Influence of Alkali Metal Cation Type on Ionization Characteristics of Carbohydrates in ESI-MS

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Alkali metal salts were introduced to enhance the ionization efficiency of glucose and maltooligoses in electrospray ionization-mass spectrometry (ESI-MS). A mixture of the same moles of glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose was used. Salts of lithium, sodium, potassium, and cesium were employed as the cationizing agent. The ionization efficiency varied with the alkali metal cation types as well as the analyte sizes. Ion abundance distribution of the $[M+cation]^+$ ions of the carbohydrates varied with the fragmentor voltage. The maximum ion abundance at low fragmentor voltage was observed at maltose, while the maximum ion abundance at high fragmentor voltage shifted to maltotriose or maltotetraose for Na, K, and Cs. Variation of the ionization efficiency was explained with the hydrated cation size and the binding energy of the analyte and alkali metal cation.

Key Words: LC/MS, Maltooligose, ESI, Cationizing agent, Binding energy

Introduction

Carbohydrates are the most abundant and structurally diverse compounds found in nature.[1] Carbohydrates, alone or as constituents of glycoproteins, proteoglycans, and glycolipids, are mediators of cellular events such as intra- and extracellular recognition, differentiation, proliferation, and even signal transduction.[2-5] Unlike linear polymers such as proteins and nucleic acids, oligo- and polymeric carbohydrates can form branched structures because linkage of the constituent monosaccharides can occur at a number of positions.

Mass spectrometry is an important tool for the structural analysis of carbohydrates, and gives precise results, analytical versatility, and very high sensitivity.[8] Soft ionization techniques such as matrix-assisted laser desorption and ionization (MALDI) and atmospheric pressure ionization (API) have been used for linkage and sequence determination of oligosaccharides.[9-23] In MALDI, carbohydrates are most often ionized by adduction of metal ions, usually sodium cation, with comparatively low efficiency.[9-11] Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the most currently used as API sources which have widely contributed to the success of liquid chromatography coupled to mass spectrometry (LC/MS) for the fast analysis of biological materials.[24-28] Analysis of carbohydrates using LC/MS have been performed mainly with ESI.[15-23] Zhu and Sato[23] analyzed underivatized monosaccharides using ESI and reported a convenient method for distinguishing underivatized isomeric monosaccharides such as glucose, galactose, and mannose. Cheng and Her[22] analyzed oligosaccharides labeled with *p*-aminobenzoic ethyl ester using negative-ion ESI-MS.

Typical product ions of carbohydrate in ESI-MS are $[M+H]^+$ and $[M+Na]^+$.[29-31] When an alkali metal cation such as Li^+, Na^+, K^+, and Cs^+ is added to carbohydrate sample, the typical product ion is $[M+cation]^+$.[29,32] In the present work, we introduced alkali metal salts as the cationizing agent to improve the ionization efficiency of carbohydrate in ESI-MS. Glucose and maltooligoses (maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose) were employed as carbohydrates and salts of Li^+, Na^+, K^+, and Cs^+ were used. The ionization efficiency depending on the analyte size and the fragmentation were investigated. A key parameter in tuning the instrument's sensitivity is the fragmentor voltage (similar to cone voltage in other instruments). At higher fragmentor voltage settings, collision-induced dissociation (CID) can be initiated in the region between the end of the transfer capillary and the first skimmer cone, so that fragmentation increases.[33-35]

Experimental

Glucose (DP1), maltose (DP2), maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6), and maltoheptaose (DP7) purchased from Aldrich Co. were employed as the carbohydrates. Lithium trifluoroacetate (LiTFA), sodium trifluoroacetate (NaTFA), potassium chloride (KCl), and cesium trifluoroacetate (CsTFA) were employed as the cationizing agents. LiTFA, NaTFA, and CsTFA were purchased from Aldrich Co. and KCl was purchased from Junsei Chemical Co. Deionized water purchased from Burdick & Jackson Inc. was employed as solvent and eluent. Acetonitrile used as one of the eluents was purchased from Aldrich Co. The carbohydrates were dissolved in deionized water and their concentrations were 5 mM. The cationizing agents were also dissolved in deionized water and their concentrations were 1 mM. We prepared the samples by mixing each 1 mL of the 5 mM seven carbohydrates solutions and 1 mL of the 1 mM cationizing agent solution.

ESI mass spectra were obtained with 1100 series LC/MSD of Agilent Co. The sample solution of 10 μL was injected and a flow rate of the eluent (deionized water/acetonitrile = 50/50)

was 1.0 mL/min. In positive ion mode, the entrance to the capillary was −4 kV relative to the needle and −500 V relative to the end cap. The spray was stabilized with nitrogen gas at 55 psi and drying gas heated 350 °C with a flow rate of 12 L/min was used to evaporate the solvent in the spray chamber. Fragmentor voltages were 50, 75, 125, and 150 V.

Results and Discussion

In order to investigate the ionization efficiency depending on the analtye size, the seven carbohydrates (DP1 - DP7) of the same concentration were mixed with the same volume. Mass spectra of the mixture sample without the salt display the $[M\text{-}OH]^+$ and $[M+Na]^-$ ions but the $[M+H]^-$ ions were not observed as shown in Figure 1. The *m/z* 163, 325, 487, 649, 811, 973, and 1135 were assigned to the $[M\text{-}OH]^+$ ions of DP1, DP2, DP3, DP4, DP5, DP6, and DP7. The molecular ions ($M^{+\cdot}$) of DP1 (*m/z* 180) and DP2 (*m/z* 342) were detected but the protonated molecular ions, $[M+H]^-$ were not observed. This indicates that the $[M\text{-}OH]^+$ ions can be formed directly from the molecular ion by loss of the hydroxyl group on C1 of glucose not the $[M+H]^+$ ions. Sodium cation of the $[M+Na]^-$ ions may come from the sugars or glassware. The $[M\text{-}OH]^-$ ions at higher fragmentor voltage were observed better than at lower fragmentor voltage. For glucose (DP1), the dehydrated ions ($[M\text{-}OH\text{-}nH_2O]^+$) of *m/z* 145, 127, and 109 for n = 1, 2, and 3, respectively, were observed.

Mass spectra of the sample containing the salt mainly show the ion distributions of the $[M+cation]^+$, where the cation is the alkali metal cation of the salt, as shown in Figures 2 - 5. And the $[M+cation]^-$ ions are much abundant than the $[M\text{-}OH]^+$ ions, which means that the alkali metal cation enhances the ionization efficiency of the carbohydrates. The mass spectra of the sample containing the lithium salt predominantly show the ion distributions of $[M+Li]^-$ (*m/z* 187, 349, 511, 673, 835, 997, and 1159 for DP1, DP2, DP3, DP4, DP5, DP6, and DP7, respectively) and the ion distribution is getting more clear by increasing the fragmentor voltage. But, for the $[M\text{-}OH]^+$ ions, only the $[DP1\text{-}OH]^+$ and $[DP2\text{-}OH]^+$ (*m/z* 163 and 325) are observed (Figure 2). The *m/z* 367 ion assigned to [DP2+Li+

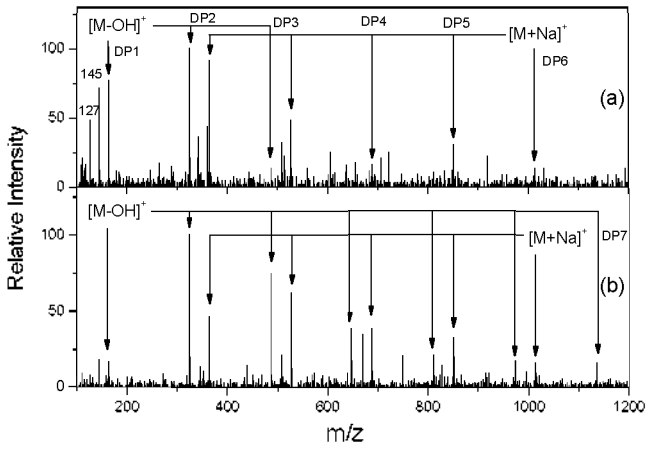

Figure 1. ESI mass spectra of the mixture of glucose and maltooligoses without salt at the fragmentor voltages of 75 V (a) and 150 V (b).

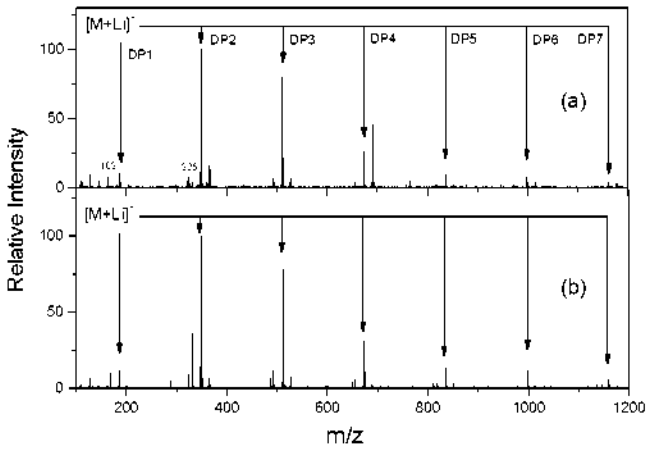

Figure 2. ESI mass spectra of the mixture of glucose and maltooligoses containing the lithium salt at the fragmentor voltages of 75 V (a) and 150 V (b).

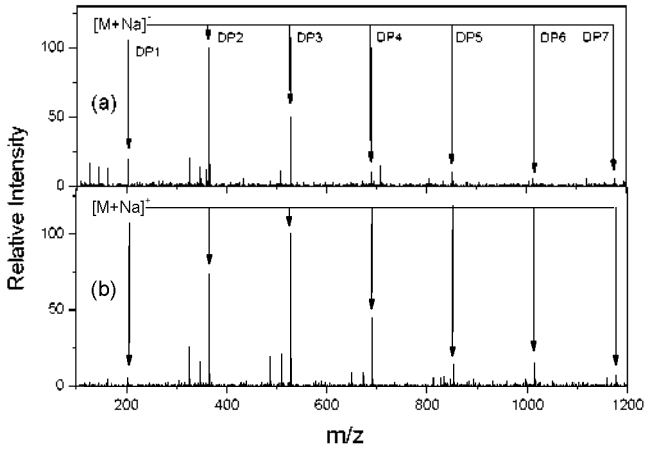

Figure 3. ESI mass spectra of the mixture of glucose and maltooligoses containing the sodium salt at the fragmentor voltages of 75 V (a) and 150 V (b).

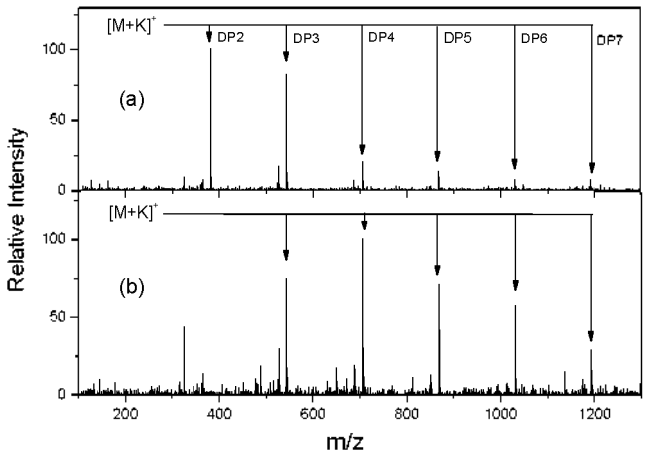

Figure 4. ESI mass spectra of the mixture of glucose and maltooligoses containing the potassium salt at the fragmentor voltages of 75 V (a) and 150 V (b).

$H_2O]^+$ was observed at low fragmentor voltage of 50 V. The mass spectra of the sample containing the sodium salt show well the ion distributions of $[M+Na]^-$ (*m/z* 203, 365, 527, 689, 851, 1013, and 1175 for DP1, DP2, DP3, DP4, DP5, DP6, and DP7, respectively) and the most abundant ion peak is shifted to the larger size from DP2 to DP3 by increasing the fragmentor voltage (Figure 3). The mass spectra of the sample containing the potassium salt show the ion distributions of $[M+K]^-$ (*m/z* 381, 543, 705, 867, 1029, and 1191 for DP2, DP3, DP4, DP5, DP6, and DP7, respectively) and the most abundant ion is shifted to the larger size from DP2 to DP4 by increasing the fragmentor voltage (Figure 4). The $[DP1+K]^-$ ion (*m/z* 219) was not observed and even the $[DP2+K]^-$ ion was also not observed at high fragmentor voltage. The most abundant ion in the mass spectra of the sample containing the cesium salt is the cesium ion (*m/z* 133) and the mass spectra show the ion distributions of $[M+Cs]^+$ (*m/z* 475, 639, 799, 961, 1123, and 1285 for DP2, DP3, DP4, DP5, DP6, and DP7, respectively) as shown in Figure 5. The $[DP1+Cs]^-$ ion (*m/z* 313) was not also observed and even the $[DP2+Cs]^-$ ion was also not observed at high fragmentor voltage.

As shown in Figures 2 - 5, the ionization efficiencies of the carbohydrates were enhanced by adding the alkali metal salt and the relative ion intensity distribution of the $[M+cation]^-$ varied with the cation type and the analyte size. In order to investigate these phenomena in detail, variation of the relative ion intensity distribution of the $[M+cation]^+$ was plotted as a function of the analyte size as shown in Figures 6 - 9. The ion

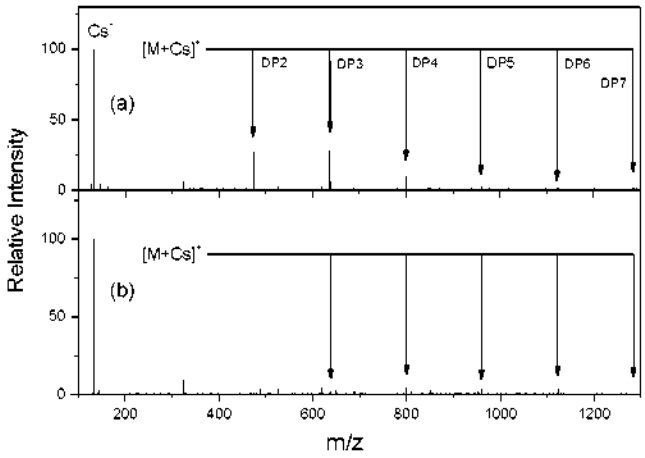

Figure 5. ESI mass spectra of the mixture of glucose and maltooligoses containing the cesium salt at the fragmentor voltages of 75 V (a) and 150 V (b).

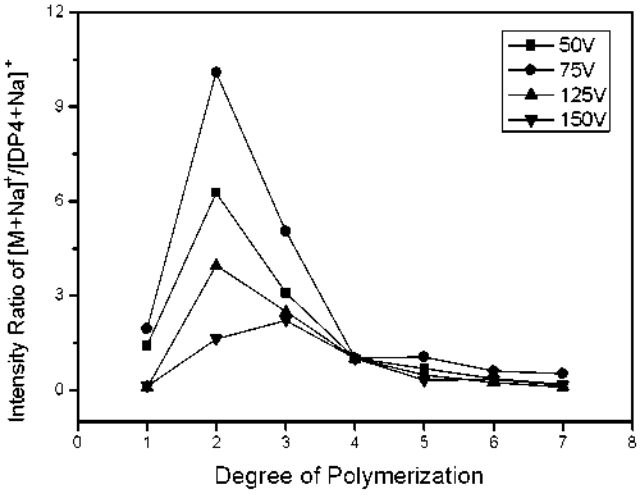

Figure 7. Variation of the relative intensity ratios of the $[M + Na]^+$s of the mixture of glucose and maltooligoses containing the sodium salt with the degree of polymerization. The ion intensity of the $[DP4+Na]^+$ was used as the reference. Squares, circles, up-triangles, and down-triangles stand for the fragmentor voltages of 50, 75, 125, and 150 V, respectively.

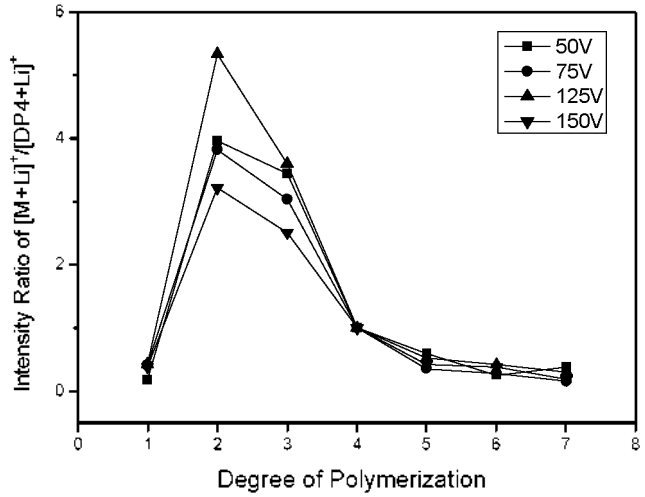

Figure 6. Variation of the relative intensity ratios of the $[M + Li]^+$s of the mixture of glucose and maltooligoses containing the lithium salt with the degree of polymerization. The ion intensity of the $[DP4+Li]^+$ was used as the reference. Squares, circles, up-triangles, and down-triangles stand for the fragmentor voltages of 50, 75, 125, and 150 V, respectively.

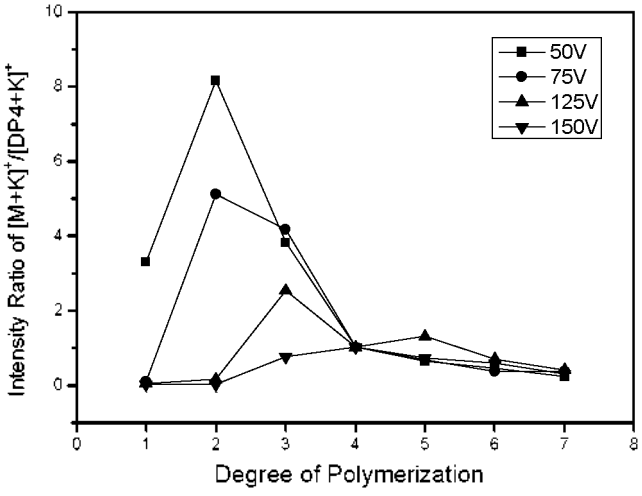

Figure 8. Variation of the relative intensity ratios of the $[M + K]^+$s of the mixture of glucose and maltooligoses containing the potassium salt with the degree of polymerization. The ion intensity of the $[DP4+K]^+$ was used as the reference. Squares, circles, up-triangles, and down-triangles stand for the fragmentor voltages of 50, 75, 125, and 150 V, respectively.

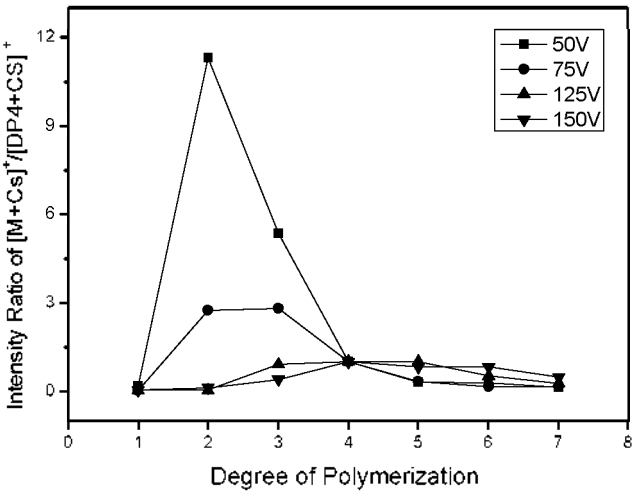

Figure 9. Variation of the relative intensity ratios of the $[M + Cs]^+$s of the mixture of glucose and maltooligoses containing the cesium salt with the degree of polymerization. The ion intensity of the $[DP4+Cs]^+$ was used as the reference. Squares, circles, up-triangles, and down-triangles stand for the fragmentor voltages of 50, 75, 125, and 150 V, respectively.

intensity of the $[DP4+Li]^+$ was used as the reference. The ion intensity ratios of the $[M+cation]^+$s varied depending on the analyte size though the mixture sample contained the 7 carbohydrates of the same concentration. This means that the ionization efficiency to form the alkali metal cation-adducted carbohydrate varies with the analyte size. The maximum ion intensity ratio is DP2, DP3, or DP4 and it varies with the alkali metal cation types and the fragmentor voltages.

The experimental results of LC/ESI-MS are very different from the MALDI-TOFMS results. Liu and coworkers analyzed underivatized oligosaccharides using LC/ESI-MS and reported that the relative abundances of oligosaccharides were different depending one the analyte size.[36] Unfortunately, detail discussion about the ionization efficiency difference with the analyte size was not performed. For MALDI, the ionization efficiency of maltooligose with alkali metal cation tends to increase by increasing the analyte size.[11] The ionization efficiency difference in ESI and MALDI may be due to the states of ion complex formation. For MALDI, the ion complex can be formed in gas phase. For ESI, however, the ion complex of maltooligose and alkali metal cation is formed in liquid phase, solvent molecules are evaporated by high voltage application and nebulization, and finally the gas phase ion complex enters to the mass spectrometer. In aqueous solution, the alkali metal cation is hydrated and the hydrated radius is much bigger than the pure cation radius.[37] Since the alkali metal cation affinity of saccharides is consistent with the multidentate coordination,[38] the distinct ionization efficiencies of the saccharides depending on the size may be due to the structural stability of the saccharide-alkali metal cation complex in water solution.

Maltooligosaccharide has a helix structure when its size is large enough.[39] One turn helix structure is composed of six monomers and hydroxyl groups are located in outer part. Maltose (DP2) can have a linear structure whereas maltotetraose (DP4) can have a semicircular structure. Since the outside of maltooligose is hydrophilic whereas the inside is hydrophobic, the hydrated alkali metal cation is located in the outside of maltooligose not the inside. Stability of the maltooligose-alkali metal cation complex will be increased by increasing the number of the coordination sites between the maltooligose and alkali metal cation. For maltooligose having a cyclic or semicircular structure, the stable structure will be changed to more strained structure to increase the coordination sites and the structural stability will decrease. Thus, the number of the coordination sites of maltooligose having a cyclic or semicircular structure is relatively small and the ionization efficiency is relatively low. One can expect that maltose has a linear structure and many coordination sites,[35] the number of coordination sites of a linear maltotriose is relatively large but that of a semicircular maltotriose is small, and maltotetraose has a semicircular structure and few coordination sites. If the size of alkali metal cation is large enough, the stable structure of pure maltotriose will not be destroyed by formation of the maltotriose-alkali metal cation complex. Thus, using a big alkali metal cation, the maximum ion abundance can be appeared at $[DP3+cation]^+$.

For the $[M+Li]^+$ ion intensity distribution, the ratio of $[DP2+Li]^+$ is the highest and then decreases with increasing the analyte size irrespective of the fragmentor voltages (Figure 6). The $[M+Li]^+$ ion intensity decreases as the analyte size increases from $[DP2+Li]^+$ to $[DP7+Li]^+$. For the $[M+Na]^+$ ion intensity distribution, the ratio of $[DP2+Na]^+$ is also the highest (except for the fragmentor voltage of 150 V) and then decreases with increasing the analyte size (Figure 7). The most abundant $[M+Na]^+$ ion at the fragmentor voltage of 150 V is the $[DP3+Na]^+$. The relative ion intensities of $[M+Na]^+$ show decreasing trends with the fragmentor voltage except for the 75 V.

For the $[M+K]^+$ ion intensity distribution, the highest ratio shifts from the $[DP2+K]^+$ to the $[DP4+K]^+$ with increasing the fragmentor voltage (Figure 8). For the small carbohydrates, the ion intensity ratios of $[M+K]^+$ decrease with the fragmentor voltage. The ion intensity ratios of $[DP2+K]^+$ at the high fragmentor voltages of 125 and 150 V are negligible. This phenomenon was also observed in the $[M+Cs]^+$ ion intensity distribution as shown in Figure 9. This indicates that the binding energies of the $[DP2+K]^+$ and $[DP2+Cs]^+$ are relatively much small. Rogatsky and coworkers studied the attachment of cesium cation to mono- and disaccharides in ESI-MS and reported the transition of $[M+Cs]^+$ to Cs^+.[32] For the $[M+Cs]^+$ ion intensity distribution, the highest ratio also shifts from the $[DP2+Cs]^+$ to the $[DP4+Cs]^+$ with increasing the fragmentor voltage (Figure 9).

Shift of the maximum ion abundance peak to the larger maltooligose depending on the fragmentor voltage (Figures 7 - 9) can be explained with the binding energy of the alkali metal cation with the maltooligose since the ion-dipole interaction increases with decreasing the alkali metal cation size.[39] The maximum peak shift was not observed for the $[M+Li]^+$ ion distributions, whereas it was appeared well for the $[M+K]^+$ and $[M+Cs]^+$ ion distributions as shown in Figures 6 - 9. If the binding energy is relatively weak, the analyte molecule can be easily separated from the $[M+cation]^+$ by high collision energy of high fragmentor voltage. According to the experimental

results, the binding energies of the $[DP2+K]^+$ and $[DP2+Cs]^+$ ions may be much lower than that of the $[DP2+Li]^+$ ion. In MALDI, large alkali metal ions also required larger oligosaccharides to produce the quasimolecular ions.[40] It can be considered that large alkali metal cations such as K^+ and Cs^+ will require larger oligosaccharides to make stronger interactions by multidentated coordinations.

Conclusions

When the alkali metal salts were employed as the cationizing agents, the ionization efficiencies of the carbohydrates were enhanced. The ion distributions of $[M+cation]^+$ showed the maximum peak which shifted depending on the alkali metal cation size and the fragmentor voltage. The $[DP2+cation]^+$ is the maximum peak at low fragmentor voltage, irrespective of the alkali metal cation types. For using Na^+, K^+, or Cs^+ as the cationizing agent, the maximum peak shifted from $[DP2+cation]^+$ to $[DP3+cation]^+$ or $[DP4+cation]^+$ by increasing the fragmentor voltage. This phenomenon was observed well when Cs^+ was used as the cationizing agent. The maximum peak shift was due to the binding energy of the analyte with the alkali metal cation and detachment of the analyte molecule from the ion complex by collision energy at high fragmentor voltage.

References

1. Lamari, F. N.; Kuhn, R.; Karamanos, N. K. *J. Chromatogr. B* **2003**, *793*, 15-36.
2. Iozzo, R. V. *Crit. Rev. Biochem. Mol. Biol.* **1997**, *32*, 141-174.
3. Bernfield, M.; Gotte, M.; Park, P. W.; Reizes, O.; Fitzgerald, M. L.; Lincecum, J.; Zako, M. *Annu. Rev. Biochem.* **1999**, *68*, 729-778.
4. Schwartz, N. *Front. Biosci.* **2000**, *5*, D649-655.
5. Sugahara, K.; Kitagawa, H. *Curr. Opin. Struct. Biol.* **2000**, *10*, 518-527.
6. Helenius, A.; Aebi, M. *Science* **2001**, *291*, 2364-2369.
7. Wells, L.; Vosseller, K.; Hart, G. W. *Science* **2001**, *291*, 2376-2378.
8. Zaia, J. *Mass Spectrom. Rev.* **2004**, *23*, 161-227.
9. Harvey, D. J. *Mass Spectrom. Rev.* **1999**, *18*, 349-450.
10. Naven, T. J. P.; Harvey, D. J. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 829-834.
11. Choi, S.-S.; Ha, S.-H. *Bull. Kor. Chem. Soc.* **2006**, *27*, 1243-1245.
12. Harvey, D. J.; Küster, B.; Naven, T. J. P. *Glycoconjugate J.* **1998**, *15*, 333-338.
13. Tseng, K.; Hedrick, J. L.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 3747-3754.
14. Price, N. P. J. *Anal. Chem.* **2006**, *78*, 5302-5308.
15. Viseux, N.; de Hiffmann, E.; Domon, B. *Anal. Chem.* **1998**, *70*, 4951-4959.
16. Weiskopf, A. S.; Vouros, P.; Harvey, D. J. *Anal. Chem.* **1998**, *70*, 4441-4447.
17. Li, D. T.; Her, G. R. *J. Mass Spectrom.* **1998**, *33*, 644-652.
18. Garozzo, D.; Impallomeni, G.; Spina, E.; Green, B. N.; Hutton, T. *Carbohydr. Res.* **1991**, *221*, 253-257.
19. Mulroney, B.; Traeger, J. C.; Stone, B. A. *J. Mass Spectrom.* **1995**, *30*, 1277-1283.
20. Yuan, J.; Hashii, N.; Kawasaki, N.; Itoh, S.; Kawanishi, T.; Hayakawa, T. *J. Chromatogr. A* **2005**, *1067*, 145-152.
21. Wan, E. C. H.; Yu, J. Z. *J. Chromatogr. A* **2006**, *1107*, 175-181.
22. Cheng, H. L.; Her, G. R. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 1322-1330.
23. Zhu, X.; Sato, T. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 191-198.
24. Liang, H. R.; Takagaki, T.; Foltz, R. L.; Bennett, P. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2284-2294.
25. Kakola, J.; Alen, R.; Pakkanen, H.; Matilainen, R.; Lahti, K. *J. Chromatogr. A* **2007**, *1139*, 263-270.
26. Lim, J. Y.; Kumar, A. P.; Kim, C.; Ahn, C.; Yoo, Y. J.; Lee, Y. I. *Bull. Kor. Chem. Soc.* **2009**, *30*, 397-401.
27. Lee, I.; Ahn, S.; Kim, B.; Hwang, E.; Seong, Y. *Bull. Kor. Chem. Soc.* **2008**, *29*, 2125-2128.
28. Cherlet, M.; de Baere, S.; Croubels, S.; de Backer, P. *Anal. Chim. Acta* **2005**, *529*, 361-369.
29. Harvey, D. J. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 900-915.
30. Harvey, D. J. *J. Mass Spectrom.* **2000**, *35*, 1178-1190.
31. Reis, A.; Coimbra, M. A.; Domingues, P.; Ferrer-Correia, A. J.; Domingues, M. R. M. *Carb. Polym.* **2004**, *55*, 401-409.
32. Rogatsky, E.; Jayatillake, H.; Goswami, G.; Tomuta, V.; Stein, D. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1805-1811.
33. Carrott, M. C.; Jones, D. C.; Davidson, G. *Analyst* **1998**, *123*, 1827-1833.
34. Alonso, M. C.; Barcelo, D. *Anal. Chim. Acta* **1999**, *400*, 211-231.
35. Choi, S.-S.; Song, M. J. *Bull. Kor. Chem. Soc.* **2008**, *29*, 1847-1849.
36. Liu, Y.; Urgaonkar, S.; Verkade, J. G.; Armstrong, D. W. *J. Chromatogr. A* **2005**, *1079*, 146-152.
37. Harris, D. C. *Quantitative Chemical Analysis*, seventh ed.; Freeman: New York, 2007.
38. Cerda, B. A.; Wesdemiotis, C. *Int. J. Mass Spectrom.* **1999**, *189*, 189-204.
39. Davis, H.; Skrzypek, W.; Khan, A. *J. Polym. Sci. A* **1994**, *32*, 2267-2274.
40. Cancilla, M. T.; Penn, S. G.; Carroll, J. A.; Lebrilla, C. B. *J. Am. Chem. Soc.* **1996**, *118*, 6736-6745.