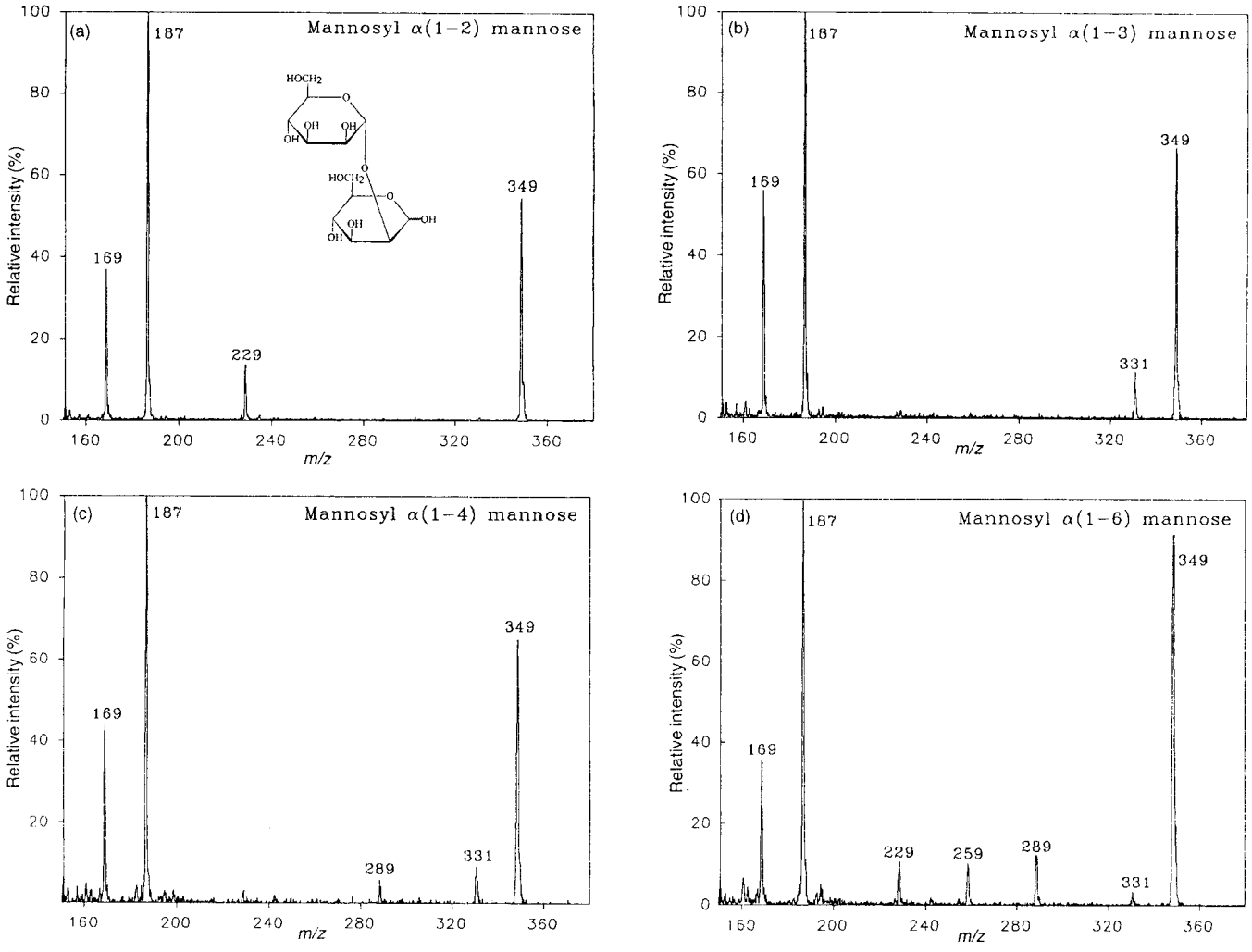

Figure 1. Surface-induced dissociation spectra of (a) mannosyl-α(1 → 2)-mannose, (b) mannosyl-α(1 → 3)-mannose, (c) mannosyl-α(1 → 4)-mannose and (d) mannosyl-α(1 → 6)-mannose at a collision energy of 30 eV on a 2-(perfluorooctyl)ethanethiol monolayer surface on gold.

higher internal energies into the precursor ions, as would be expected.

Acknowledgements. This research was funded by NSF (CHE-9224719).

Yours

ASHOK R. DONGRÉ and VICKI H. WYSOCKI*
Department of Chemistry,
Virginia Commonwealth University,
Richmond, Virginia 23284-2006,
USA

References

1. Md. A. Mabud, M. J. Dekrey and R. G. Cooks, *Int. J. Mass Spectrom. Ion Processes* **67**, 285 (1985).
2. B. E. Winger, R. K. Julian Jr, R. G. Cooks and C. E. D. Chidsey, *J. Am. Chem. Soc.* **113**, 8967 (1991).
3. V. H. Wysocki, J. L. Jones and J.-M. Ding, *J. Am. Chem. Soc.* **113**, 8969 (1991).
4. Á. Somogyi, T. E. Kane, J.-M. Ding and V. H. Wysocki, *J. Am. Chem. Soc.* **115**, 5275 (1993).
5. M. Morris, D. E. Riederer Jr, B. E. Winger, R. G. Cooks, T. Ast and C. E. D. Chidsey, *Int. J. Mass Spectrom. Ion Processes* **122**, 181 (1992).
6. T. E. Kane, Á. Somogyi and V. H. Wysocki, *Org. Mass Spectrom.* **28**, 1665 (1993).
7. M. E. Bier, J. C. Schwartz, K. L. Schey and R. G. Cooks, *Int. J. Mass Spectrom. Ion Processes* **103**, 1 (1990).
8. A. L. McCormack, J. L. Jones and V. H. Wysocki, *J. Am. Soc. Mass Spectrom.* **3**, 859 (1992).
9. A. L. McCormack, Á. Somogyi, A. R. Dongré and V. H. Wysocki, *Anal. Chem.* **65**, 2859 (1993).
10. J. L. Jones, A. R. Dongré, Á. Somogyi and V. H. Wysocki, *J. Am. Chem. Soc.*, in press.
11. R. B. Cole, S. LeMeillour and S.-C. Tabet, *Anal. Chem.* **64**, 365 (1992).
12. W. Aberth, *Anal. Chem.* **62**, 609 (1990).
13. E. R. Williams, K. D. Henry, F. W. McLafferty, J. Shabanowitz and D. F. Hunt, *J. Am. Soc. Mass Spectrom.* **1**, 413 (1990).
14. R. G. Cooks, T. Ast and Md. A. Mabud, *Int. J. Mass Spectrom. Ion Processes* **100**, 209 (1990).
15. V. H. Wysocki, H. I. Kenttämäa and R. G. Cooks, *Int. J. Mass Spectrom. Ion Processes* **75**, 181 (1987).
16. M. J. DeKrey, H. I. Kenttamaa, V. H. Wysocki and R. G. Cooks, *Org. Mass Spectrom.* **21**, 193 (1986).
17. L. Blok-Tip, J. W. Dallinga, W. Heerma and J. Haverkamp, *Biol. Mass Spectrom.* **21**, 331 (1992).
18. M. L. Coates and C. L. Wilkins, *Anal. Chem.* **59**, 197 (1987).
19. B. Spengler, J. W. Dolce and R. J. Cotter, *Anal. Chem.* **62**, 1731 (1990).
20. J. A. Carroll and C. B. Lebrilla, *Org. Mass Spectrom.* **27**, 639 (1992).
21. R. A. Laine, K. M. Pamidimukkala, A. D. French, R. W. Hall, S. A. Abbas, R. K. Jain and K. L. Matta, *J. Am. Chem. Soc.* **110**, 6831 (1988).
22. F. W. Rollegen, U. Giessmann, F. Borchers and K. Levsen, *Org. Mass Spectrom.* **13**, 459 (1978).

23. J. J. Conboy and J. Henion, *Biol. Mass Spectrom.* **21**, 397 (1992).
24. Z. Zhou, S. Ogden and J. A. Leary, *J. Org. Chem.* **55**, 5444 (1990).
25. G. E. Hofmeister, Z. Zhou and J. A. Leary, *J. Am. Chem. Soc.* **113**, 5964 (1991).
26. J. A. Leary, Z. Zhou, S. Ogden and T. D. Williams, *J. Am. Soc. Mass Spectrom.* **1**, 473 (1990).
27. D. Garozzo, M. Giuffrida and G. Impallomeni, *Anal. Chem.* **62**, 279 (1990).
28. V. H. Wysocki, J.-M. Ding, J. L. Jones, J. H. Callahan and F. L. King, *J. Am. Soc. Mass Spectrom.* **1**, 473 (1990).
29. C. W. White and E. S. Jacobson, *Can. J. Microsc.*, submitted for publication.