Analysis of Mixture of Maltooligoses Using MALDI-TOFMS: Influence of Cationizing Agent Types

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Carbohydrates are the most abundant and structurally diverse compounds found in nature and serve the dual purpose of being structural components and key players of the energy metabolism.[1] Carbohydrates, either 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.[2-7] Unlike proteins and nucleic acids, which are linear polymers of amino acids and nucleotides respectively, carbohydrates can adopt complex branched structures with individual monomeric units linked at one of several sites.[8] Mass spectrometry is an important tool for the structural analysis of carbohydrates, and offers precise results, analytical versatility, and very high sensitivity.[9] 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.[10-24] In MALDI, carbohydrates most often ionize by adduction of metal ions in contrast to the more usual $[M + H]^+$ ion produced by proteins.[10-12,25] MALDI has been found to be from 10 to 100 times more sensitive than fast atom bombardment (FAB) or plasma desorption (PD) mass spectrometry for carbohydrate and glycoprotein analysis.[26-29] Several researches[12,30-34] reported that the ion abundances of carbohydrates were varied in accordance with the molecular size. In the present work, we analyzed maltooligosaccharides using MALDI and the sample was prepared with a cationizing agent to enhance the ionization efficiency. Nine alkali metal salts were employed as the cationizing agents and the differences in the ionization efficiencies, depending on the cationizing agents, were compared.

We prepared the sample by mixing 1 aliquot of the 5 mM carbohydrate mixture solution with 5 aliquots of the 100 mM matrix solution, which yields an analyte to matrix ratio of 1:100. Concentrations of the alkali metal salts were 1 mM. Figure 1 shows MALDI-TOF mass spectra of the samples containing LiTFA, NaTFA, and KTFA as the cationizing agent, respectively. The $[M + \text{cation}]^+$ ions of the alkali metal cations were clearly observed in the mass spectra but the protonated molecules, $[M + H]^+$ were not detected. The cation-adducted ions of maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6), and maltoheptaose (DP7) were detected, but those of glucose (DP1) and maltose (DP2) were not observed. The relative intensity of the $[M + \text{cation}]^+$ increases as the maltooligose size increases. The reason for no detection of the $[M + \text{cation}]^+$ ions of DP1 and DP2 may be due to the matrix suppression. Bashir and coworkers[35] studied MALDI analysis of glucose and reported that $[M + Na]^+$ of glucose was produced at high analyte concentration. The increasing cationization efficiency of large maltooligose can be explained with the multidentate coordination. Cerda and Wesdemiotis[36] suggested that for Na^+ coordinated saccharides the Na^+ affinity was consistent with the saccharides being multidentate ligands to Na^+. Lee and coworkers[37] reported that Na^+ coordinated with all four sugar moieties of maltotetraose. Thus, the peak intensity of $[M + \text{cation}]^+$ will enhance as the sample size increases since the number of coordination sites increases.

For the sample containing lithium cation, the product ions, besides the $[M + Li]^+$ ions of maltooligoses (DP3 − DP7), the $[M + Na]^+$ and $[M + K]^+$ ions were also observed. The sodium and potassium cations may come from the sugars or glassware

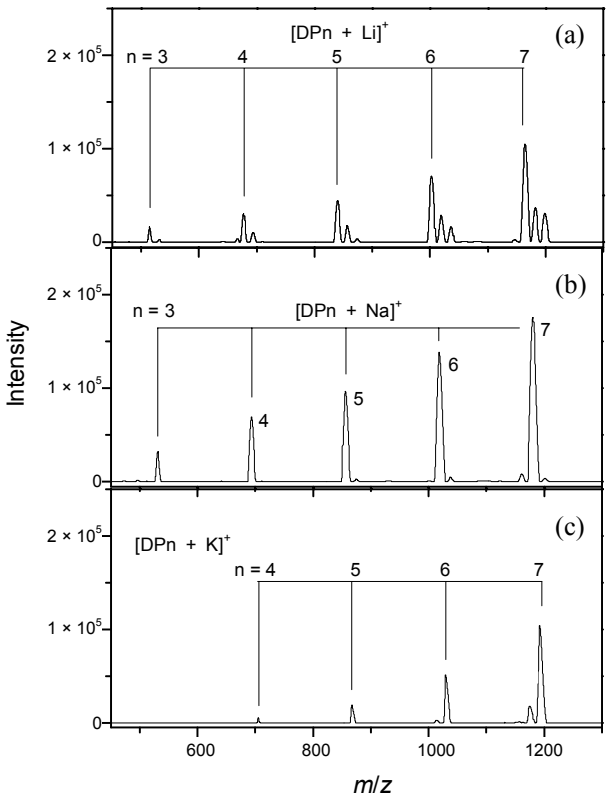

Figure 1. MALDI-TOF mass spectra of mixture of glucose and maltooligoses containing LiTFA (a), NaTFA (b), and KTFA (c) as a cationizing agent.

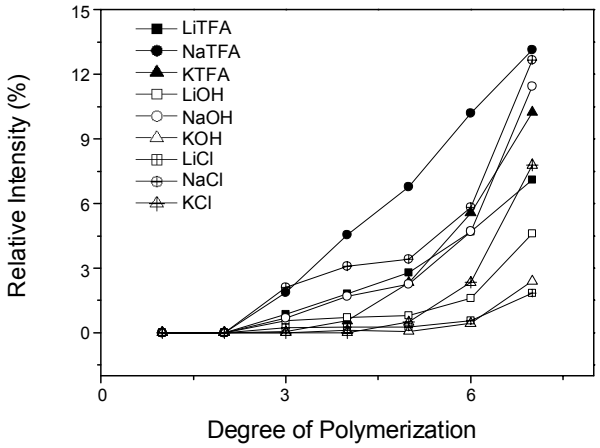

Figure 2. Variation of the relative intensities of $[M + cation]^+$ with the degree of polymerization of saccharides. The peak intensities were normalized with $[DHB + H - H_2O]^+$. Squares, circles, and triangles, and down-triangles stand for the $[M + Li]^+$, $[M + Na]^+$, and $[M + K]^+$, respectively. Solid, open, and crossed symbols indicate the CatTFA, CatOH, and CatCl, respectively.

because MALDI mass spectra of oligosaccharides without any cationizing agents displayed only $[M + Na]^+$ and $[M + K]^+$ ions.[38] Formation of the abundant $[M + Na]^+$ and $[M + K]^+$ ions in the sample containing lithium cation means that the adduction capability of Li^+ to the oligosaccharides is relatively much lower than those of Na^+ and K^+. The mass spectrum of the sample containing sodium cation displays the ion distributions of $[M + Na]^+$ from DP3 to DP7. Of the mass spectra of Li^+, Na^+, and K^+, the peak intensities of $[M + cation]^+$ ions of Na^+ are larger than the others. Dehydrated products of $[DP6 + Na - H_2O]^+$ and $[DP7 + Na - H_2O]^+$ ions formed from the $[DP6 + Na]^+$ and $[DP7 + Na]^+$ ions were observed. The $[DP6 + Na - H_2O]^+$ and $[DP7 + Na - H_2O]^+$ ions were particularly observed in the mass spectrum of the sodium cation. In the previous work,[12] we reported that the two terminal monomers of the heptamer overlapped and the dehydration reaction could occur between two hydroxyl groups of the both terminal monomers. The mass spectrum of the sample containing potassium cation displays the ion distributions of $[M + K]^+$ ions and the $[DP6 + Na]^+$ and $[DP7 + Na]^+$ ions are also observed. The $[DP3 + K]^+$ ion was detected by trace.

We investigated not only the alkali metal types but also the counter anion types on the ionization efficiencies of maltooligosaccharides using CatTFA, CatOH, and CatCl. The relative intensity of $[M + cation]^+$ was compared in terms of the cation and anion types as well as the analyte size. The relative intensity of $[M + cation]^+$ was normalized with $[DHB + H - H_2O]^+$ because the $[DHB + H - H_2O]^+$ is the most abundant product ion. The relative intensity of $[M + cation]^+$ varies with the cation and anion types as well as the analyte size as shown in Figure 2. All the relative intensities of $[M + cation]^+$ increase with increasing the sugar size irrespective of kinds of cations and anions. Likewise, the relative intensities of $[M + Na]^+$ are much larger than those of the $[M + Li]^+$ and $[M + K]^+$ ions irrespective of the kind of anions. In ESI-MS, the ion distributions of $[M + cation]^+$ showed a maximum peak at $[DP2 + cation]^+$, $[DP3 + cation]^+$, or $[DP4 + cation]^+$.[34]

The relative intensities of $[M + cation]^+$ generated using CatTFA salts are larger than those using CatOH and CatCl salts. It is very interesting that the ionization efficiency of saccharide depends on the anion type of the salt as well as the cation type. It can be expected that anion of the salt affects state of the sample preparation, protonation to the matrix, and formation of cation-maltooligose complex. We first examined the crystalline state of the MALDI sample, but did not find something different. Next, we considered the anion behaviors in water solution. Water solutions of CatTFA, CatOH, and CatCl are weak alkaline, strong alkaline, and neutral, respectively. The salts are fully dissolved and some of the anions can be changed to their conjugate acids. Conjugate acids of TFA^-, OH^-, and Cl^- are HTFA, H_2O, and HCl, respectively. However, only HTFA can be formed because HCl is strong acid and H_2O is solvent. By the formation of HTFA, the metal cation of CatTFA can easily adduct to maltooligose.

The order of ion intensities of $[M + cation]^+$ with different cations shows some difference according to the anion type of salt. For CatTFA and CatCl, the order of $[M + cation]^+$ intensity (M = DP6 and DP7) is $[M + Na]^+ > [M + K]^+ > [M + Li]^+$. For CatOH, the order is $[M + Na]^+ > [M + Li]^+ > [M + K]^+$. Our results were not consistent with binding affinities proposed by Mohr and coworkers.[39] Mohr and coworkers analyzed maltoheptaose with high concentration (0.25 M) of salt mixture and suggested that based on the relative abundance of alkali metal doped maltoheptaose, the order of binding affinities for carbohydrates was as follows: Li < Na < K. This may be due to the state of alkali metal cation in the solid MALDI sample and the capacity to form gas phase alkali metal cation by laser ablation. Ion intensity distributions of $[M + cation]^+$ using CatCl vary with the maltooligose size as well as the cation type. The order of $[DP7 + cation]^+$ intensity using CatCl is the same trend with the ion intensity order using CatTFA: $[DP7 + Na]^+ > [DP7 + K]^+ > [DP7 + Li]^+$. From the experimental results, we found that CatTFA enhanced ionization efficiencies of maltooligoses more than CatOH and CatCl. This was explained with the role of anion of the salt to improve the sample state, protonation to the matrix, and the formation of cation-maltooligose complex.

Experimental Section

Glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose were purchased from Aldrich Co. (St. Louis, USA). 2,5-Dihydroxybenzoic acid (DHB) used as the matrix was also purchased from Aldrich Co. (St. Louis, USA). Lithium trifluoroacetate (LiTFA), sodium trifluoroacetate (NaTFA), potassium trifluoroacetate (KTFA), lithium hydroxide (LiOH), sodium hydroxide (NaOH), potassium hydroxide (KOH), lithium chloride (LiCl), sodium chloride (NaCl), and potassium chloride (KCl) were employed as the cationizing agents, and they were purchased from Aldrich Co. (St. Louis, USA). The each saccharide (5 mM), matrix (100 mM), and cationizing agent (1 mM) were dissolved in deionized water. The seven saccharide solutions of glucose to maltoheptaose were mixed with the same concentration (5 mM) and volume (1 μL). The mixtrue of carbohydrates, matrix, and cationizing agent solutions were mixed (mixture of carbohydrates : matrix : cationizing reagent = 1 : 5 : 1 by volume ratio). The mixed solution

of 1 μL was spotted onto the sample plate and was dried.

MALDI mass spectra were obtained with Axima-LNR MALDI-TOFMS (Kratos-Shimadzu Co. of Japan). Ions were produced by irradiation of the sample with nitrogen laser (337 nm). Profiling of product ions was achieved in the positive mode using linear TOF. The accelerating voltage was 20 kV. The sum of 100 shots was collected for each spectrum.

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