Characterization of Ionized Maltooligosaccharides by Sodium Cation in MALDI-TOFMS Depending on the Molecular Size

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Carbohydrates are the most abundant and structurally diverse compounds found in nature. 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. With such a wide range of structural types, carbohydrate analysis by mass spectrometry can involve a large number of techniques with no single method being ideal for all compounds. Electron impact ionization, for example, is only applicable to the smaller molecules. Matrix-assisted laser desorption/ionization (MALDI),[1-3] on the other hand, is more versatile because most compounds give signals in their native states. As with other types of mass spectrometry, MALDI can provide valuable information on several aspects of structural analysis, such as the determination of sequence, branching, and linkage. Saccharides are more difficult to analyze than proteins. The hydrophilic nature of oligosaccharides and the lack of a chromophore have presented problems for their analysis, particularly with respect to detection. In addition, the absence of a basic site inhibits protonation in MALDI mass spectrometry. Carbohydrates most often ionize by adduction of metal ions, usually sodium cation, with comparatively low efficiency.[4,5]

Molecular weight distributions of materials can be obtained using MALDI mass spectrometry.[6-9] In order to measure accurate molecular weight distributions of an analyte, ionization efficiencies of all molecules in the analyte must be corrected. Several researches[10-13] reported that the ion abundances of carbohydrates were varied with the molecular size, but the detailed study and discussion have not been reported. In the present work, we investigated the variation of ionization efficiency of maltooligose with its size. The sample was ionized in the presence of sodium ion. Concentrations of the maltooligose and sodium ion were varied. Fragmentation of the $[M + Na]^+$, where the M is maltooligosaccharide, was also studied.

The mass spectra of the samples containing maltooligosaccharides, NaTFA, and 2,5-DHB are composed of the matrix-related ions and the maltooligose-related ions as shown in Figure 1. The m/z 137, 154, 155, and 177 can be assigned to $[m–OH]^+$, m^+, $[m + H]^+$, and $[m + Na]^+$ (m = 2,5-DHB), respectively. The m/z 537, 689, 851, 1013, and 1175 are assigned to $[M + Na]^+$ ions for M = maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose,

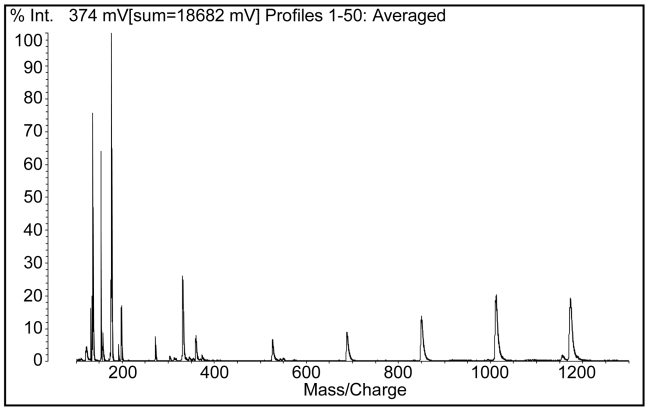


Figure 1. MALDI-TOF mass spectrum of 0.01 M maltooligoses and 0.01 M NaTFA.

respectively. The peak intensity of $[M + Na]^+$ increases notably until the hexamer by increasing the maltooligose size and then decreases. Of the four matrix-related ions, the $[m + Na]^+$ is the most abundant one and the second is the $[m – OH]^+$. The peak intensities of the $[M + Na]^+$ ions were normalized with $[m + Na]^+$ and $[m – OH]^+$ to investigate the difference in the ionization efficiencies of the maltooligoses. Figures 2, 3, and 4 show variations of the $[M + Na]^+/[m + Na]^+$ and $[M + Na]^+/[m – OH]^+$ ratios as a function of the molecular size for the NaTFA concentrations of 0.001, 0.005, and 0.01 M, respectively.

By increasing the molecular size, the $[M + Na]^+/[m + Na]^+$ and $[M + Na]^+/[m – OH]^+$ ratios increase continuously until the hexamer. The ion intensity ratios also increase with increse of the maltooligose concentration. The enhanced ion intensity with the molecular size may be due to the stable ion-molecule complex formed between sodium cation and maltose. The sodium cation can be easily adducted to hydroxyl groups of the maltose. The ion-molecule complex with two hydroxyl groups of the neighboring maltose units is more stable than that with only one.[14] By increasing the maltooligose size, the ion-molecule complex with two hydroxyl groups of the neighboring units will increase (Scheme 1).

For the maltoheptaose, the $[M + Na – 18]^+$ ion was also detected. The $[M + Na – 18]^+$ ions were not observed in the maltotriose to maltopentaose and it was detected only by

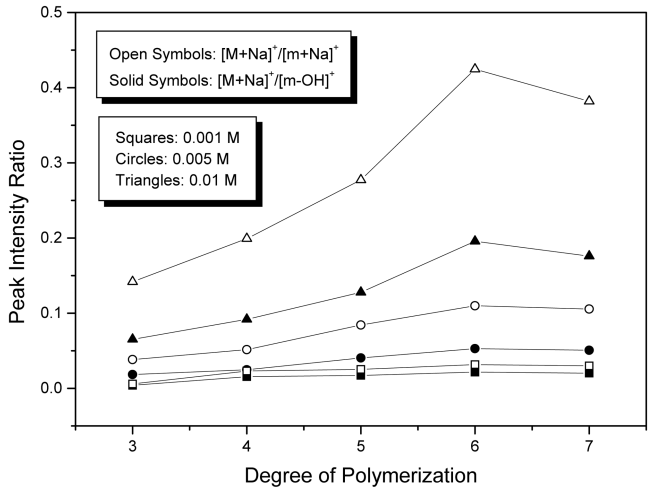


Figure 2. Variations of the peak intensity ratios as a function of the degree of polymerization for the NaTFA 0.001 M. Squares, circles, and triangles indicate the maltooligoses concentrations of 0.001, 0.005, and 0.01 M, respectively. Open and solid symbols stand for the ratios of $[M + Na]^+/[m + Na]^+$ and $[M + Na]^+/[m - OH]^+$ respectively, where m and M are matrix and maltooligose, respectively.

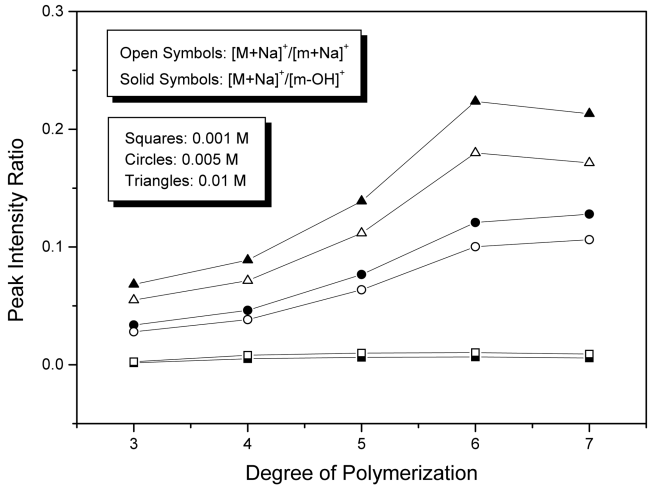


Figure 3. Variations of the peak intensity ratios as a function of the degree of polymerization for the NaTFA 0.005 M. Squares, circles, and triangles indicate the maltooligoses concentrations of 0.001, 0.005, and 0.01 M, respectively. Open and solid symbols stand for the ratios of $[M + Na]^+/[m + Na]^+$ and $[M + Na]^+/[m - OH]^+$ respectively, where m and M are matrix and maltooligose, respectively.

trace in the maltohexaose. The ratios of $[M + Na - 18]^+/[M + Na]^+$ are 0.084 and 0.023 for maltoheptaose and maltohexaose, respectively, when the concentrations of sodium cation and maltooligoses are 0.01 M. The $[M + Na - 18]^+$ ion can be assigned to $[M + Na - H_2O]^+$ formed from the $[M + Na]^+$ ion by dehydration. The maltooligose has helix structure when its size is large enough.[15] The hexamer has one turn helix structure as shown in Scheme 2 and the two terminal monomers of the heptamer are overlapped. The sodium cation can be located between the terminal units of the maltoheptaose. Dehydration reaction can occur between two hydroxyl groups of 3- and 6-carbons of the maltoheptaose as

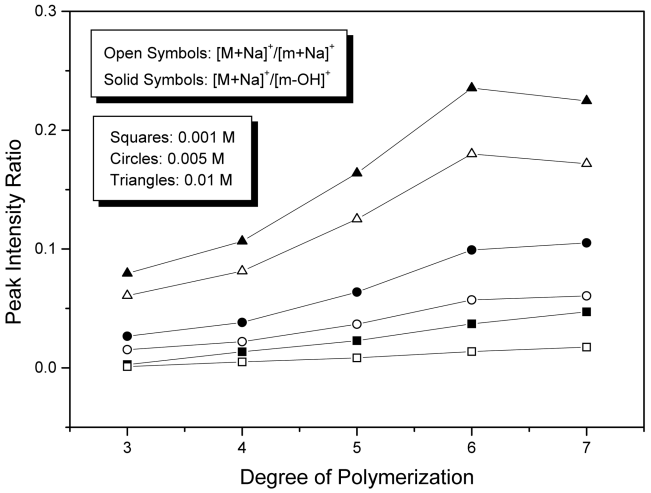
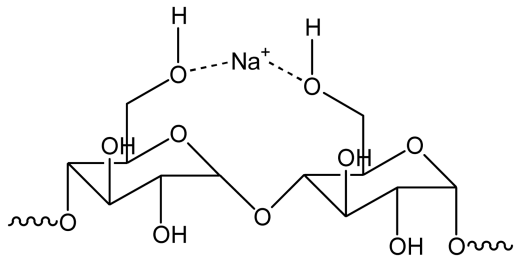
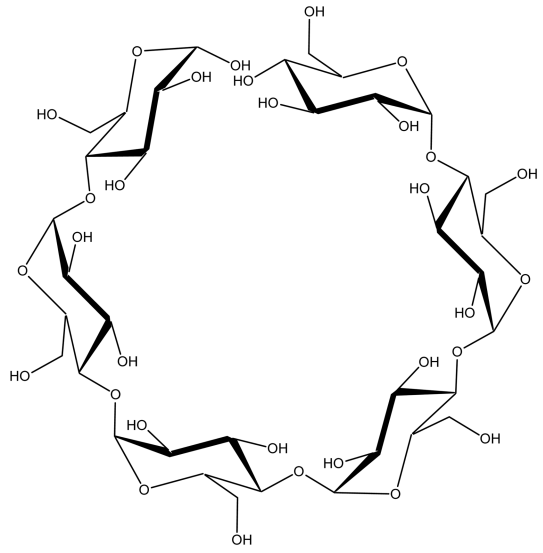


Figure 4. Variations of the peak intensity ratios as a function of the degree of polymerization for the NaTFA 0.01 M. Squares, circles, and triangles indicate the maltooligoses concentrations of 0.001, 0.005, and 0.01 M, respectively. Open and solid symbols stand for the ratios of $[M + Na]^+/[m + Na]^+$ and $[M + Na]^+/[m - OH]^+$ respectively, where m and M are matrix and maltooligose, respectively.



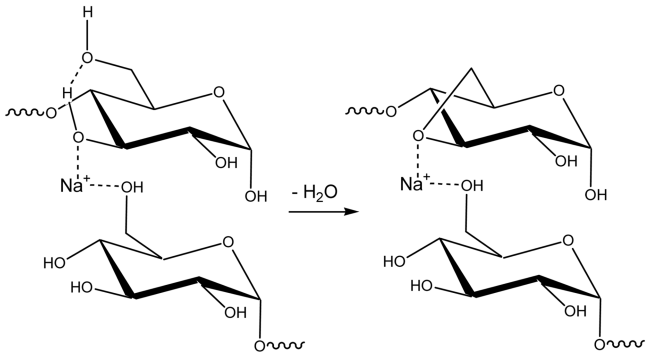
Scheme 1. Sodium cation-maltose complex.



Scheme 2. Structure of maltohexaose.

shown in Scheme 3. Further study for the dehydration reaction is needed.

From the experimental results, it can lead to a conclusion



Scheme 3. Plausible mechanism for formation of [maltoheptaose + Na – H_2O]$^+$.

that ionization efficiency of maltooligose is varied with the molecular size and the difference becomes larger as the sample concentration increases.

Experimental Section

Maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose purchased from Aldrich Co. were employed as maltooligosaccharides. 2,5-Dihydroxybenzoic acid (2,5-DHB) and sodium trifluoroacetate (NaTFA) purchased from Aldrich Co. were used as matrix and cationizing agent, respectively. The matrix, maltooligosaccharides, and cationizing agent were dissolved in distilled water. The maltooligosaccharides (trimer-heptamer) with the same moles were dissolved. Concentration of 2,5-DHB was constant of 0.1 M and concentrations of NaTFA were varied of 0.001 M, 0.005 M, and 0.01 M. Concentrations of maltooligose mixture of the trimer-heptamer were also varied of 0.001 M, 0.005 M, and 0.01 M. The maltooligoses, cationizing agent, and matrix solutions were mixed (maltooligoses : cationizing reagent : matrix = 1 : 1 : 5). The mixed solution of 2 μL was spotted onto the sample plate and dry.

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 50 shots was collected for each spectrum.

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