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Comparison of ionization behaviors of ring and linear carbohydrates in MALDI-TOFMS

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ABSTRACT

Ionization efficiencies of cyclodextrins and their linear compounds in matrix-assisted laser desorption and ionisation (MALDI) analysis were compared, and differences in the ionization efficiencies of α- and β-cyclodextrins were also studied. The mass spectra showed a series of the $[M+cation]^+$ ions but not the $[M+H]^+$ ions. Alkali metal salts of Li^+, Na^+, K^+, and Cs^+ were used as the cationizing agents to enhance the ionization efficiency. Relative ion intensities of the ring compounds (α- and β-cyclodextrins) were much larger than those of the linear ones (maltohexaose and maltoheptaose), and the difference showed an increasing trend with the size of the alkali metal cation. β-Cyclodextrin had higher ionization efficiency than α-cyclodextrin and the difference increased by increasing the size of the alkali metal cation. It was also found that the ionization efficiency was affected by the counter anion of the salt. The higher ionization efficiencies of cyclodextrins were explained with the number of coordination sites and the binding energies.

1. Introduction

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, alone or as constituents of glycoproteins, proteoglycans, and glycolipids, are mediators of cellular events such as intra- and extra-cellular recognition, differentiation, proliferation, and even signal transduction [2–7]. Mass spectrometry is an important tool for the structural analysis of carbohydrates, which 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]. 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 since 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. In MALDI, carbohydrates most often ionize by adduction of metal ions, usually sodium cation, with comparatively low efficiency [9–11]. Cerda and Wesdemiotis [24] suggested that for Na^+ coordinated saccharides the Na^+ affinity was consistent with the saccharides being multidentate ligands to Na^+. Lee et al. [25] reported that Na^+ coordinated with all four sugar moities of maltotetraose. Cancilla et al. [26] studied relationship between the size of the alkali metal ion and the yields of fragment ions of oligosaccharides, and reported that the smallest alkali metal ion produced the greatest amount of fragment ions.

Ion abundances of carbohydrates varied with the molecular size [11,27–30]. In general, the ionization efficiencies of carbohydrates tend to be enhanced by increasing the size. In order to perform a quantitative analysis of an analyte, ionization efficiencies of all molecules in the analyte must be corrected. Carbohydrates can exist either as linear or as cyclic compounds. For example, α- and β-cyclodextrins are the ring compounds of maltohexaose and maltoheptaose, respectively. One can expect that ionization efficiencies of the ring and linear compounds will be different since it is known that cyclodextrins have good inclusion capacity to form complex with different compounds [31,32]. However, the direct comparison of the ionization efficiencies of linear and cyclic carbohydrates seems not well studied. In the present work, we analyzed linear and ring carbohydrates using MALDI and their ionization efficiencies were compared. The MALDI sample was prepared with a cationizing agent to enhance the ionization efficiency. Twelve alkali metal salts were employed as the cationizing agents and differences in

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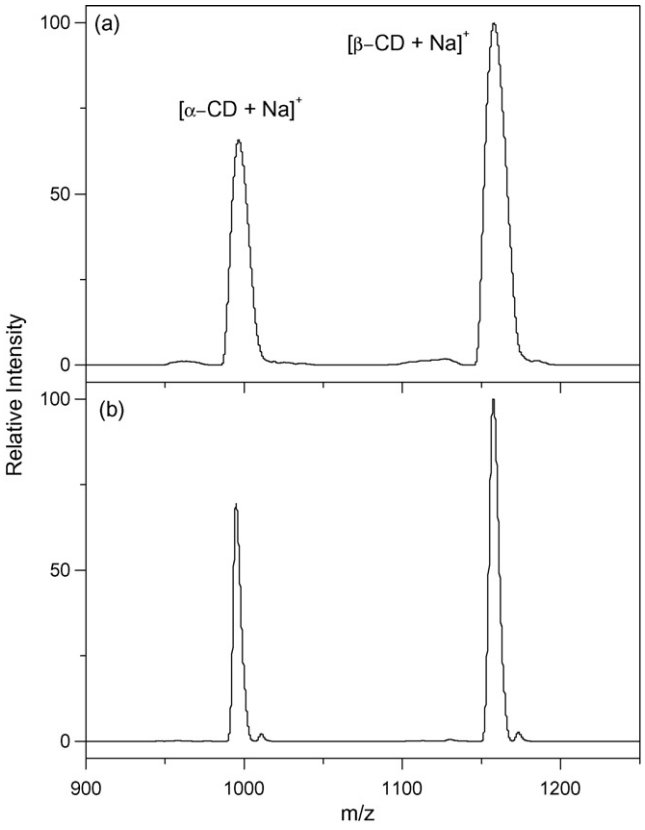


Fig. 1. MALDI-TOF mass spectrum of mixture of α- and β-cyclodextrins without a cationizing agent (a) and containing NaTFA as a cationizing agent (b).

the ionization efficiencies depending on the cationizing agents were compared. Coordinated structures and binding energies of the carbohydrates with sodium cation were calculated to explain the difference in the ionization efficiencies of linear and ring carbohydrates.

2. Experimental

Maltohexaose, maltoheptaose, and α- and β-cyclodextrins were purchased from Aldrich Co. 2,5-dihydroxybenzoic acid (DHB) used as the matrix was also purchased from Aldrich Co. Lithium trifluoroacetate (LiTFA), sodium trifluoroacetate (NaTFA), potassium trifluoroacetate (KTFA), cesium trifluoroacetate (CsTFA), lithium hydroxide (LiOH), sodium hydroxide (NaOH), potassium hydroxide (KOH), cesium hydroxide (CsOH), lithium chloride (LiCl), sodium chloride (NaCl), potassium chloride (KCl), and cesium chloride (CsCl) were employed as the cationizing agents. The alkali metal salts were purchased from Aldrich Co. except for CsCl which was purchased from Yakuri Pure Chemicals Co. The carbohydrates (5 mM), matrix (100 mM), and cationizing agents (1 mM) were dissolved in deionized water. Mixtrue of the carbohydrates, matrix, and cationizing agent solutions was prepared by mixing them (carbohydrates:matrix:cationizing reagent = 1:5:1 by volume ratio). The sample solution of 1 μL was spotted onto the sample plate and 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 the 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.

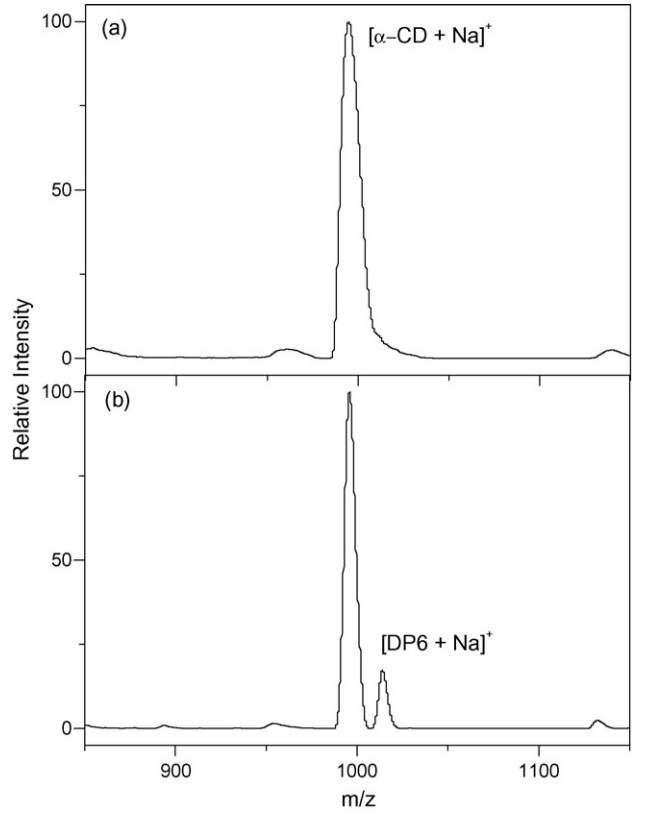


Fig. 2. MALDI-TOF mass spectrum of mixture of α-cyclodextrin and maltohexaose without a cationizing agent (a) and containing NaTFA as a cationizing agent (b).

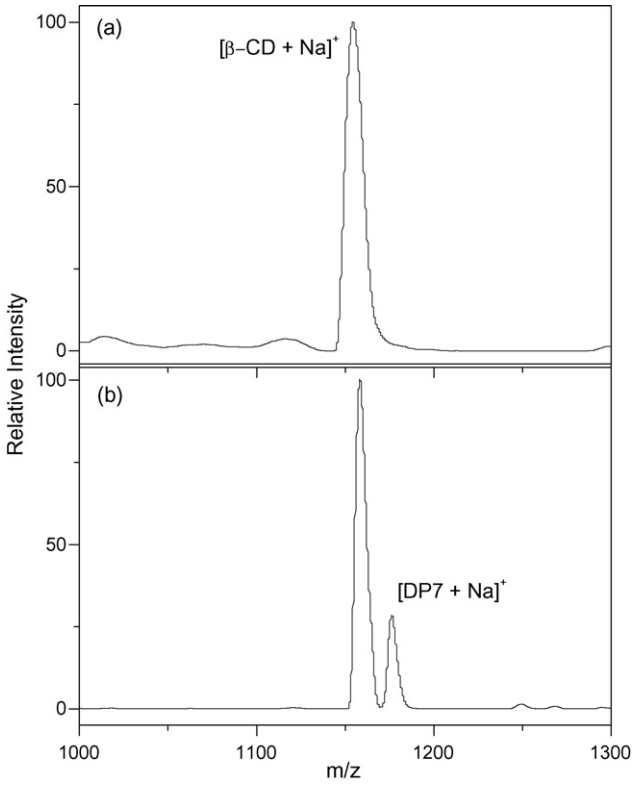


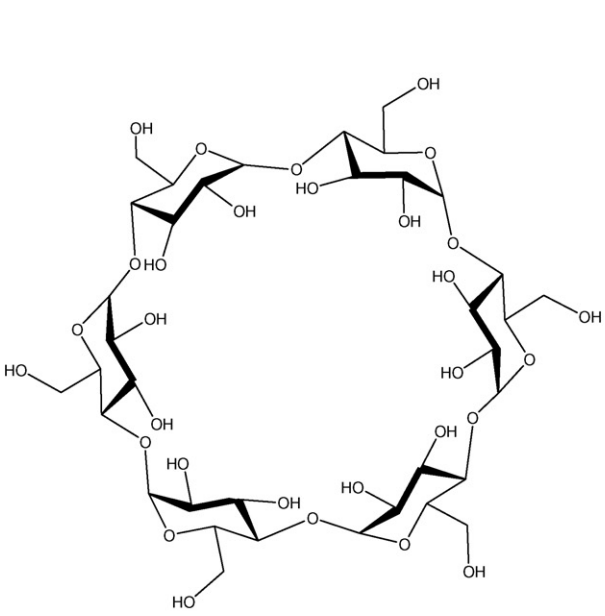
Fig. 3. MALDI-TOF mass spectrum of mixture of β-cyclodextrin and maltoheptaose without a cationizing agent (a) and containing NaTFA as a cationizing agent (b).

Table 1
Bond distances between the sodium cation and oxygen atoms of the carbohydrates. Unit is Å.

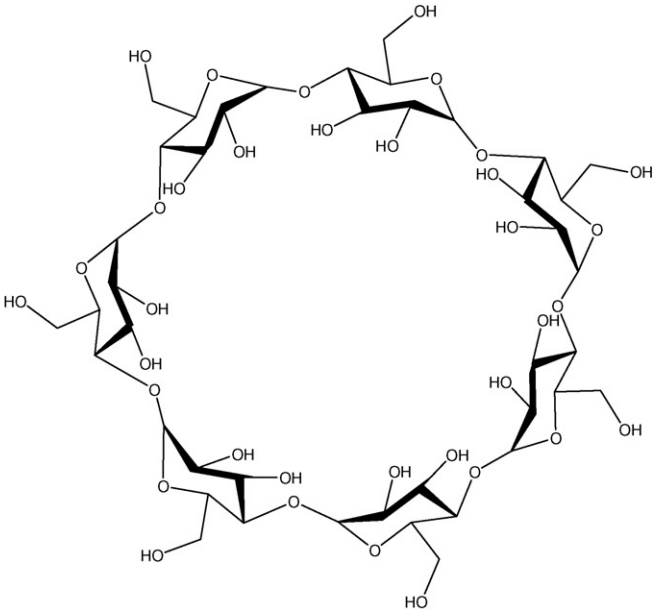
Conformation	α-Cyclodextrin	Maltohexaose
A	2.049, 2.024, 2.019	2.057, 2.001, 2.167, 2.041, 2.029
B	2.163, 2.187, 2.140, 2.235, 2.232, 2.293, 2.042	2.127, 2.060, 2.071, 2.043, 2.013
C	2.076, 2.125, 2.098, 2.104, 2.151, 2.155	1.999, 2.078, 2.019, 2.078
D	2.212, 2.102, 2.160, 2.160, 2.144, 2.124	2.090, 2.002, 2.010, 2.038

3. Results and discussion

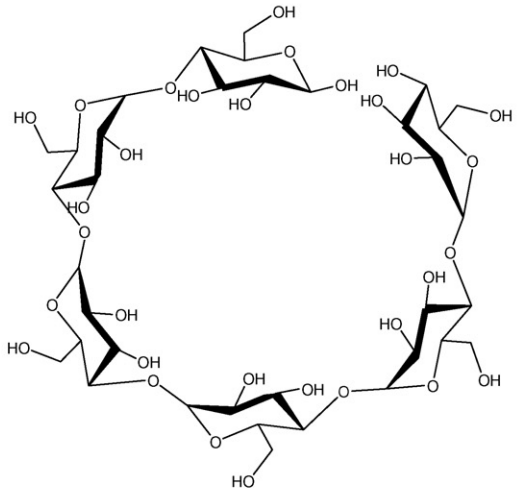
Differences in the ionization efficiencies of α- and β-cyclodextrins, α-cyclodextrin and maltohexaose, and β-cyclodextrin and maltoheptaose were investigated by analyzing the mixture samples of the two compounds. Alkali metal salts were employed as the cationizing agent to enhance the ionization efficiency. The influence of the kind of alkali metal cations and the counter anion of salt on the ionization efficiency was also investigated. The mass spectra of the mixture sample containing NaTFA display the $[M+Na]^+$ ions as shown in Figs. 1–3. The $[M+Na]^+$ ions are also typically observed in the sample conditions without a cationizing agent since sodium cation is a ubiquitous contaminant from glassware or aqueous mobile phases. Ion abundances of the $[M+Na]^+$ are enhanced by adding sodium salt. By adding the alkali metal salts of Li^+, K^+, and Cs^+, the mass spectra typically display the $[M+Li]^+$, $[M+K]^+$,



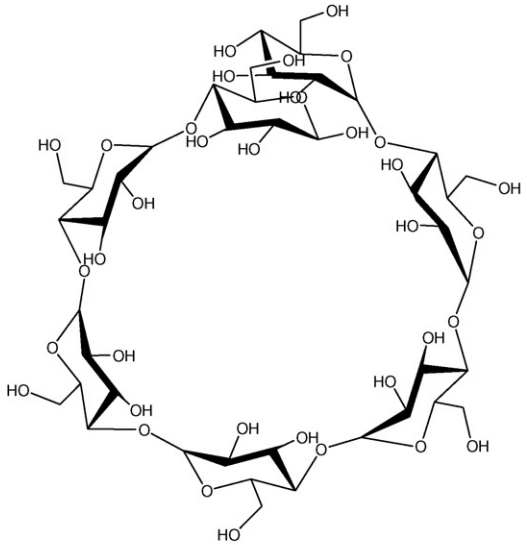
α-cyclodextrin



β-cyclodextrin



maltohexaose



maltoheptaose

Scheme 1. Chemical structures of α- and β-cyclodextrins, maltohexaose, and maltoheptaose.

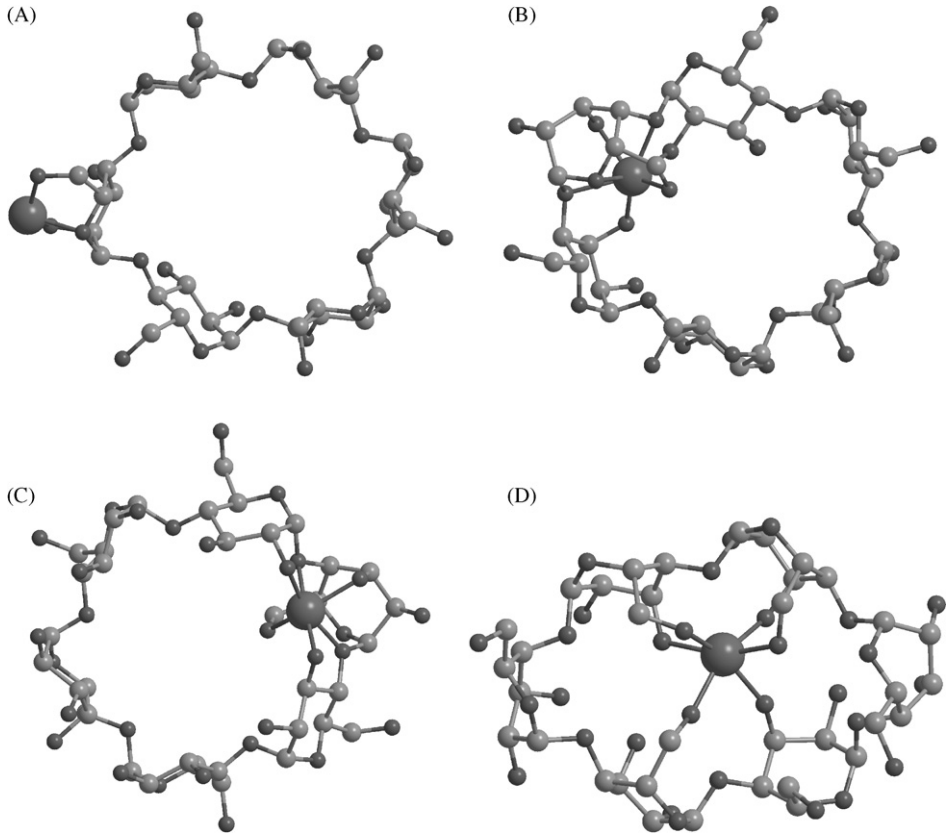


Fig. 4. Possible coordinated conformers of α-cyclodextrin and sodium cation.

and $[M+Cs]^+$ ions, respectively. For the mixture sample of α- and β-cyclodextrins, the ion abundance of β-cyclodextrin is higher than that of α-cyclodextrin as shown in Fig. 1. Increasing ionization efficiency of large maltooligose was reported in the previous work [11] and can be explained with multidentate coordination [24,25]. Alkali metal cation is coordinated to hydroxyl groups of the carbohydrate and the complex is increasingly stabilized by increasing the number of coordination sites. β-Cyclodextrin has four more hydroxyl groups than α-cyclodextrin (Scheme 1).

The $[M+Na]^+$ ions were observed in the MALDI-TOFMS spectrum of the mixture sample of α- and β-cyclodextrins without the cationizing agent. For the mixture samples of ring and linear compounds such as α-cyclodextrin/maltohexaose and β-cyclodextrin/maltoheptaose without the alkali metal salt, the mass spectra showed the $[M+Na]^+$ ions of α- and β-cyclodextrins but did not display the $[M+Na]^+$ ions of the linear compounds such as maltohexaose and maltoheptaose (Figs. 2 and 3). When NaTFA was added to the analyte, the $[M+Na]^+$ ions of maltohexaose and maltoheptaose were observed but their relative intensities were much smaller than those of α- and β-cyclodextrins. The primary difference in the ion abundances of the ring and linear compounds implies that the ring compounds form the ion complex with alkali metal cation much better than the linear compounds. This can be explained with the binding energy and the number of coordination sites of the ion complex between the analyte molecule and alkali metal cation.

In an attempt to explain a possible reason behind the stabilization of sodium cation-bound cyclodextrin over sodium cation-bound maltooligose, we performed *ab initio* electronic structure calculation of both α-cyclodextrin and maltohexaose before and after sodium binding. The geometry optimization was performed using HF/STO-3G with Gaussian 03 [33]. The initial structures are prepared by placing the sodium cation near the "possible" coordination sites with oxygen atoms. Figs. 4 and 5 show some possible coordinated conformations for α-cyclodextrin and maltohexaose, respectively. The number of the oxygen atoms coordinated to the sodium cation is 3, 6, and 7 for α-cyclodextrin conformations, whereas it is 4 and 5 for maltohexaose. The bond distances between the sodium cation and oxygen atoms are from 2.00 to 2.29 Å depending on the oxygen sites and conformations (Table 1). The energy differences after and before sodium cation binding are listed in Table 2 for each conformers. The energy difference means the stabilization energy for formation of the sodium cation–carbohydrate complex. The main feature from Table 2 is that the difference of sodium cation binding energy among different conformers is minimal for α-cyclodextrin unlike maltohexaose, which shows significant difference (conformer B). Thus, it can be suggested that the large number of sodium cation binding sites rather than the binding energy could be responsible for high ionization efficiency for α-cyclodextrin. It should be noted, however, that the current calculation level is rather low compared to other methods such as B3LYP/6-31G(d,p). Furthermore, finding stable conformers after sodium binding is a daunting task, and we have not explored all the possible conformers. Therefore, the current calculation results should be considered as a rough measure of overall binding tendency.

Table 2
The energy differences of each conformer after and before sodium cation binding. Energy unit is in hartree.

Conformation	A	B	C	D
α-Cyclodextrin	160.06	160.19	160.17	160.18
Maltohexaose	86.27	160.13	86.21	86.21

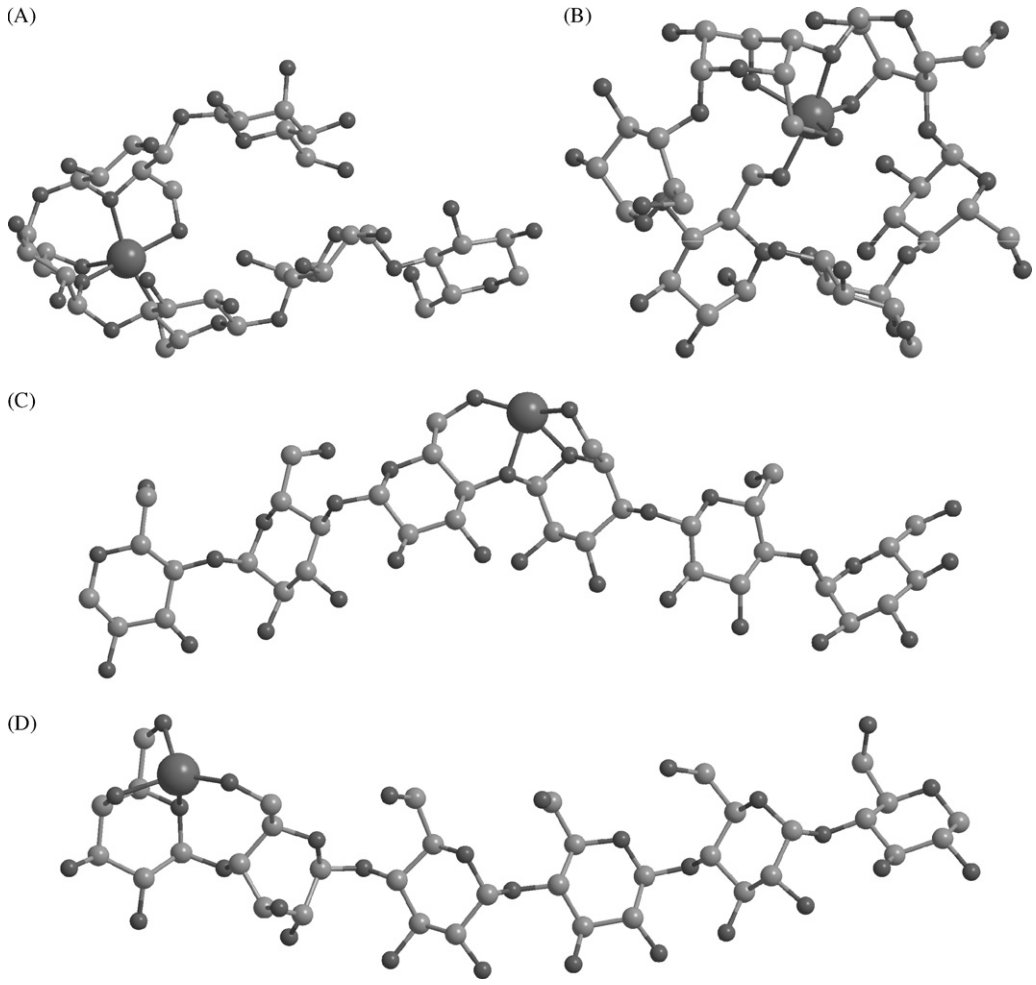


Fig. 5. Possible coordinated conformers of maltohexaose and sodium cation.

Differences in the ionization efficiencies of α- and β-cyclodextrins vary with the kind of alkali metal cations as shown in Fig. 6. Values of the [α-cyclodextrin+cation]$^+$/[β-cyclodextrin+cation]$^+$ ratios are less than 1.0. This implies that the ionization efficiency of β-cyclodextrin is always higher than that of α-cyclodextrin irrespective of the kind of alkali metal cations. The higher ionization efficiency of β-cyclodextrin can be explained with the number of hydroxyl groups to bind with alkali metal cation as discussed previously. Differences in the ionization efficiencies of α- and β-cyclodextrins notably increase with increasing the alkali metal cation size. This can be explained with the hole size of cyclodextrin. The alkali metal cation in the complex is mainly located in the cyclodextrin hole as shown in Fig. 4. Thus, formation of the cyclodextrin–alkali metal cation complex is more favorable when the hole size is substantial. The variation of the [α-cyclodextrin+cation]$^+$/[β-cyclodextrin+cation]$^+$ ratio was also affected by the counter anion of salt. The [α-cyclodextrin+cation]$^+$/[β-cyclodextrin+cation]$^+$ ratios for the chloride salts (CatCl) are higher than those for the hydroxide and TFA salts (CatOH and CatTFA, respectively). This can be explained by the acidity of MALDI sample. Since the CatOH is a strong base, the CatTFA is a weak base, and the CatCl is almost neutral, the order of the sample acidity is CatCl > CatTFA > CatOH. Since the matrix DHB is a weak acid (pK_a = 3.0), some of DHB molecules are dissociated to form [DHB–H]$^-$. The [DHB–H]$^-$ can form a complex with the alkali metal cation during solvent evaporation, which reduces the formation of analyte–alkali metal cation complex. By using the basic alkali metal salt, formation of the [DHB–H]$^-$ will be enhanced while the formation of analyte–alkali metal cation complex will be reduced.

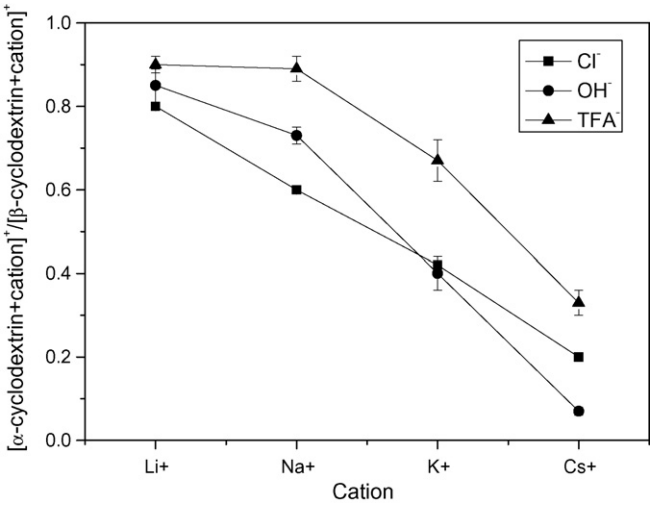


Fig. 6. Variation of the peak intensity ratio of [α-cyclodextrin+cation]$^+$/[β-cyclodextrin+cation]$^+$ with the alkali metal cation. Squares, circles, and triangles stand for the TFA^-, OH^-, and Cl^- as the counter anions, respectively.

The [cyclodextrin+cation]$^+$/[maltooligose+cation]$^+$ ratios also vary with the kind of alkali metal cation as shown in

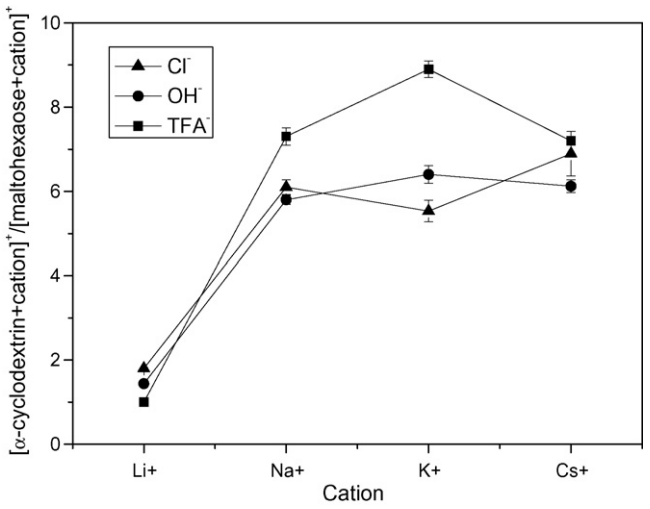


Fig. 7. Variation of the peak intensity ratio of [α-cyclodextrin+cation]$^+$/[maltohexaose+cation]$^+$ with the alkali metal cation. Squares, circles, and triangles stand for the TFA^-, OH^-, and Cl^- as the counter anions, respectively.

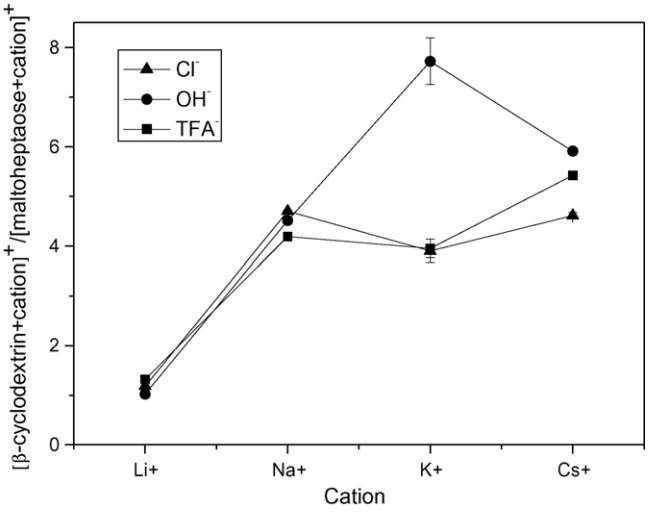


Fig. 8. Variation of the peak intensity ratio of [β-cyclodextrin+cation]$^+$/[maltoheptaose+cation]$^+$ with the alkali metal cation. Squares, circles, and triangles stand for the TFA^-, OH^-, and Cl^- as the counter anions, respectively.

Figs. 7 and 8. There is no specific trend depending on the anion type. The [cyclodextrin+cation]$^+$/[maltooligose+cation]$^+$ ratio remarkably increases by increasing the cation size from Li^+ to Na^+. The [cyclodextrin+cation]$^+$/[maltooligose+cation]$^+$ ratios for the lithium salts are 1.0–1.8 and 1.0–1.3 for α- and β-cyclodextrins, respectively, whereas those for sodium salts are 5.8–7.3 and 4.2–4.7, respectively. The relatively lower [cyclodextrin+cation]$^+$/[maltooligose+cation]$^+$ ratios for the lithium salts may be due to the strained structure. For cyclodextrins, the molecular structure will be strained as shown in Fig. 4 when several glucose units participate in the formation of the cyclodextrin–cation complex. Thus, the complex formation is less favorable when the cation size is small.

4. Conclusion

When the alkali metal salts were employed as the cationizing agents, the ionization efficiencies of carbohydrates were enhanced. The ionization efficiencies of cyclodextrins were much larger than those of the maltooligoses. The binding energy of the cyclodextrin–cation complex was higher than that of the maltooligose–cation complex and the number of the coordination sites of the former was larger than that of the latter. The ionization efficiency of β-cyclodextrin was higher than that of α-cyclodextrin. The alkali metal cation size and the counter anion type of salt were found to affect the ionization efficiencies. Differences in the ionization efficiencies of cyclodextrin and maltooligose decreased when the lithium cation was used, but the differences were rather significant when the other cations were used.

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