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Fast Atom Bombardment Mass Spectroscopic Investigation of Inclusion Complexes of Cyclodextrins with 4-Nitrophenol and Benzoic Acid

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Abstract. Mixtures of α-, β-, and γ-cyclodextrins and 4-nitrophenol (or benzoic acid) were investigated by means of fast atom bombardment mass spectroscopy. In the gas phase, inclusion complexes of α-, β-, and γ-cyclodextrins with 4-nitrophenolate and benzoate, which have a 1 : 1 cyclodextrin–guest stoichiometry, were observed in negative-ion mode. In addition, guest anions, clusters of two and three guest molecules, and guest–matrix complexes were observed.

Key words: cyclodextrins, inclusion complexes, FAB mass spectroscopy, 4-nitrophenol, benzoic acid.

1. Introduction

Cyclodextrins are cyclic oligomers composed of six, seven, and eight D-glucopyranose units, which are called α-, β-, and γ-cyclodextrins, respectively [1]. Because they are shaped like a truncated cone and have a hydrophobic cavity, various kinds of organic compounds are accommodated into the cavities to form inclusion complexes.

Inclusion complexes of cyclodextrins in aqueous solutions have usually been investigated by use of spectrophotometry, fluorimetry, phosphorimetry, NMR spectroscopy, chromatography, and so on [2–8]. Recently, however, inclusion complexes of cyclodextrin have been detected in the gas phase by means of electrospray ionization mass spectrometry and ionspray ionization (and tandem) mass spectrometry [9–20], although Cunniff and Vouros have noted that complexes of β-cyclodextrin and amino acids, detected by mass spectrometry, are probably electrostatic in nature rather than having a hydrophobic inclusion nature [21]. In aqueous

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solutions, water molecules around cyclodextrin and guest molecules likely participate in the inclusion complexation between cyclodextrin and guest molecules. In order to understand the roles and actions of water molecules surrounding inclusion complexes of cyclodextrins, therefore, it is significantly important to examine the complexation in the gas phase where water molecules are absent around the cyclodextrin and guest molecules.

Fast atom bombardment (FAB) mass spectroscopy has the advantage of being able to effectively ionize non-volatile and highly polar substances. In FAB mass spectroscopy, ionized fragments, produced by a FAB ionization process, as well as the ionized substance itself, have been analyzed to identify the molecular structure of a substance. Consequently, it is likely that the FAB ionization process confers excess kinetic energy on non-covalent complexes, dissociating them into component molecules. For this reason, it would seem to be difficult to investigate complexation by means of FAB mass spectroscopy, although complexes of polysaccharide with 3-*O*-alkyltrimethylammonium ions have been observed [22]. Very recently, Mele and Selva have found a 1 : 1 adduct of β-cyclodextrin with piroxicam using FAB mass spectrometry [23]. Furthermore, Davey *et al.* have observed enantioselective inclusion complexation between methylated cyclodextrins and methyl esters of amino acids [24]. Mele *et al.* have found a ternary inclusion complex of β-cyclodextrin, 2-acetyl-1-pyrroline (2-propionyl-1-pyrroline), and a matrix molecule (3-mercaptopropane-1,2-diol) [25]. Peaks due to the 1 : 1 β-cyclodextrin–pyrroline derivative inclusion complexes were noticeably weaker than those for the ternary inclusion complexes. Ionspray ionization and tandem mass spectrometry have been used to observe ternary inclusion complexes of β-cyclodextrin, drug, and diethanolamine [26].

In mass spectroscopic studies on inclusion complexes of cyclodextrins in the gas phase, only cationic guests incorporated into the cyclodextrin cavities have been observed until now, except for inclusion complexes of cyclodextrin–1-anilinonaphthalene-8-sulfonate, cyclodextrin–2-toluidinylnaphthalene-6-sulfonate [19, 20], and cyclodextrin–β-carotene [11]. Thus, we have tried to examine the inclusion complexation of cyclodextrins with anionic guests (4-nitrophenolate and benzoate) in the gas phase by use of FAB mass spectrometry.

2. Experimental

α- and γ-Cyclodextrins purchased from Nacalai Tesque, Inc. were used as received. β-Cyclodextrin from Nacalai Tesque, Inc. was twice recrystallized from water. 4-Nitrophenol obtained from Tokyo Kasei Kogyo Co. Ltd. was twice recrystallized from ethanol. Benzoic acid from Wako Pure Chemical Industries, Ltd. and diethanolamine from Tokyo Kasei Kogyo Co. Ltd. were used without further purification. Mixtures of cyclodextrin (about 300 mg), 4-nitrophenol (or benzoic acid), and a very small amount of water were kneaded in an agate mortar with an agate pestle. 4-Nitrophenol or benzoic acid was mixed with α-, β-, or γ-cyclodextrin.

After all the mixtures thus obtained were dried, they were solubilized in diethanolamine, which was employed as a matrix in negative-ion mode, and were used as samples for FAB mass spectrometry. Glycerin was employed as a matrix in positive-ion mode.

FAB mass spectra were acquired with a JMS-BU20 (GCmate) double focused high resolution gas chromatography mass spectrometer (JEOL Ltd.). Ions were generated by bombardment of a neutral Xe atom operated at 3 kV. In measurements of the α- and β-cyclodextrin–guest mixtures, scan times for the m/z range of 50–1500 were 5 s at 5 s intervals under an ion-source accelerating potential of 1.67 kV. For the γ-cyclodextrin–guest mixtures, a smaller accelerator potential of 1.25 kV was applied to scan the wider m/z range of 50–2000 compared to the α- and β-cyclodextrin–guest mixtures.

3. Results and Discussion

3.1. FAB MASS SPECTRA OF α-, β-, AND γ-CYCLODEXTRINS

In negative-ion mode, the FAB mass spectrum of α-cyclodextrin alone showed a peak at m/z 971 which is assigned to a species of (α-cyclodextrin $-$H)$^-$ (not shown). Similarly, peaks at m/z 1133 and 1295 were observed for β- and γ-cyclodextrins, respectively. They are ascribed to β- and γ-cyclodextrin anions [(β-cyclodextrin $-$H)$^-$ and (γ-cyclodextrin $-$H)$^-$], respectively. In positive-ion mode, a peak at m/z 973 was detected for α-cyclodextrin, indicating the existence of a species of (α-cyclodextrin +H)$^+$ (not shown).

3.2. INCLUSION COMPLEXES OF α-, β-, AND γ-CYCLODEXTRINS WITH 4-NITROPHENOL IN THE GAS PHASE

Figure 1 shows the FAB mass spectrum of a kneaded 1 : 1 mixture of α-cyclodextrin and 4-nitrophenol in negative-ion mode. As noted previously, a peak at m/z 971 represents anionic α-cyclodextrin. A peak at m/z 1110, which has nearly the same intensity as that at m/z 971, evidently shows the presence of a 1 : 1 inclusion complex [(inclusion complex $-$ H)$^-$] between α-cyclodextrin and 4-nitrophenolate. This finding indicates that the non-covalent binding force between α-cyclodextrin and a 4-nitrophenolate anion is fairly strong, since it can form a 1 : 1 inclusion complex in the gas phase, even though excess kinetic energy has been given to the inclusion complex through the FAB ionization process. In positive-ion mode, no peak due to the 1 : 1 inclusion complex [(inclusion complex + H)$^+$] was observed, providing additional evidence that α-cyclodextrin forms the 1 : 1 inclusion complex with 4-nitrophenolate. These findings are reasonable because 4-nitrophenol is not readily protonated, but rather deprotonated.

As stated previously, Cunniff and Vouros have suggested that the complexes between β-cyclodextrin and amino acids are the result of the relatively strong electrostatic bonds that arise from interactions of the positively charged ammonium

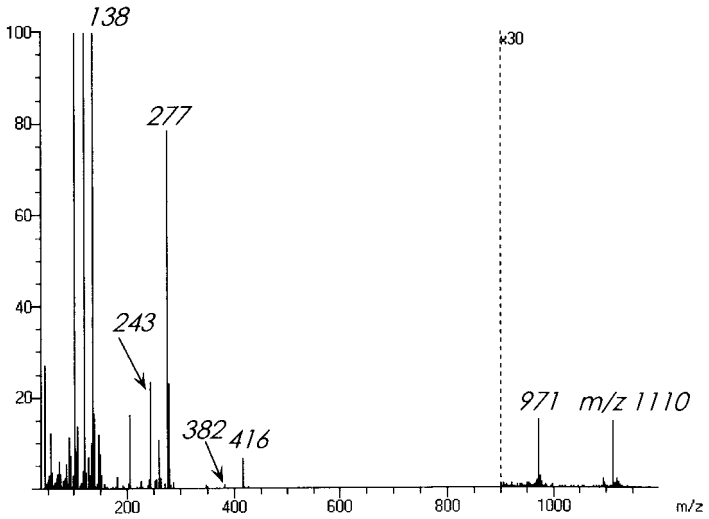

Figure 1. Negative-ion FAB mass spectrum of a kneaded 1 : 1 mixture of α-cyclodextrin and 4-nitrophenol.

termini of the amino acids with the electronegative oxygen atoms of β-cyclodextrin [21]. Consequently, the possibility may exist that the α-cyclodextrin–4-nitrophenolate complex is a 1 : 1 electrostatically bound adduct. However, 4-nitrophenolate is negatively charged. Therefore, it is unlikely that 4-nitrophenolate is bound to α-cyclodextrin without incorporation in the α-cyclodextrin cavity, although protonated α-cyclodextrin has been observed in positive-ion mode.

A signal of m/z 243 is seen in Figure 1. This signal can be assigned to a complex $[(G + Mx - H)^-]$ between a guest and a matrix molecule, where G and Mx stand for a 4-nitrophenol molecule and a matrix molecule, respectively. In addition, there is a very weak signal of m/z 382, which is due to a complex $[(2G + Mx - H)^-]$ of two 4-nitrophenol molecules and a matrix molecule. Furthermore, there are peaks of m/z 138, 277, and 416, which are assigned to 4-nitrophenolate $[(G - H)^-]$, a cluster $[(2G - H)^-]$ of two 4-nitrophenol molecules, and a cluster $[(3G - H)^-]$ of three 4-nitrophenol molecules, respectively. These findings suggest that 4-nitrophenol readily associates with itself.

Figure 2 exhibits a negative-ion FAB mass spectrum of a kneaded 1 : 1 mixture of β-cyclodextrin and 4-nitrophenol. As in the case of the α-cyclodextrin mixture, a signal of a 1 : 1 β-cyclodextrin–4-nitrophenolate inclusion complex [(inclusion complex $-H)^-$] is observed at m/z 1272. However, the intensity of this peak is about half that at m/z 1133, which is due to a β-cyclodextrin anion. In aqueous solutions, an equilibrium constant (75 ± 10 mol^{-1} dm^3) for the formation of the 1 : 1 inclusion complex of β-cyclodextrin with 4-nitrophenolate is one fourth of that (300 ± 40 mol^{-1} dm^3) of α-cyclodextrin [7, 27]. The small ratio of the peak intensity for the 1 : 1 inclusion complex to that for β-cyclodextrin, compared to the

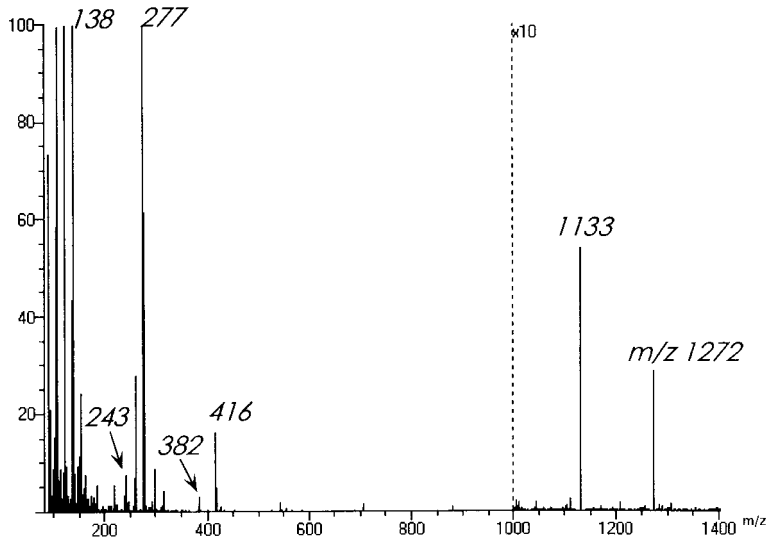

Figure 2. Negative-ion FAB mass spectrum of a kneaded 1 : 1 mixture of β-cyclodextrin and 4-nitrophenol.

peak ratio regarding the α-cyclodextrin mixture, may suggest that in the gas phase the inclusion complex of 4-nitrophenolate with α-cyclodextrin is readily formed compared to β-cyclodextrin, although, in the gas phase, an equilibrium may not be established between a free guest and an inclusion complex. In Figure 2, one can see signals due to 4-nitrophenolate [$(G - H)^-$], the 4-nitrophenol–matrix complexes [$(G + Mx - H)^-$ and $(2G + Mx - H)^-$], and the clusters [$(2G - H)^-$ and $(3G - H)^-$]. This finding is reasonable because these species can be generated irrespective of the presence and kinds of cyclodextrins. The clusters of guest–guest and guest–matrix are probably formed in the gase phase. Consequently, FAB mass spectra do not seem to directly reflect the species existing in a sample (matrix), although part of the inclusion complex in a matrix may be detected in the gas phase.

In addition to a very small quantity of a host–guest inclusion complex, a ternary inclusion complex containing a matrix molecule has been observed for the systems of β-cyclodextrin, 2-acetyl-1-pyrroline (2-propionyl-1-pyrroline), and a matrix (3-mercaptopropane-1,2-diol) [25]. In contrast to these systems, a ternary inclusion complex containing a guest molecule (4-nitrophenol) and a matrix molecule (diethanolamine) is not seen in Figure 2.

Figure 3 shows the FAB mass spectrum of a kneaded 1 : 2 mixture of γ-cyclodextrin and 4-nitrophenol in the negative-ion mode. As described previously, a peak at m/z 1295 represents a signal of a γ-cyclodextrin anion, whereas a peak at m/z 1434 is assigned to a 1 : 1 γ-cyclodextrin–4-nitrophenolate inclusion complex [(inclusion complex $- H)^-$]. The ratio of the peak intensity for the γ-cyclodextrin–4-nitrophenolate inclusion complex to that for γ-cyclodextrin is the smallest of the cyclodextrins examined. Because the γ-cyclodextrin cavity is too wide to snugly

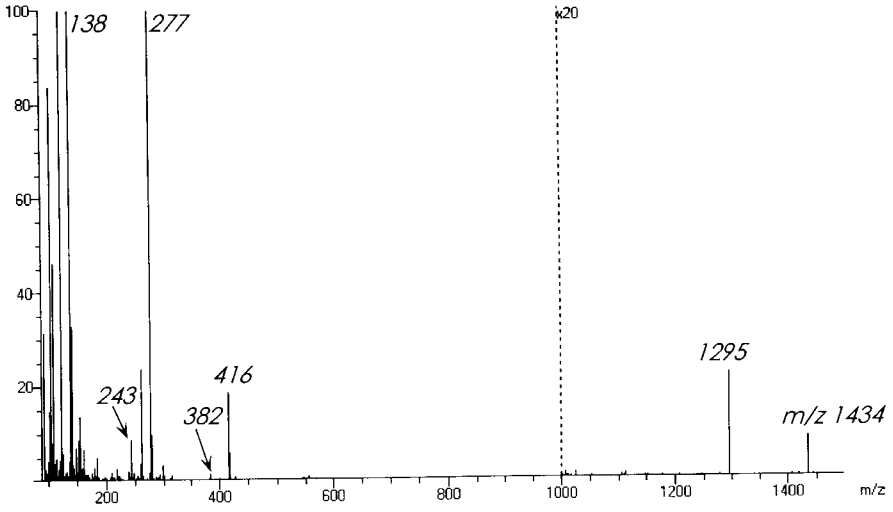

Figure 3. Negative-ion FAB mass spectrum of a kneaded 1 : 2 mixture of γ-cyclodextrin and 4-nitrophenol.

accommodate a 4-nitrophenolate anion, the formation of the inclusion complex with γ-cyclodextrin is disfavored, resulting in the smallest peak intensity ratio for γ-cyclodextrin. In spite of a 1 : 2 molar ratio for the γ-cyclodextrin–4-nitrophenol mixture, no signal due to a 1 : 2 γ-cyclodextrin–4-nitrophenol inclusion complex was observed. As in the cases of the α- and β-cyclodextrin mixtures, 4-nitrophenolate, the 4-nitrophenolate–matrix complexes, and the clusters were observed for the γ-cyclodextrin mixtures.

3.3. INCLUSION COMPLEXES OF α-, β-, AND γ-CYCLODEXTRINS WITH BENZOIC ACID IN THE GAS PHASE

In the negative-ion mode we further examined α-, β-, and γ-cyclodextrin–benzoic acid mixtures, which had a 1 : 1 host–guest molar ratio. For γ-cyclodextrin, 1 : 1.5, 1 : 2, and 1 : 4 host–guest mixtures were also investigated. In addition to signals due to cyclodextrin anions themselves [(cyclodextrin $-$ H)$^-$], peaks at *m/z* 1093, 1255, and 1417, which are assigned to the 1 : 1 inclusion complexes [(inclusion complex $-$ H)$^-$] of benzoate with α-, β-, and γ-cyclodextrins, respectively, were observed for the α-, β-, and γ-cyclodextrin–benzoic acid mixtures. As in the case of 4-nitrophenol, a signal assignable to the 1 : 1 α-cyclodextrin–benzoic acid inclusion complex was not detected in the positive-ion mode, supporting the notion that the 1 : 1 α-cyclodextrin–benzoate inclusion complex is formed in the gas phase. Signals due to benzoate [(G $-$ H)$^-$], the benzoate–matrix complexes [(G + Mx $-$ H)$^-$ and (2G + Mx $-$ H)$^-$], and the clusters [(2G $-$ H)$^-$ and (3G $-$ H)$^-$] were observed for all the cyclodextrin–benzoic acid mixtures as well as the cyclodextrin–4-nitrophenol mixtures.

In contrast to 4-nitrophenol, in the negative-ion mode, the intensity ratio of the peak for the β-cyclodextrin–benzoate inclusion complex to that for β-cyclodextrin was rather greater than that for α-cyclodextrin, although, in aqueous solution, the equilibrium constant (370 mol^{-1} dm^3) for the formation of the 1 : 1 β-cyclodextrin–benzoate inclusion complex is less than half of that (910 mol^{-1} dm^3) for the 1 : 1 α-cyclodextrin–benzoate inclusion complex [29]. This finding may imply that in aqueous solutions, water molecules surrounding cyclodextrin and benzoate contribute to the inclusion complexation between them. A 1 : 2 γ-cyclodextrin–benzoate inclusion complex has been observed in aqueous solution [29]. However, only a 1 : 1 γ-cyclodextrin–benzoate inclusion complex was observed in FAB mass spectrometry, even when 1 : 1.5, 1 : 2, and 1 : 4 γ-cyclodextrin–benzoic acid mixtures were employed.

As in the case of 4-nitrophenol, ternary inclusion complexes including a matrix molecule were not observed for the cyclodextrin–benzoic acid mixtures, indicating that the interactions between cyclodextrin and a matrix molecule are weaker than those between cyclodextrin and benzoic acid. However, signals of m/z 121, 243, and 365 were observed. They are assigned to benzoate [$(G - H)^-$], a cluster [$(2G - H)^-$] of two benzoic acid molecules, and a cluster [$(3G - H)^-$] of three benzoic acid molecules, respectively. These findings suggest that the self-association of benzoic acid readily occurs in the gas phase.

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