THE UNITED GRADUATE SCHOOL OF
AGRICULTURAL SCIENCES, TOTTORI UNIVERSITY

A Thermodynamic Study on the Inclusion Equilibria of Guanidino Modified Cyclodextrins with *p*-Nitrophenolate Ion

（グアニジノ基修飾シクロデキストリン誘導体と
p-ニトロフェノレートイオンの包接平衡に関する熱力学的研究）

THESIS BY

Keita Takezawa

As Partial Fulfillment of the Requirement
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At

Department of Bioresources Science
The United Graduate School of Agricultural Sciences,
Tottori University

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Chapter 1.

Introductions

1. 1 Cyclodextrin

Cyclodextrin (CD) is a cyclic oligomer composed of six (α-CD), seven (β-CD), eight (γ-CD), or more α-D-glucopyranose units linked 1→4 as amylose. CDs are soluble to water, since all the secondary hydroxyl groups (C2- and C3-OH) are located at the wider edge of the ring, and the primary hydroxyl groups (C6-OH) are at the narrower edge (Figure 1-1). On the other hand, the interior cavities of CDs are hydrophobic in nature due to the presence of apolar hydrogens (C3- and C5-H), together with the glucoside oxygen atoms (Figure 1-2). The size of CD differs depending on the number of glucose units that compose the ring (Table 1-1). The most important characteristic of CD is that this molecule forms inclusion complexes with specific guest molecules. There are many intermolecular interactions, which work cooperatively to stabilize the inclusion complexes [1-3].

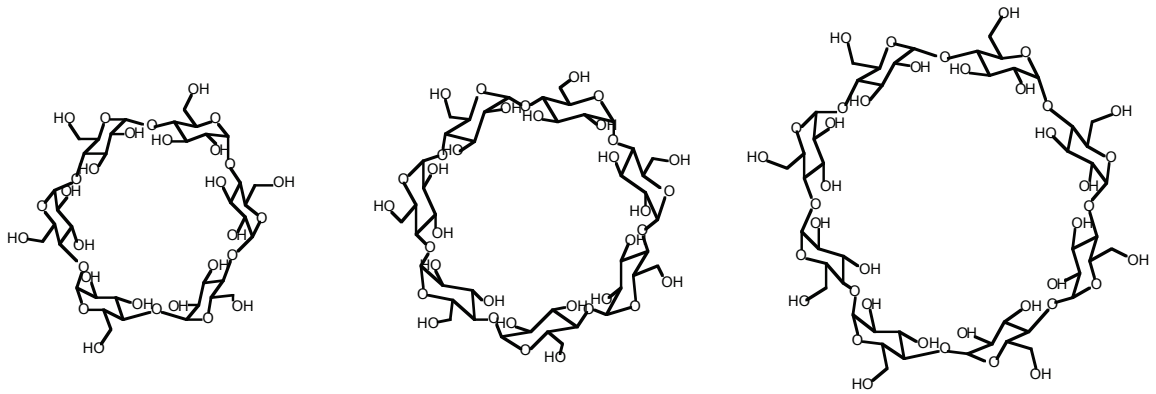


Figure 1-1. Structures of α-CD, β-CD, and γ-CD.

Table 1-1 Physicochemical Properties of Cyclodextrins

	α-CD	β-CD	γ-CD
Number of glcopyranose	6	7	8
Molecular weight	973	1135	1297
Aqueous solubility, 25℃/g l^{-1}	145	18.5	232
Angle of rotation $[a]_D^{25}$/ °	150.5 ± 0.5	162.5 ± 0.5	17.4 ± 0.5
Cavity diameter/ nm	0.47 - 0.53	0.60 - 0.65	0.75 - 0.83
Diameter of outer periphery/ nm	1.46	1.56	1.75
Height of torous/ nm	0.79	0.79	0.79
Cavity volume/ nm^3	0.174	0.262	0.427

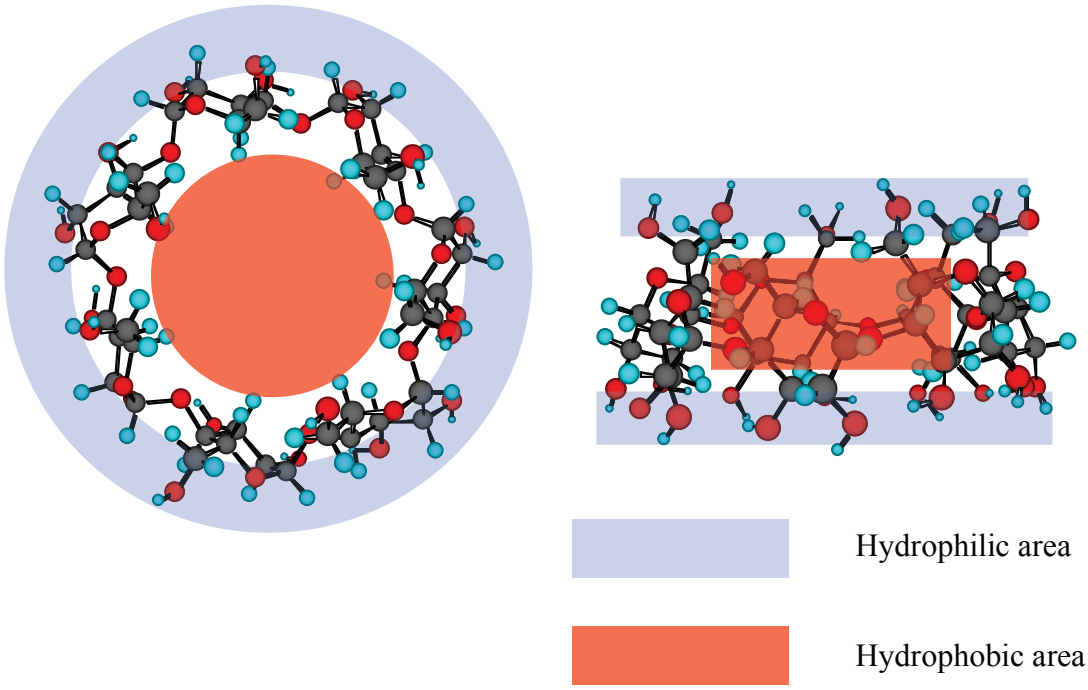


Figure 1-2. Hydrophobic and Hydrophilic area of β-CD.

1. 2 Binding Forces Contributing to Inclusion Complex Formation

Stability of a CD inclusion complex is generally represented by the value of association constant (K_a) for the inclusion complexation equilibrium. A large value of K_a expresses the strong affinity of a guest to the CD. The value of K_a widely varies depending on the physicochemical properties of guests such as size, structure, and hydrophobicity and on the size of the CD cavity, providing CDs. Such kinds of selectivity bring about the molecular recognition ability of CD. Other famous molecules with this ability are crown ether, cryptand, cyclophane, calixarene, etc. [4]. Molecular recognition plays an important role in such life activities as in neurotransmitter and taste-bud receptors, antibody, enzyme, etc. Therefore, the reproduction of molecular recognition by using artificial molecules is of great interest in the field of applied science and industry.

There are many intermolecular interactions which contribute to molecular recognition. Because CD is a neutral molecule, following interactions are mainly responsible for the formation of CD-guest inclusion complexes in aqueous solutions [5-6].

1. Hydrophobic interaction
2. van der Waals interaction
3. Hydrogen bonding

Hydrophobic interaction plays an important role for the inclusion complexation of CD with guest complex formation as follows: in aqueous solution. When a

hydrophobic molecule is dissolved in water, the network of hydrogen bonding of water molecules around the hydrophobic one is forced to change the form to entropically favorable “iceberg structure”. Once the hydrophobic molecule is included into the hydrophobic CD cavity, the iceberg structure is disrupted and the water molecules trapped in the CD cavity are released to a bulk solution. The net entropy of water molecules greatly increases through the process, resulting in the formation of inclusion complexes. This process is thus accompanied by an increase in the degree of freedom of water molecules, so that the hydrophobic interaction essentially involves a favorable positive entropy change ($\Delta S° > 0$), together with slightly positive enthalpy change ($\Delta H° \geq 0$) [5-7].

Van der Waals interaction involves two different terms. One is the interaction arising from the polarity of molecules such as dipole-dipole interaction and dipole-induced dipole interaction. Another is the London dispersion force that works between apolar molecules. The former interaction is very weak in water with a large dielectric constant, because the strength of such interaction is inversely proportional to the dielectric constant of solvent. However, in the CD cavity, the interaction become fairly strong since the dielectric constant of CD cavity is significantly small. The London dispersion force works between molecules locating at very short distance to be in contact with each other. It becomes very large in the case that the steric structure of the guest well fits to the CD cavity. The van der Waals interaction is accompanied by a change in thermodynamic parameters ($\Delta H° < 0$ and $\Delta S° < 0$), which are opposite to the hydrophobic interaction. When the contribution of van der Waals interaction for

the complexation is smaller than the contribution of the hydrophobic interaction, the thermodynamic changes accompanied by complexation are $\Delta H° \geq 0$ and $\Delta S° > 0$. In contrast, when the van der Waals interaction is stronger than hydrophobic interaction, thermodynamic changes are $\Delta H° < 0$ and $\Delta S° < 0$ [7]. The importance of these forces in the CD complexation has been emphasized through thermodynamic and theoretical investigations [8-13].

The hydrogen bonding is also one of the primary binding forces for CD complexation, because CD molecules has many primary and secondary hydroxyl groups capable of forming hydrogen bonding with guest molecules [14-15]. Several X-ray crystallographic investigations have shown that the primary hydroxyl groups of CD are hydrogen bonding to polar guest molecules [3, 16-20]. However, in aqueous solutions, such hydrogen bonding between host and guest is prevented because the water molecules have much stronger ability to construct hydrogen bonding with polar guests. Therefore, it is not clear how much the hydrogen bonding contributes to the stability of the inclusion complexes.

The intermolecular interactions described above work simultaneously and cooperatively. The extent to which these interactions contribute to the complexation depend on the nature of host and guest molecules.

1. 3 Modification of Cyclodextrins

Since native CDs have only hydroxyl groups as functional groups, the ability of molecular recognition and enzyme mimic catalyst are limited. The chemical modification or substitution of some or all of the hydroxyl groups would give CDs enhanced molecular recognition abilities. Introduction of charged groups such as amino, pyridinio or guanidino groups into CDs are of great interest, as the introductions give CDs new recognition sites. A pyridinio derivative of α-CD where two pyridinio groups are symmetrically introduced at the primary hydroxy side, is reported to restrict molecular rotation of the guest inside its hydrophobic cavity [21]. Guanidino group, which is known as a side chain of arginine, has a stable positive charge over a wide pH range, plays a key role in cell penetrating property of a specific cell penetrating peptides (CPPs). Some CD derivatives having 8 guanidino groups at the primary hydroxy side are reported to penetrate cell membrane with the guest molecules included in the [22, 23].

1. 4 Objective of This Thesis

As mentioned above, there are many studies on the substitution of the primary hydroxy groups of CDs to improve the molecular recognition ability and guest binding affinity. However, not much is reported on the modification on the secondary hydroxyl side of CDs or on the detailed investigation on the mechanism for the inclusion complexations of the derivatives having deformed cavities with guests. As the CD derivatives with deformed cavities are expected to be excellent candidate molecules that would mimic the induced-fit property of substrates binding by proteins, the detailed investing on the thermodynamic parameters for the inclusion complexations are considered to be of great importance.

In this study, three types of α-CD derivatives, one with a positively charged guanidino group on the secondary hydroxy side, others on the secondary hydroxy side, are synthesized to investigate the effect of the position of the substituents on the affinity toward the negatively charged guest molecule. Further investigation on the inclusion property of one of the three hosts was also conducted to examine the effect of the macroring deformation on the molecular motion of the guest to reveal the inter molecular forces contributing to the inclusion complexation.

This report consists of the following two parts;

1. Inclusion complexation of three structural Isomers of mono(deoxyguanidino)-α-cyclodextrin with the *p*-Nitrophenolate Ion

2. Thermodynamic and structural studies on the complexation of

guanidino appended α-cyclodextrin derivatives with *p*-nitrophenolate ion

In chapter 2, the synthesis of three types of guanidino appended α-CD is reported and the binding constants (K_a) for the complexation of *p*-nitrophenolate ion (*p*-NP^-) were measured in buffered solutions with different ionic strength to investigate the effect of electrostatic interaction on the inclusion complexation.

In chapter 3, the structure of the complex and intermolecular forces for the inclusion complexation between the guanidino derivative of α-CD that has a deformed macroring and *p*-NP^- is discussed based on the experimental results of thermodynamic parameters for the inclusion complexation.

Chapter 2.

Inclusion Complexation of Three Structural Isomers of Mono(deoxyguanidino)-α-Cyclodextrin with the *p*-Nitrophenolate Ion

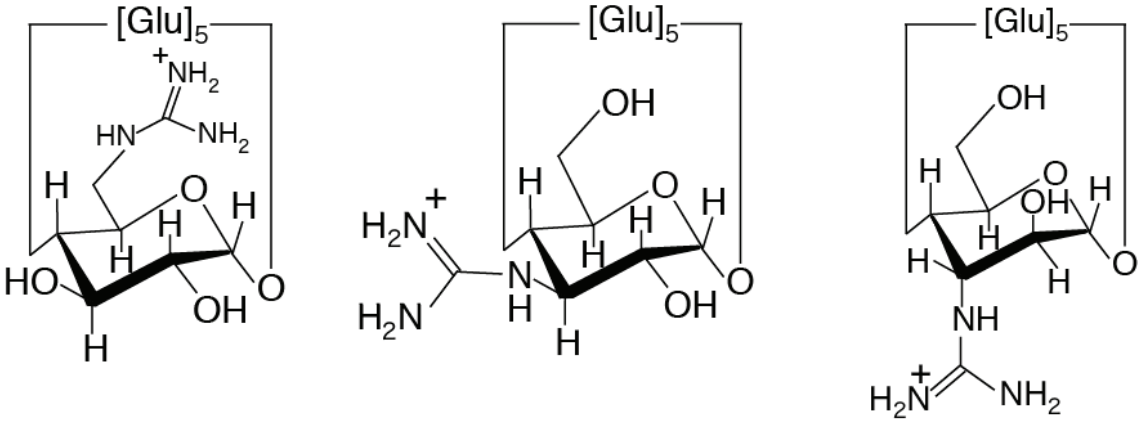
2. 1 Introduction

Inclusion ability of CDs can be improved by the substitution of the primary and/ or secondary hydroxy groups of CDs with various functional groups [27]. Among such functional groups, positively or negatively charged substituents are of great interest because they provide electrically neutral CDs with new recognition sites for electrostatic interaction, which can be controlled by changing pH or ionic strength (μ) of the solutions[28]. In particular, the guanidino group has drawn attention due to its highly stable positive charge over a wide range of pH and to the strong ion pairing with biologically important carboxylate and phosphate groups [29]. One example is a α-CD derivative bearing a guanidino group on the primary hydroxy side, for which the complexation equilibrium with phosphotyrosine is reported [30]. Some studies have focused on the interactions of guanidino-modified CDs with biologically important molecules such as ATP [31] ADP [31], DNA [22, 23] or biological membranes [23]. These reports show that the guanidino-modified CDs can be utilized as molecular capsules which can selectively bind guests bearing carboxylate or phosphate groups, or as drug carriers which penetrate biological membranes with drug molecules included inside their cavities. However, detailed mechanisms for the formation of inclusion complexes of guanidino-modified CDs with guests have not been much reported [32]. In the present study, we investigated the effect of the substitution position of a guanidino group on the binding constants (K_a's) of the equilibria and molecular

orientations for the inclusion complexes of three monoguanidino-modified α-CDs with the *p*-nitrophenolate ion (*p*-NP^-).

One of the isomers has the guanidino group on the primary hydroxy side of α-CD, mono(6-deoxy-6-guanidino)-α-CD (**1**), and the others on the secondary hydroxy side, mono(3-deoxy-3-guanidino)-α-CD (**2**) and mono(3-deoxy-3-guanidino-*altro*)-α-CD (**3**) (Figure 2-1). The isomers **2** and **3** have different configurations at C(2)-OH and C(3) substituents as depicted in Figure 2-1. It should be noted that the cavity of 3 is distorted by the conversion of one of six glucoses of α-CD to an altrose [33].

In this study, the K_a values for the complexation of the three hosts and the native α-CD with *p*-NP^- were determined by UV-Vis titration experiments at pH 10.6. The guest ion *p*-NP^- has often been studied for complexation with CDs [34-36] and is an appropriate ion for the investigation by means of UV-Vis and NMR spectroscopy. The molecular orientations of the inclusion complexes of **1**, **2**, **3**, and α-CD with *p*-NP^- were estimated using rotating frame nuclear overhauser effect spectroscopy (ROESY).



1 **2** **3**

Figure 2-1. Chemical structures of **1**, **2**, and **3**. Counter ions and C(6)-H are omitted for simplicity.

2. 2 Experimental

2.2.1 General Procedures.

α-CD was supplied by Ensuiko Sugar Refining Co., Ltd. and dried overnight in vacuo at 110 °C before use. Other reagents used for the synthesis of the hosts and UV-Vis titration experiments were of special grade, purchased from Wako Pure Chemical Industries, Ltd. UV-Vis spectra were recorded using a Shimadzu UV-2100 UV-Vis spectrophotometer equipped with a temperature-controlled cell holder. D_2O (Isotec, 99.8 atom % D) and DMSO-d_6 (Isotec, 99.9 atom % D) were used as solvents for ^1H NMR spectrum measurements. NMR spectra were recorded on a JEOL JNM- A400 FT NMR spectrometer (400 MHz) with sample tubes of 5.0 mm diameter at 298 K.

2.2.2 Synthesis of Mono(deoxyguanidino)-α-CDs. Mono(6-deoxy-6-guanidino)-α-CD (1): Mono(6-deoxy-6-amino)-α-CD (0.450 g, 0.463 mmol), prepared according to a literature procedure [30] was dissolved in H_2O (3 cm^3), followed by the addition of *N,N*-diisopropylethylamine (2 cm^3) and an excess of 1*H*-pyrazole-1-carboxamidine hydrochloride (0.500 g, 3.41 mmol). The mixture was allowed to react overnight at 313 K and precipitated with 500 cm^3 of acetone to give solid crude product. The crude product was purified with a carboxymethyl cellulose column using aqueous NH_4HCO_3 (0.01-0.05 mol dm^{-3}) as eluent to give pure **1** as HCO_3^- salt (0.232g, 0.216 mmol, yield; 48%). ^1H NMR (D_2O, 298 K): 5.09-5.04 (m, 6H, C(1)-H), 4.00-3.78 (m, 22H), 3.69-3.50 (m, 14H). ^{13}C NMR (D_2O, 298K): δ

152.9 (guanidino C), 96.5, 77.5, 76.7, 76.6, 76.3, 68.4, 68.3, 68.2, 67.1, 66.7, 66.6, 55.8, 55.5, 55.3, 37.4.

Mono(3-deoxy-3-guanidino)-α-CD (2): Mono(3-deoxy-3-amino)-α-CD (0.451g, 0.464mmol), prepared according to a literature procedure [37], was guanidinylated by the same procedure as described in the synthesis of **1** to give **2** as HCO_3^- salt (0.190g, 0.177mmol, yield; 38.1%). ^{1}HNMR (D_2O, 298K): 5.05-5.00 (m, 6H, C(1)-H), 4.00-3.82 (m, 24H), 3.65-3.44 (m, 11H), 3.17 (t, 1H, J = 10.0 Hz). ^{13}C NMR (D_2O, 298 K): δ 153.4 (guanidino C), 96.7, 96.4, 96.3, 96.2, 95.6, 76.7, 76.3, 76.3, 76.1, 73.9, 68.3, 68.2, 67.6, 67.5, 67.1, 67.0, 66.9, 66.7, 66.6, 66.5, 65.7, 55.4.

Mono(3-deoxy-3-guanidino-*altro*)-α-CD (3): Mono(3-deoxy-3-amino-*altro*)-α-CD (0.542 g, 0.558 mmol), prepared according to a literature procedure [37] was guanidinylated by the same procedure as described in the synthesis of **1** to give **3** as HCO_3^- salt (0.186 g, 0.177 mmol, yield; 31.0%). ^{1}H NMR (D_2O, 298K): 5.13-5.00 (m, 6H, C(1)-H), 4.20 (dd, 1H, J = 4.4, 10.3 Hz), 4.09-3.83 (m, 25H), 3.70-3.54 (m, 10H). ^{13}C NMR (D_2O, 298 K): δ 155.4 (guanidino C), 102.4, 100.0, 99.9, 99.4, 98.9, 98.6, 79.4, 79.0, 77.9, 71.6, 71.4, 71.2, 70.9, 70.4, 70.0, 69.8, 69.7, 69.6, 69.4, 69.2, 68.8, 58.9, 58.7, 58.5, 58.4.

2.2.3 Determination of Binding Constants.

UV-Vis titration experiments of *p*-NP^- with the hosts were carried out at 298 K in Na_2CO_3/$NaHCO_3$ buffer solutions at pH 10.6 at which **1**, **2**, and **3** exist as positively charged ions and α-CD as uncharged (pK_a = 12.3) [38]. The ionic strength

(μ) of the buffer solution was adjusted to be 0.0230, 0.115, 0.230, or 1.15 mol dm^{-3} by changing the concentration of the buffers. The absorption maximum at 420 nm for *p*-NP^- (5.00×10^{-2} mmol dm^{-3}) decreased with the addition of **1**, **2**, **3**, or the native α-CD (up to 1.38 mmol dm^{-3}) to give isosbestic points for each of them, confirming the formation of 1:1 inclusion complex. Changes in the absorbance (ΔA) for *p*-NP^- at 420 nm accompanied by the addition of each hosts were analyzed by means of a nonlinear curve-fitting method upon an assumption of the formation of 1:1 inclusion complex to give K_a values. As a typical example, UV-Vis spectral change for *p*-NP^- by the addition of **1** and plots of ΔA versus concentration of **1** are shown in Figures 2-2 and 2-3. The concentrations of the added hosts are considerably lower than those of the buffer salts (10 mmol dm^{-3} for the buffer solution with μ = 0.0230 mol dm^{-3}) in order to prevent the change in the pH of the buffer solutions during the titrations.

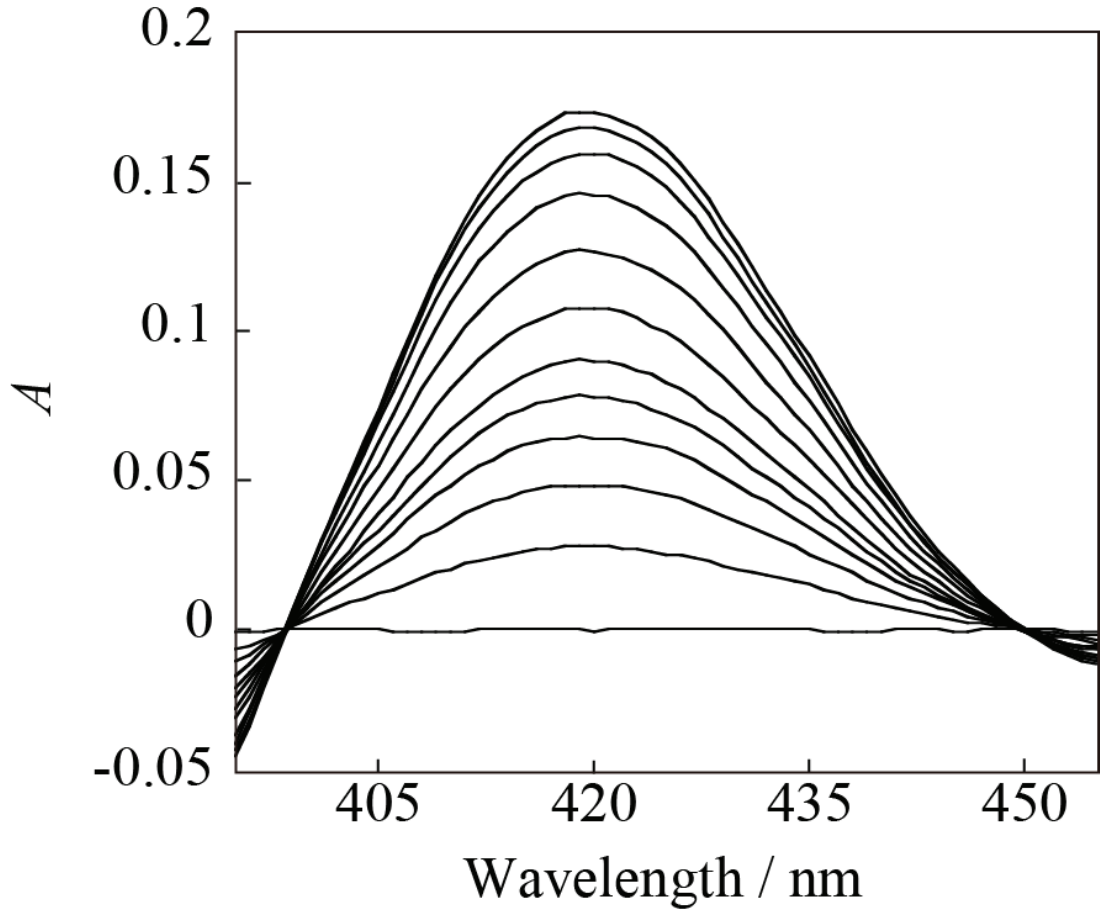


Figure 2-2. Spectral change of *p*-NP^- with the addition of **1**. The absorbance maximum of *p*-NP^- at 420 nm decreased with the addition of **1** (0- 1.38 mmol dm^{-3}).

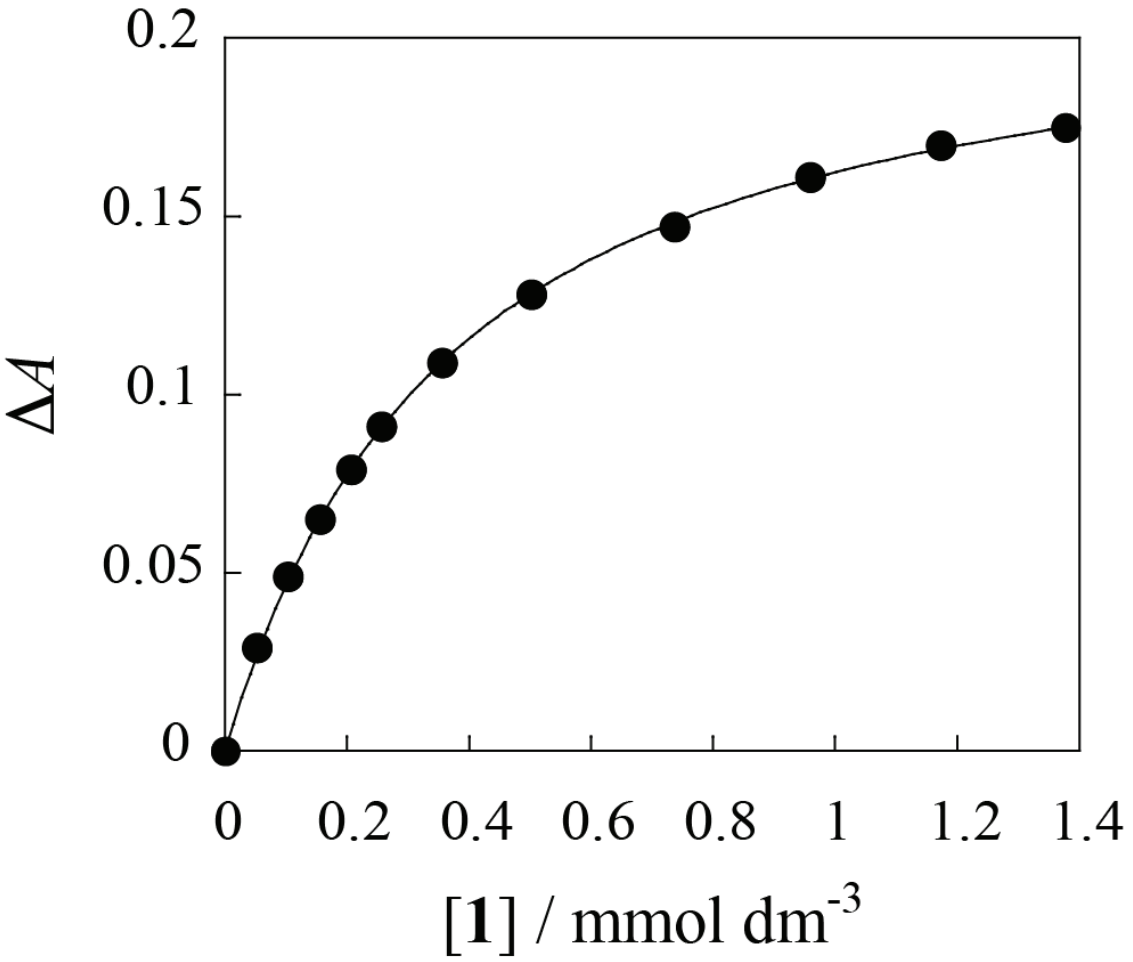


Figure 2-3. Plots of versus concentration of **1**. The solid line was calculated with a nonlinear curve-fitting method upon an assumption of the formation of 1:1 inclusion complex.

2.2.4 ROESY Spectra Measurements.

The molecular orientations of the inclusion complex of the three hosts or α-CD with *p*-NP^- were estimated by means of ROESY, which gives information on the proton correlations at a distance less than 5 Å [39]. The ROESY spectra were measured in a $Na_2CO_3/NaHCO_3$ buffered D_2O solution at pD 11.0 (pD = pH + 0.4), at 298 K. The concentrations of the host and the guest were 10 and 30 mmol dm^{-3}, respectively.

2. 3 Results

2.3.1 Binding Constants in the Buffer Solution at μ = 0.230 mol^{-1} dm^3.

The K_a values for the complexation of **1**, **2**, **3**, or the native α-CD with *p*-NP^- in the buffer solution at μ = 0.230 mol dm^{-3} were determined by UV-Vis titration experiments as described in the experimental section. The K_a value for the complexation of **1** with *p*-NP^- was 3120±160 mol^{-1} dm^3, which is 1.7 times larger than that for the native α-CD under the same experimental conditions (1810±70 mol^{-1} dm^3, Table 2-1). The greater K_a value for **1** than that for α-CD indicates that substituting the positively charged guanidino group for one of six C(6)-OH of α-CD contributes to the stabilization of the inclusion complex with *p*-NP^-. On the other hand, the K_a values for the complexation of **2** and **3** with *p*-NP^- were 1240±70 and 680±30 mol^{-1} dm^3, respectively, which are smaller than that for α-CD. These facts show that the introduction of the positively charged guanidino group into an electrically neutral α-CD does not always contribute to the stabilization of the inclusion complexes with negatively charged guests.

Table 2-1. K_a Values ($mol^{-1}dm^3$) for the Complexation of **1**, **2**, **3**, or α-CD with *p*-NP^- in the Buffer Solutions of Different μ at pH 10.6, 298 K

	K_a / $mol^{-1}dm^3$			
Hosts	I / mol dm^{-3}			
	0.0230	0.115	0.230	1.15
1	4250 ± 60	3520 ± 180	3120 ± 160	2400 ± 70
2	1690 ± 30	1390 ± 20	1240 ± 70	940 ± 60
3	840 ± 70	750 ± 30	680 ± 30	560 ± 60
α-CD	1900 ± 60	1740 ± 40	1810 ± 70	1810 ± 50

2.3.2 Influence of μ of the Buffer Solution on K_a Values.

The K_a values for the complexation of **1**, **2**, or **3** with *p*-NP^- decreased with an increase in μ of the buffer solution from 0.0230 to 1.15 mol dm^{-3}. The ratio of the decrease in K_a values accompanied by the increase in μ of the buffer solution from 0.0230 to 1.15 mol dm^{-3} is 44 % for **1** and **2**, and 33 % for **3**. On the other hand, the K_a values for the inclusion of α-CD with *p*-NP^- was virtually constant, irrespective of μ of the buffer solutions.

2.3.3 Estimation of Molecular Orientation of the Inclusion Complexes by Means of ROESY.

A part of the ROESY spectrum of a solution containing **1** and *p*-NP^- is depicted in Figure 2-4. The F1 and F2 axes cover aromatic protons of *p*-NP^- and aliphatic protons of **1**, respectively. Strong cross peaks were observed for the C(3, 5)-H signal of *p*-NP^- at 8.3 ppm and aliphatic protons of **1**, while those for the C(2, 6)-H signal of *p*-NP^- at 6.6 ppm and aliphatic protons of **1** were weak. As the primary hydroxyl side of CD is narrower than the secondary hydroxyl side, the stronger cross peaks between C(3, 5)-H of *p*-NP^- and aliphatic protons of **1** suggest that *p*-NP^- is included into the cavity of **1** to direct the nitro group to the primary hydroxyl side of **1**. The proposed molecular orientation of the inclusion complex of **1** with *p*-NP^- is depicted in Figure 2-5. The ROESY spectra of the complexes of **2**, **3**, and native α-CD with *p*-NP^- also gave strong cross peaks between the C(3,5)-H of *p*-NP^- and aliphatic protons of the hosts, indicating that *p*-NP^- is included within the cavities of the hosts in the same manner as in **1**. Thus, it seems that the molecular orientation of

p-NP^- in the inclusion complexes is not affected by the position of the guanidino group.

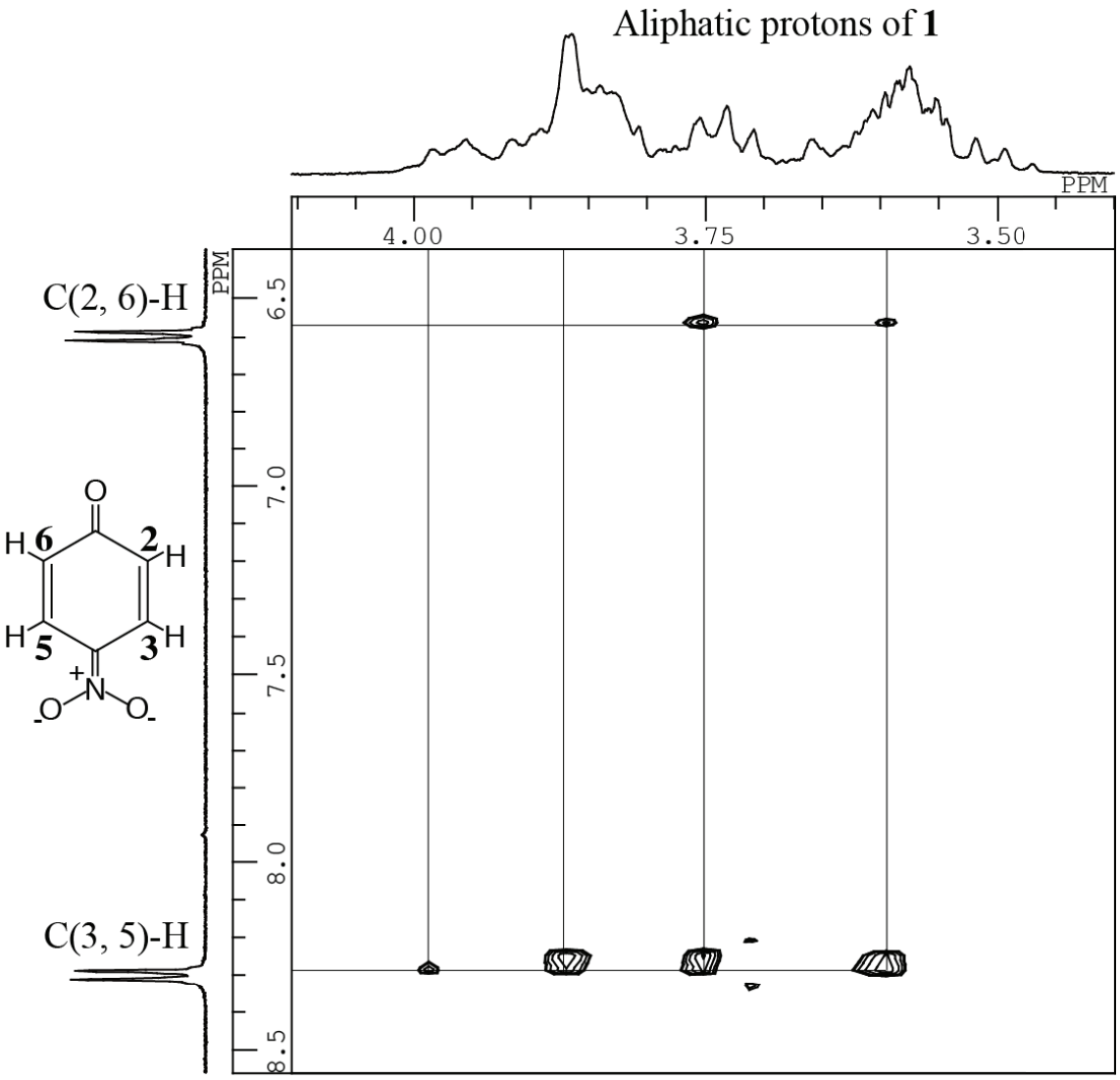


Figure 2-4. A partial ROESY spectrum of *p*-NP^- (5 mmol dm^{-3}) and **1** (5 mmol dm^{-3}) in D_2O at 298K, covering the aromatic protons of *p*-NP^- in F1 axis and aliphatic protons of **1** in F2 axis.

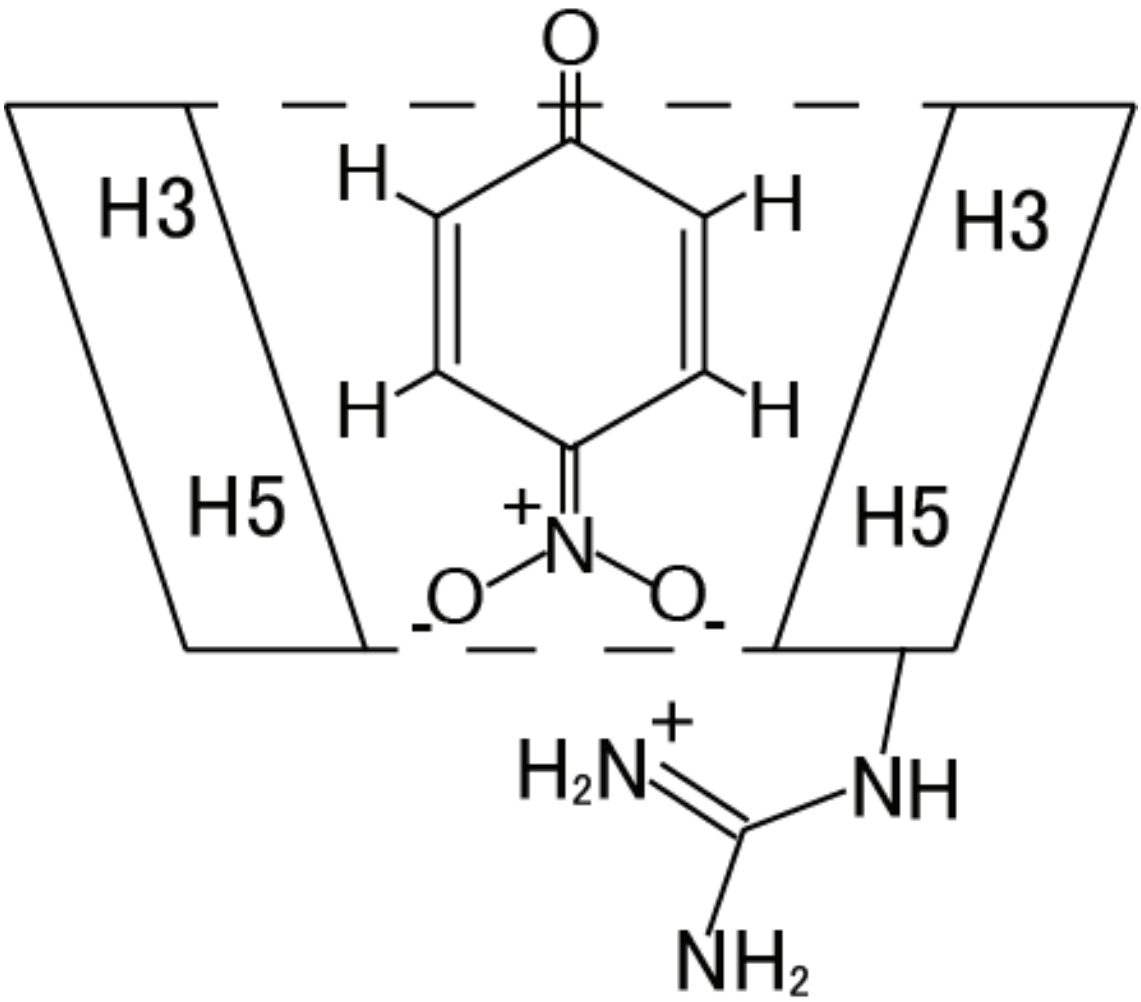


Figure 2-5. A proposed molecular orientation of the inclusion complex of **1** with *p*-NP^-. The letters H3 and H5 on **1** represent the approximate positions of C(3)- and C(5)-H of **1**, which locate inside the cavity of the macroring.

2. 4 Discussion

The negative charge on the phenolate oxygen of *p*-NP^- is delocalized to the nitro group as a result of resonance as depicted in Figure 2-6, with the stronger electronegativity on the nitro group. At first assumption, the greater K_a values for the complexation of **1** with *p*-NP^- was attributed to the stronger charge-charge interaction between the guanidino group of **1** and the nitro group of *p*-NP^-, and the smaller K_a values for **2** and **3** were supposed due to the longer distance between those functional groups. According to this assumption, the K_a values for **1** with *p*-NP^- should decrease to a larger extent than that for **2** or **3** with increasing μ of the buffer solutions, due to the larger contribution of electrostatic interaction to the complexation. However, the K_a values for the complexation of **1** and **2** with *p*-NP^- decreased to 44 % with increasing μ of the buffer solutions in both the cases. This fact indicates that electrostatic interaction contributes to the stabilization of the inclusion complexes to the same extent for **1** and **2**. That is, charge-charge interaction between the guanidono group of **2** and the phenolate oxygen of *p*-NP^- will stabilize the inclusion complex as in **1** with *p*-NP. The structural difference between **1**, **2**, and **3** was also discussed as a factor that influences the complexation with *p*-NP^-, since substituents on the secondary hydroxyl side of CD might sterically interfere with the inclusion of guests from the secondary hydroxyl side of CD, the more open and a preferential locus for the binding of guests. However, the analysis on the energy-minimized structures for **2** and **3** using MMFF94 force field in

Avogadro [40] revealed that the substituents of **2** and **3** do not interfere with the opening of the secondary hydroxyl side.

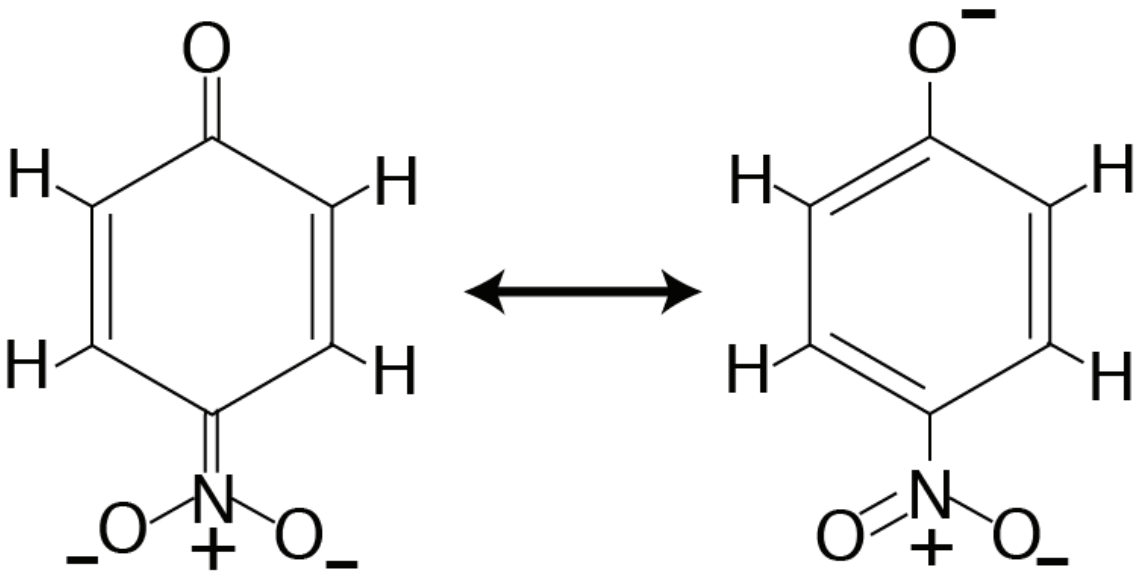


Figure 2-6. Resonance structures of *p*-NP^-.

In order to understand the factors that make the K_a value for the complexation of **2** with *p*-NP^- smaller than that for the native α-CD, the influence of the guanidino group on the dipole moment of the native α-CD was taken into consideration. It is reported that the dipole moment of the native α-CD directs from the secondary (negative) to the primary hydroxyl side (positive), and plays important roles in the formation and the determination of molecular orientations of inclusion complexes [41]. Therefore, the introduction of a positively charged guanidino group to the primary hydroxyl side of the native α-CD, as in **1**, strengthens the dipole moment of the native

α-CD and works favorably for the complexation with *p*-NP^-. On the contrary, the introduction of the same functional group to the secondary hydroxyl side of the native α-CD, as in **2** or **3**, weakens the dipole moment of the native α-CD, and works unfavorably for the complexation with *p*-NP^-.

The small K_a values for the complexation of **3** with *p*-NP^- will be due to the distortion of the macroring of **3**, which is caused by the conversion of one of six glucoses to an altrose. It is reported that the molecular rotation of the guest in such a distorted macroring is restricted [26]. The ^1H NMR signals for such a host are largely shifted by the inclusion of aromatic guests due to the anisotropic ring current effect of the guests [21, 25]. The ^1H NMR signals for **3** are largely shifted on the complexation with *p*-NP^- to give well-separated signals (Figure 2-7). Therefore, the smallest K_a values for **3** for the complexation with *p*-NP^- will be due to the weakened dipole moment by the guanidino group on the secondary hydroxyl side and the distortion of the macroring by the conversion of a glucose to an altrose in **3**.

The K_a values for the native α-CD remained constant thorough the change in the μ of the buffer solutions. Hydrophobic interaction is known to increase with increasing μ of solutions [42]. Thus, the constant K_a values indicate that hydrophobic interaction contributes to the stabilization of the inclusion complex together with the dipole-charge interaction between the native α-CD and *p*-NP^-.

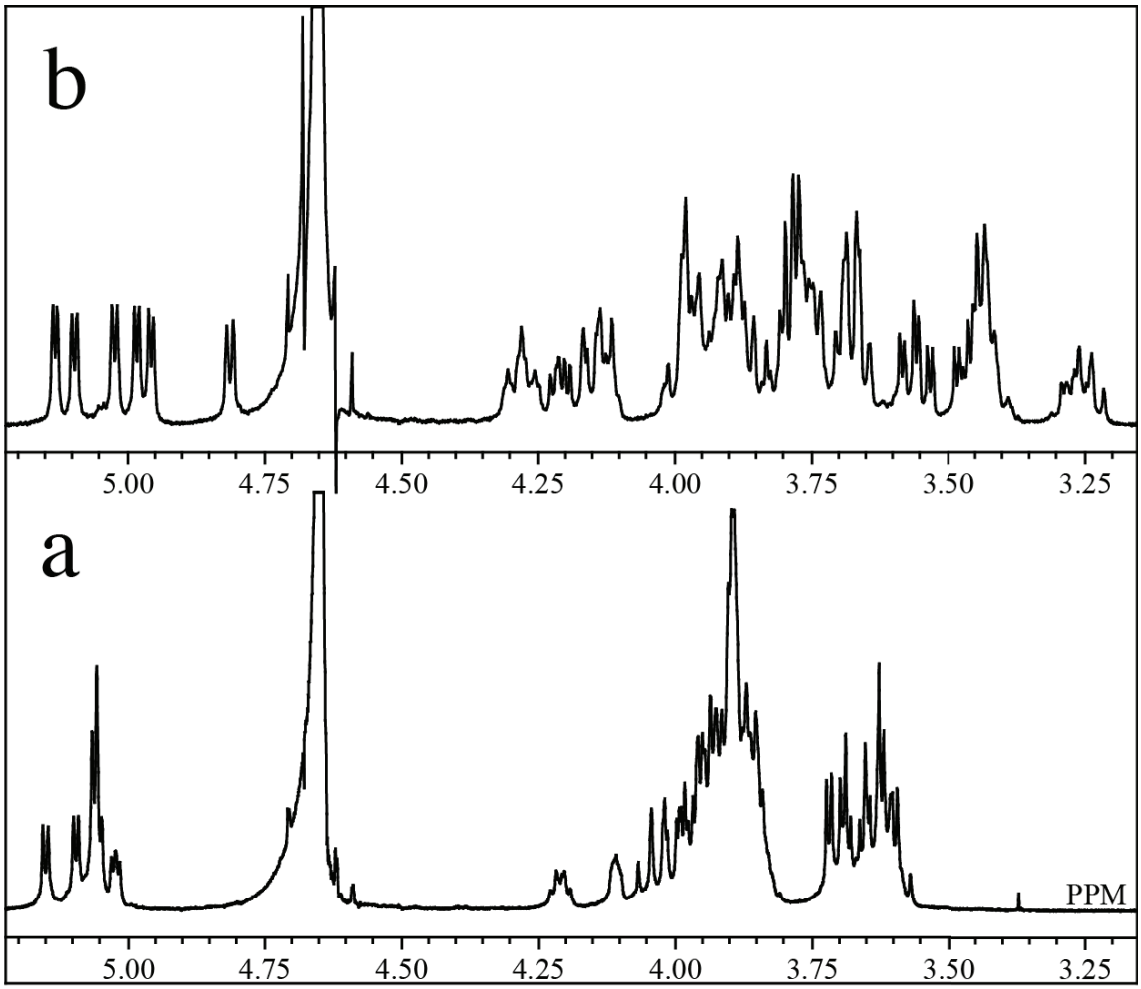


Figure 2-7. ^{1}H NMR spectra of **3** in D_2O at 313K; (a) 22.1 mmol dm^{-3} of **3**; (b) 10.1 mmol dm^{-3} of **3** with 50.0 mmol dm^{-3} of *p*-NP^{-}. The large signal at 4.65 ppm is due to HDO.

2. 5 Summary

Three structural isomers of monoguanidino-modified α-cyclodextrin (CD), i.e., mono(6-deoxy-6-guanidino)-α-CD (**1**), mono(3-deoxy-3-guanidino)-α-CD (**2**), and mono(3-deoxy-3-guanidino-*altro*)-α-CD (**3**), were synthesized to study equilibria for the formation of inclusion complexes with the *p*-nitrophenolate ion (*p*-NP^-). The binding constants (K_a's) for the equilibria, determined by UV-Vis titration at 298 K and pH 10.6, showed that **1** with a positively charged guanidino group on the primary hydroxyl side of α-CD binds the negatively charged *p*-NP^- more strongly than the native α-CD. On the contrary, either **2** or **3**, with the same functional group on the secondary hydroxyl side, binds *p*-NP^- less strongly than the native α-CD. The K_a values for **1**, **2**, and **3** decreased, while that for the native α-CD remained virtually constant, with increasing ionic strength of the solutions. The two-dimensional ^1H NMR spectra of rotating frame nuclear overhauser effect spectroscopy showed that *p*-NP^- included in the cavity of **1**, **2**, **3**, or α-CD directs the nitro group toward the primary hydroxyl side of the host. These results were explained on the basis of electrostatic interaction, including charge-charge, charge-dipole, and dipole-dipole interaction, between the hosts and the guest.

Chapter 3.

Thermodynamic and structural studies on the complexation of guanidino-appended α-cyclodextrin derivatives with *p*-nitrophenolate ion

3. 1 Introduction

CDs have long been utilized to give model systems for artificial enzymes because of its inclusion ability [14, 43-46]. The glucopyranose units composing the macroring of native CDs have a within $^4C_1$ chair conformation. The macroring of CD has a C_n symmetry where n is the number of glucopyranose units. Such macrorings with a C_n symmetry are more rigid than those of the CD derivatives whose C_n symmetry is broken by chemical modifications [47]. Among them, the derivatives with a chemically modified sugar unit as to more prefer $^1C_4$ chair conformation than the original $^4C_1$ type have macrorings with the more flexibility than the original one. Such sugar unit is in the equilibrium as $^4C_1 \leftrightarrow {}^\circ C_2 \leftrightarrow {}^1C_4$ conformations [25, 48, 49].

Mono(3-deoxy-3-guanidino-*altro*)-α-CD (**1**) is an α-CD derivative, in which one of the six glucopyranose is chemically converted to 3-deoxy-3-guanidino-altrose (sugar unit A in Figure 3-1, left) [50]. As a result, the molecule has a more flexible macroring than the native α-CD. The more flexible macroring may give a better artificial enzyme model with induced-fit property than the native α-CD. The molecule has a positively charged ganidino group at the secondary hydroxyl side. The charge might work as the one in the side chain of arginine, which is frequently found in the active site of native enzymes. The property of arginine, keeping a stable positive charge in a wide range of pH, is supposed to contribute to hold negatively charged substrates in the proper orientation by electrostatic interaction [51-53]. By thinking as above, we have come up with utilizing **1** as a host with a flexible macroring. However, in

comparison with the number of reports on the structure of CDs with flexible macroring, not much is reported on thermodynamics for their inclusion complexation or on the molecular orientation of their inclusion complexes [26]. In this report, binding constant (K_a) and thermodynamic parameters (ΔG, ΔH, and $T\Delta S$) for the inclusion equilibrium of **1** or mono(3-deoxy-3-guanidino)-α-CD (**2**) with *p*-nitrophenolate ion (*p*-NP^-) were studied. **2** was studied as a structural isomer of **1**, which has a more rigid macroring with six within $^4C_1$ chair conformation sugar units than **1**. Thus, we investigated the effect of the macroring flexibility of **1**, on the inclusion complexation with a planar and negatively charged guest. The molecular orientation of the inclusion complex of **1** with the guest is also determined by means of 1D and 2D ^{1}H NMR spectroscopy.

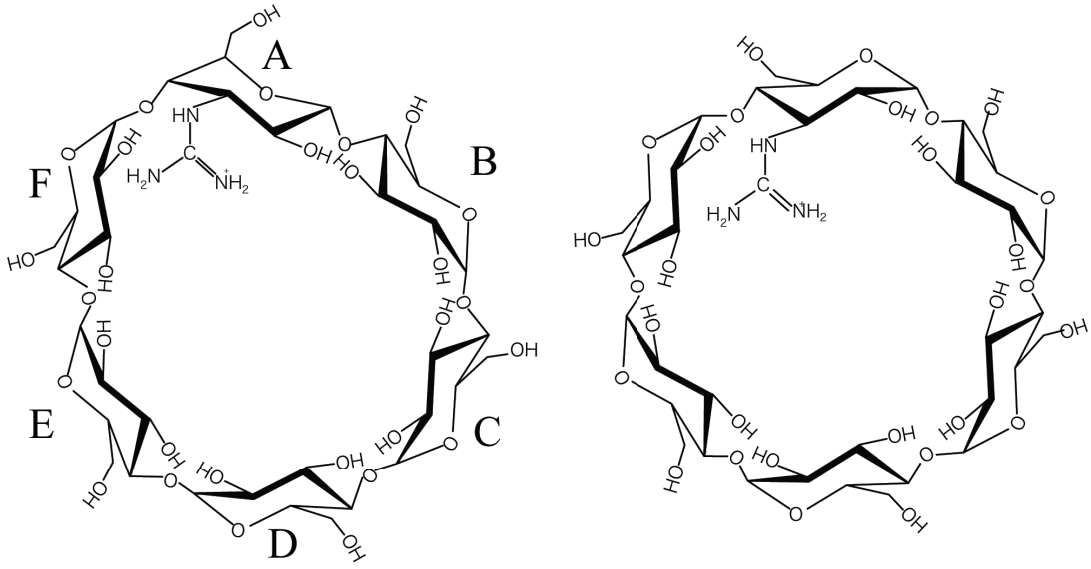


Figure 3-1. Structure of mono(3-deoxy-3-guanidino-3-*altro*)-α-CD (**1**) and mono(3-deoxy-3-guanidino)-α-CD (**2**). Letters **A** – **F** represent each sugar unit of **1**. Hydrogen atoms bonded to carbon atoms are omitted for simplicity.

3. 2 Experimental

3.2.1 Materials.

Compound **1** and **2** were synthesized according to the procedure in the literature [50]. The *p*-NP^-, $NaHCO_3$ and Na_2CO_3 were of reagent grade (Wako Pure Chem. Ind., Ltd) and used as received. D_2O (Isotec, 99.9 atom % D) and dimethyl-d_6 sulfoxide (DMSO-d_6) containing 0.05 % v/v tetramethylsilane (Cambridge Isotope Laboratiories, Inc., 99.9 atm % D) were used for the ^1H NMR measurements.

3.2.2 Apparatus.

All NMR spectra were recorded on a model JEOL JNM-A400 with the sample tube of 5.0 mm diameter. The ROESY spectra of **1** and *p*-NP^- were acquired with a mixing time of 500 ms and 512 x 256 data points, followed by zero-filling. The 1D HOHAHA spectra were obtained with mixing time of 50- 150 ms.

Binding constants and thermodynamic parameters. K_a for the complexation of **1** or **2** with *p*-NP^- were determined by nonlinear least squares analysis on $\Delta\delta$ of *p*-NP^- as a function of the concentration of the guests at 298, 308, 318, and 328 K, on an assumption of 1:1 inclusion complexation [55]. The thermodynamic parameters were calculated from the slope and intercept of the linear regression line for the plots of natural logarithm of K_a against 1/T, where T is temperature in K (van't Hoff plot) [26].

3.3 Results and Discussions

3.3.1 Binding constant and thermodynamic parameters

Binding constant (K_a) for the complexation of **1** or **2** with *p*-NP^- were determined by ^1H NMR titration experiments at 298, 308, 318, and 328 K as described in the experimental section. The final concentration of **2** (10 mmol dm^{-3}) in the titration experiment was smaller than that of **1** (30 mmol dm^{-3}) because of the low yield of the product [50]. The ^1H NMR chemical shift (δ) of aromatic protons of *p*-NP^- shifted downfield on the addition of **1** or **2** (Figure 3-2). Those protons gave a set of single peak under all host concentrations indicating that the equilibrium reaction is faster than the time scale of NMR measurements. Figure 3-3 shows the plot of the changes in δ ($\Delta\delta$) against the guest concentration. The K_a values determined in this study well coincided with those determined by UV-Vis titration experiments although the concentrations of host and guest were different [50]. Thermodynamic parameters were determined from the slope and intercept of Van't Hoff plot (Figure 3-4) as described in the experimental section to give Table 3-1. The enthalpy change for the complexation of **1** with *p*-NP^- was -39.6 kJ mol^{-1}. The magnitude of its absolute value is larger than that for the complexation of **2** with the guest, ΔH = -27.6 kJ mol^{-1}. Thermodynamic studies on the driving forces to stabilize inclusion complexes have revealed that stronger van der Waals interaction gives more exothermic process for the formation of inclusion complexes to result in the decrease in enthalpy [55]. Therefore, the larger negative value in enthalpy

change, for the complexation of **1** with the guest than that for **2**, suggests that the planar guest is bound more tightly by van der Waals interaction in the deformed macroring of **1**. The smaller negative value for the case of **2** indicates that the host also binds the guest exothermically, however, with a less contribution of van der Waals interaction, into the more rigid macroring of **2**.

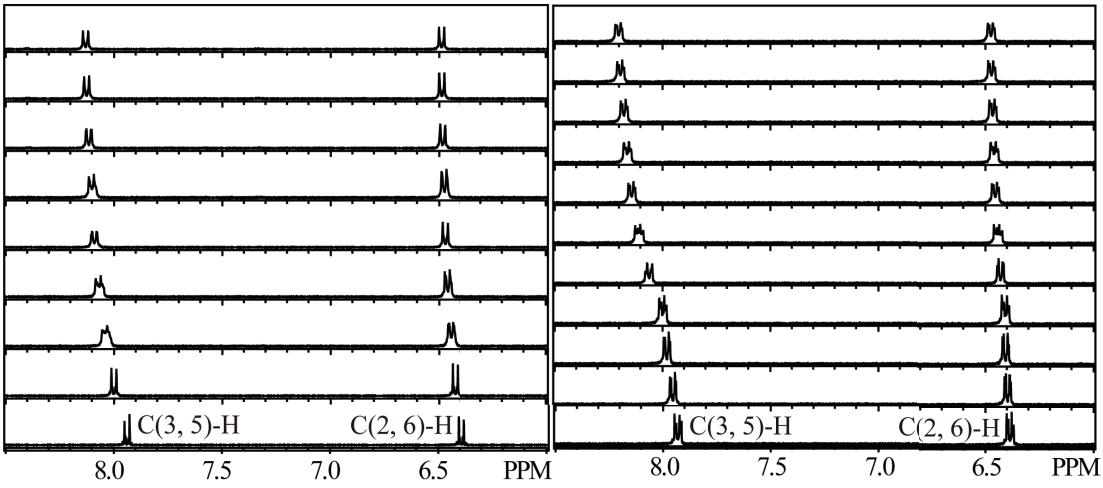


Figure 3-2. ^{1}H NMR spectra of *p*-NP^{-} (5.00 mmol dm^{-3}) at different concentrations of **1** (left; [**1**] =0-30.2 mmol dm^{-3}) or **2** (right; [**2**]= 0-10.1 mmol dm^{-3}).

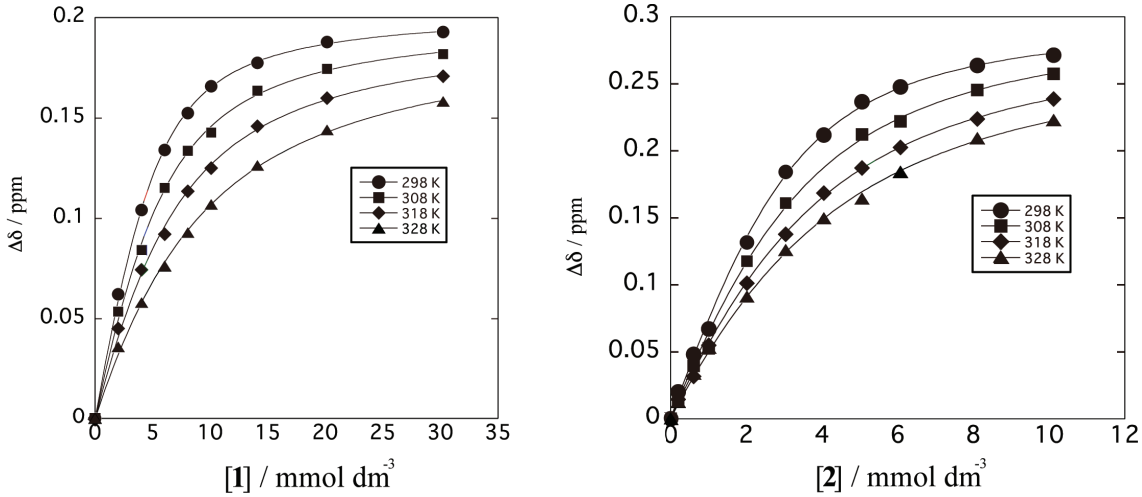


Figure 3-3. Plots of $\Delta\delta$ of *p*-NP^- against the concentrations of **1** (left) or **2** (right) at different temperatures. The solid lines show the theoretical values obtained by nonlinear least squares analysis on an assumption of 1:1 inclusion complexation.

Table 3-1 K_a and Thermodynamic Parameters for the Complexation of **1** or **2** with *p*-NP^- in D_2O containing 0.10 mol dm^{-3} Na_2CO_3-$NaHCO_3$ at 298, 308, 318, and 328 K

Hosts	Temp / K	K_a / mol^{-1}dm^3	ΔG / kJ mol^{-1}	ΔH / kJ mol^{-1}	$T\Delta S$ / kJ mol^{-1}
1	298	715	-16.3	-39.6	-23.3
	308	420	-15.5		-24.1
	318	266	-14.8		-24.8
	328	164	-13.9		-25.7
2	298	1200	-17.6	-27.6	-10.0
	308	730	-16.9		-10.7
	318	540	-16.6		-11.0
	328	430	-16.5		-11.1

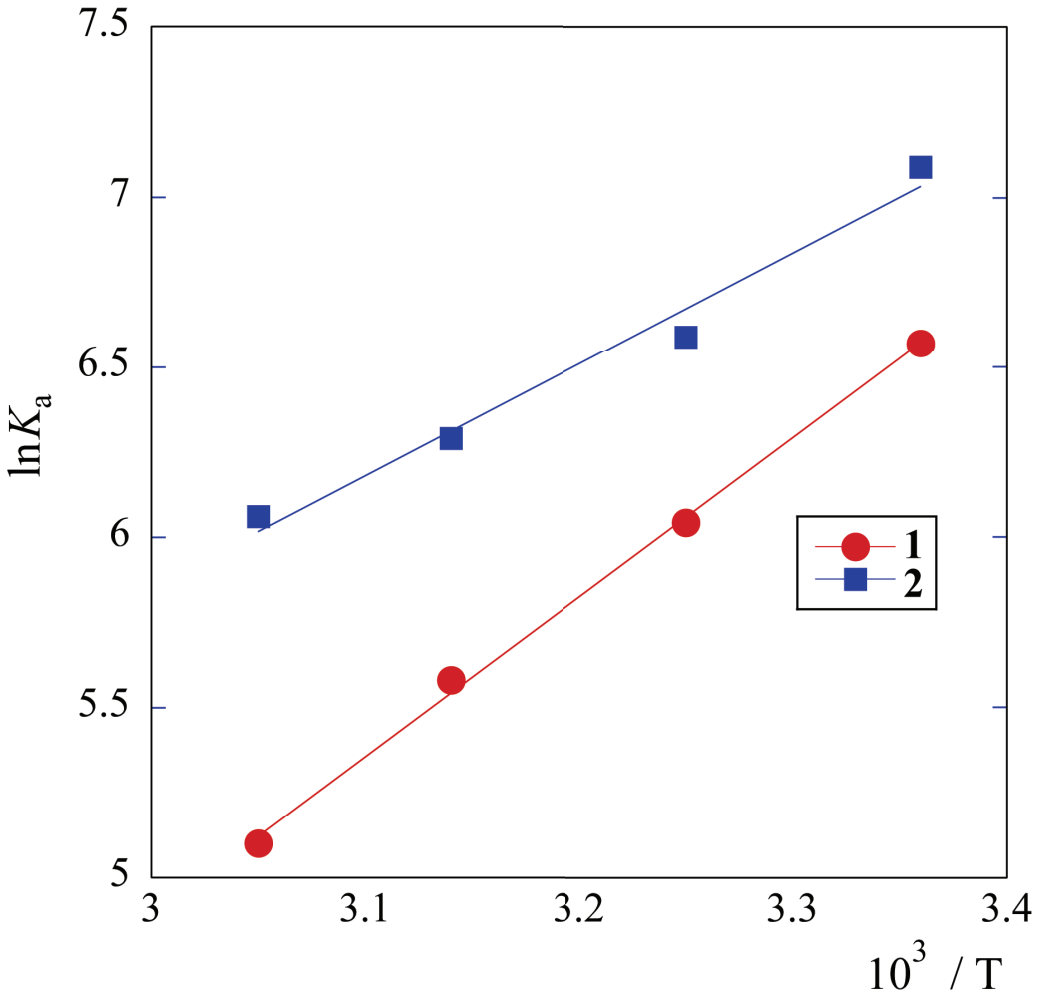


Figure 3-4. Plots of natural logarithm of K_a for the complexation of **1** or **2** with *p*-NP^- against 10^3/T, where T is temperature in K (van't Hoff plot).

The decrease in entropy term ($T\Delta S$) for the complexation of **1** with *p*-NP^- (-23.3 kJ mol^{-1} at 298 K) was apparently larger than that for **2** (-10.0 kJ mol^{-1} at 298 K). The larger decrease in for **1** can be attributed to the decrease in the degree of freedom of the molecular motion of the guest in the deformed macroring, which is caused by the restriction of the conformational changes of the altrose type sugar unit within ${}^4C_1 \leftrightarrow {}^\circ C_2 \leftrightarrow {}^1C_4$ forms. The reduced flexibility of the macroring itself is also considered to take part in the decrease in for **1**. The smaller decrease in for **2** indicates the absence of strong restriction of molecular motion of *p*-NP^- in **2**.

Based on the thermodynamic parameters obtained here, we can say that the complexation of **1** with *p*-NP^- is more driven by enthalpy than entropy in which electrostatic [50] and van der Waals interaction contribute to stabilize the inclusion complex. Molecular motions in the inclusion complex with **1** were more restricted than that with **2**.

3.3.2 Molecular orientation of *p*-NP$^-$ in the inclusion complex with 1

It is impossible to make it clear what motion is restricted for the *p*-NP$^-$ in the inclusion complex with **1**, only by the values in thermodynamic parameters. Thus, we have conducted 1D and 2D ^{1}H NMR measurements, which may give the information on the relative position of the guest against the host in the inclusion complex.

3.3.2.1 Assignment of signals of 1 in the presence of *p*-NP$^-$

The ^{1}H NMR spectrum of **1** alone gave a complicated feature as shown in Figure 3-5a. However, the addition of five equivalent amount of *p*-NP$^-$ to **1** caused the separation of six C(1)-H signals for each sugar unit which were tentatively named as from ① to ⑥ (Figure 3-5b). On the other hand, other signals for **1** that appeared in the region around δ= 3.2-4.3 ppm still gave complicated feature. In order to assign these signals to the sugar units ① ~ ⑥ , we measured HOHAHA (homo-nuclear Hartmann-Hahn) spectra. HOHAHA spectra were measured by irradiating each C(1)-H for six sugar units, one by one, to investigate the correlation of the protons through spin-spin coupling on bonds [56]. In each spectrum, the C(2)-H signal appeared to be more intense than those for other protons on the same sugar unit, when C(1)-H was irradiated with an appropriate mixing time (Figure 3-6) [56]. We also measured a H-H COSY (correlated spectroscopy) spectrum to get information on correlations between neighboring protons. The C(1)-H signal for the sugar unit ① that appeared at the higher magnetic field (δ= 4.8 ppm) was

assigned to that for altrose type sugar unit by the fact that it had apparently the larger coupling constant (J= 4.64 Hz) than those for others (J= 3.17 ~ 3.66 Hz) [57], and named as sugar unit A.

The order of sequence in six sugar units was determined by means of ROESY (rotating-frame overhauser effect spectroscopy) experiments, in which cross peaks appear between the protons in the vicinity within approximately 5 Å [39]. The C(1)-H of the sugar unit A (or ⑥) gave two cross peaks, one with C(2)-H of the same sugar unit, another with C(4)-H of the sugar unit ③. Then, the sugar unit ③ was revealed to be the sugar unit B (Figure 3-7). Successive analyses on the ROESY spectrum allowed us to determine the order of sequence of sugar units ① ~ ⑥. Assignments of the signals for C(1~5)H of **1** are given in Figure 3-8.

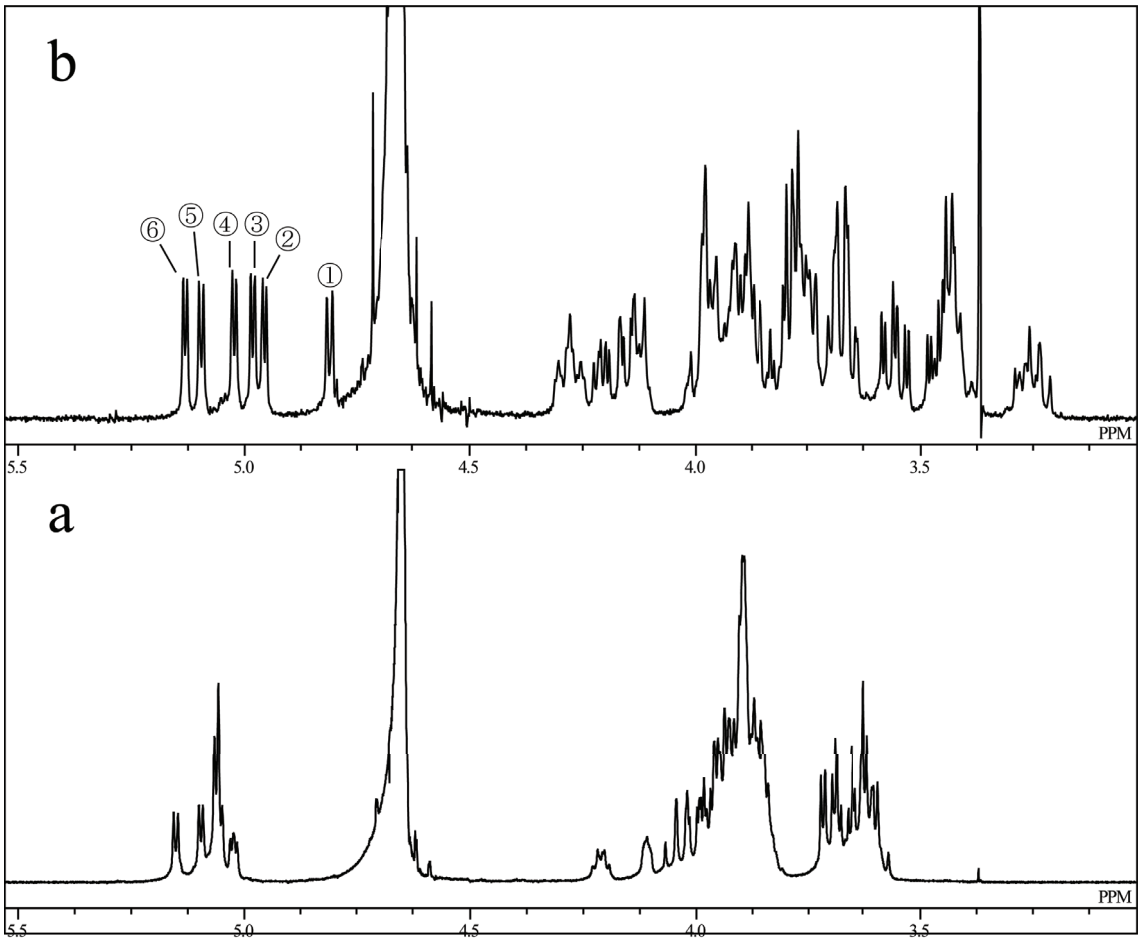


Figure 3-5. ^{1}H NMR spectra of **1** (10 mmol dm^{-3}) alone (a), and that in the presence of 50 mmol dm^{-3} of *p*-NP^{-} (b) at 313K, in D_2O.

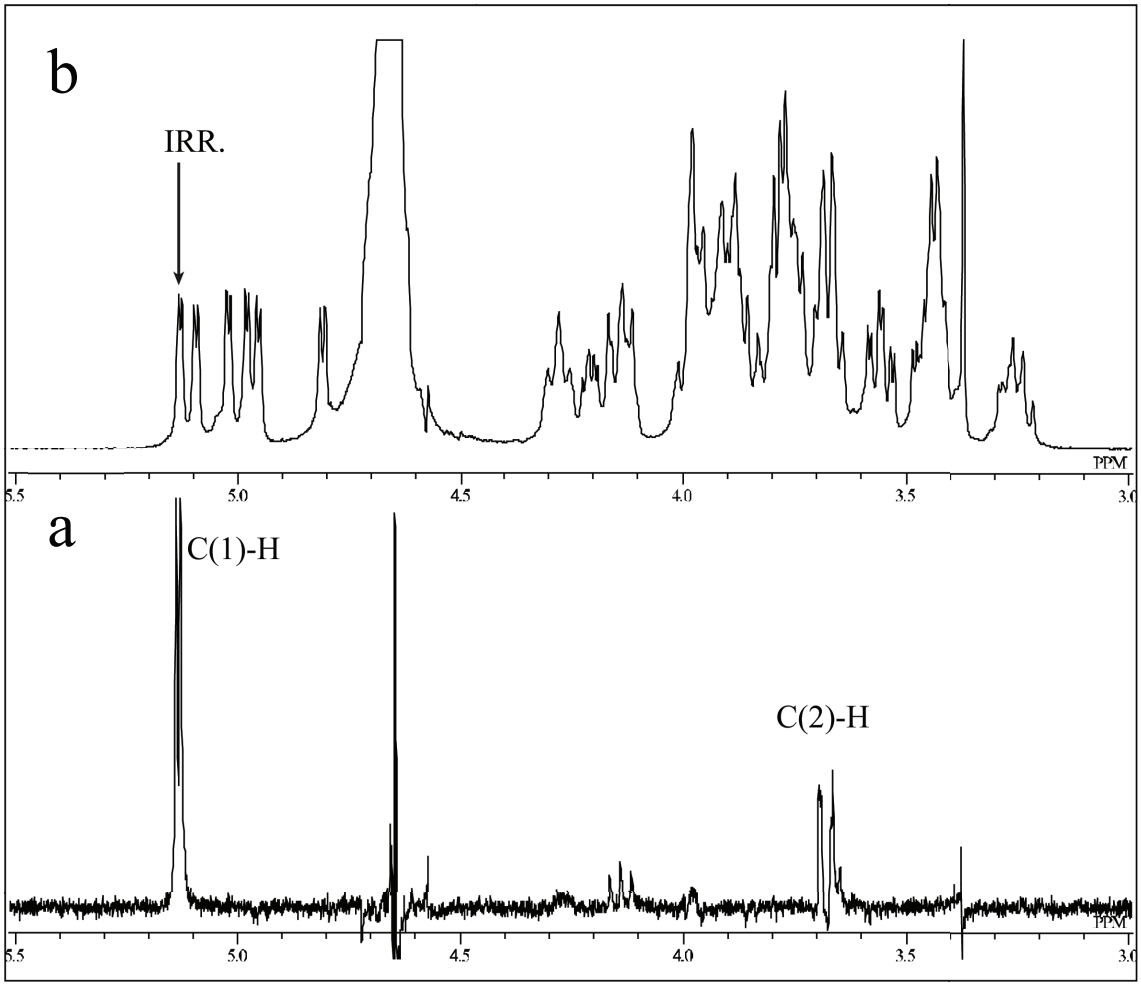


Figure 3-6. 1D HOHAHA spectrum of **1** (10 mmol dm^{-3}) in the presence of *p*-NP^- (50 mmol dm^{-3}) at 313K. a): 1D HOHAHA spectrum obtained by irradiating at δ= 5.1290 ppm (C1-H of C); b): normal 1D spectrum showing an irradiating peak with an arrow head.

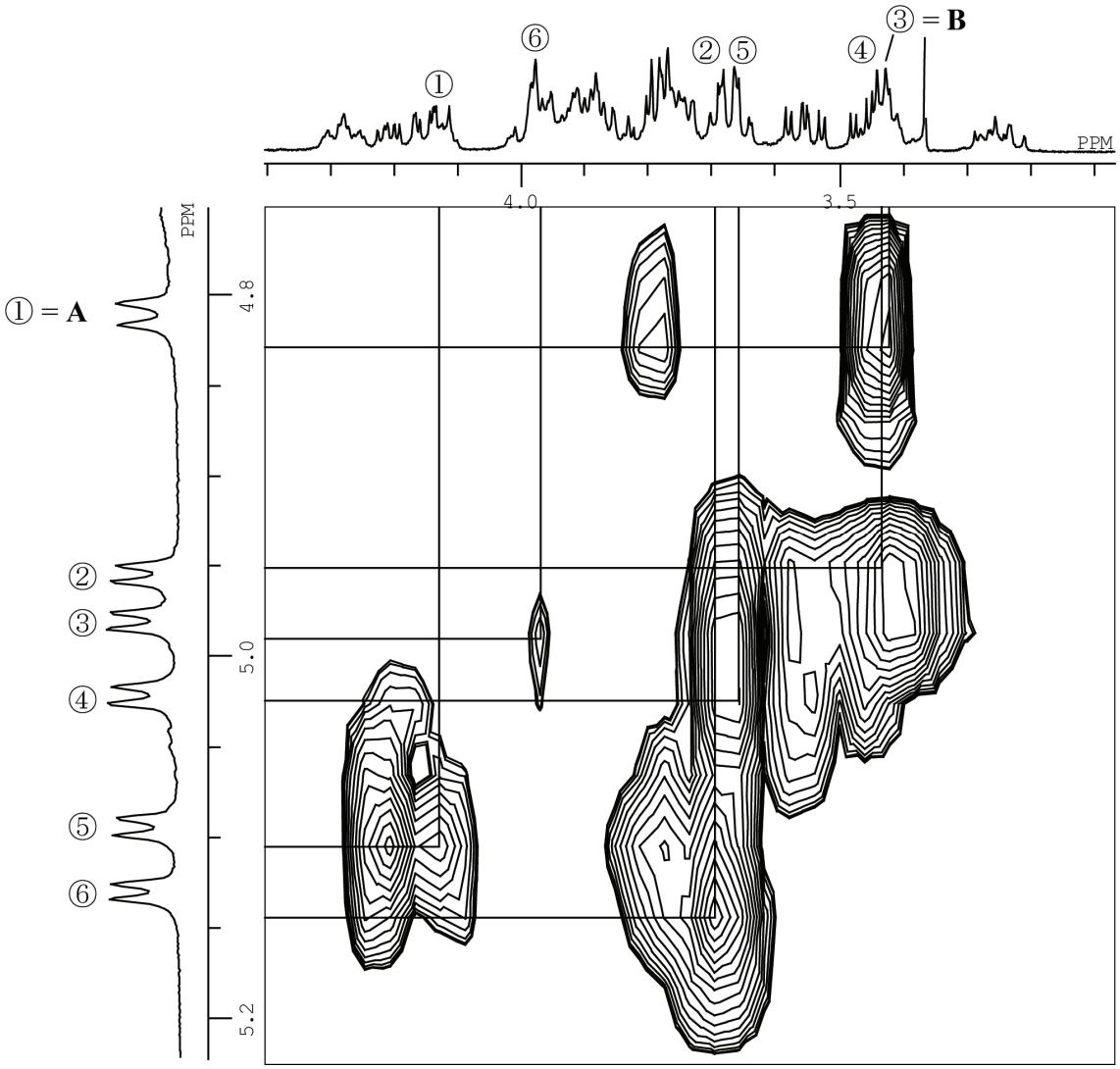


Figure 3-7. 2D ROESY spectrum of **1** (10 mmol dm^{-3}) and *p*-NP^- (50 mmol dm^{-3}) in D_2O containing 0.1 mol dm^{-3} $NaHCO_3/Na_2CO_3$ at 313 K, showing the region of C(1)-H (F1) and aliphatic protons of 1 (F2). C(4)-H signals for sugar units ① ~ ⑥ are annotated in F2.

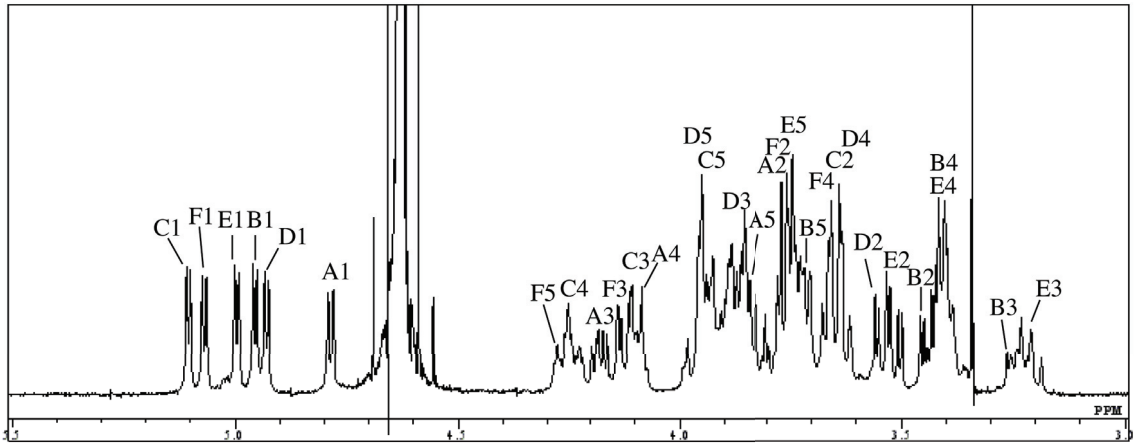


Figure 3-8. A ^{1}H NMR spectrum of **1** (10 mmol dm^{-3}) in the presence of *p*-NP^- (50 mmol dm^{-3}) in D_2O containing 0.1 mol dm^{-3} $NaHCO_3/Na_2CO_3$ at 313 K. C(1～5)-H signals are assigned.

3.3.2.2 Determination of the molecular orientation of *p*-NP^- in the inclusion complex with 1

The ROESY spectrum of **1** with *p*-NP^- has shown several cross peaks between the host and guest protons, which gave important information on the molecular orientation of inclusion complexes. The C(3,5)-H of *p*-NP^- gave two strong cross peaks, one with C(3)-H of the sugar unit C, another with C(5)-H of sugar unit F of **1**. They also gave a weak cross peak with C(5)-H of the sugar unit E (Figure 3-9a). On the other hand, C(2,6)-H of *p*-NP^- gave only one cross peak with C(3)-H of sugar unit F of **1**. The sugar units C and F are located in the opposite position in the macroring. The existence of strong cross peaks with these sugar units indicates that *p*-NP^- is bound in the macroring of **1** having a certain orientation, directing the aromatic protons to the sugar units C and F. This observation well explains the results of thermodynamic experiments, where the tight binding of the guest and the restriction of molecular motion of the complex were suggested from large negative values for both ΔH and $T\Delta S$. The nitro group of the guest in the cavity of **1** was considered to be directed to the primary hydroxy side of **1**, based on the facts that C(3,5)-H of *p*-NP^- gave stronger cross peaks with inner cavity protons of **1** than C(2,6)-H did, and that C(3)- and C(5)-H of sugar unit F gave cross peaks with C(2,6)- and C(3,5)-H of *p*-NP^-, respectively. For the comparison, we have also measured a ROESY spectrum for **2** with *p*-NP^- under the same experimental condition (Figure 3-9b). Several cross peaks were observed on the spectrum showing correlations between C(3,5)-H of *p*-NP^- and aliphatic protons of **2**,

however, no peak was observed for C(2,6)-H of *p*-NP^-. This fact suggests that *p*-NP^- is included into the cavity of **2** directing the nitro group to the primary hydroxy side in the same direction as for **1**. The aliphatic protons of **2** did not separate from each other after the addition of *p*-NP^-, indicating the inexistence of anisotropic ring current effect of *p*-NP^-. Therefore, it is reasonable to say that the rotation of *p*-NP^- bound in the cavity of **2** is not restricted.

We have also examined the direction and magnitude of the chemical shift changes ($\Delta\delta$) of the ^{1}H NMR signals for the inner protons, C(3)- and C(5)-H, for each sugar unit of **1**, after the addition of five equivalent amount of *p*-NP^- to **1** (Figure 3-10). The signals for A, C, and F of **1** were downfield shifted, while those of B and E were upfield shifted. The downfield or upfield shifts of the ^{1}H NMR signals indicate that the protons are more deshielded or shielded, respectively, after the complexation with *p*-NP^-. The results suggest that the sugar units A, C, and F are located at the lateral area of the plane of the aromatic ring of *p*-NP^-, while B and E are above or below the ring (anisotropic ring current effect) [26]. On the basis of these experimental results, we have elucidated the molecular orientation of *p*-NP^- in the inclusion complex with **1** as shown in Figure 3-11.

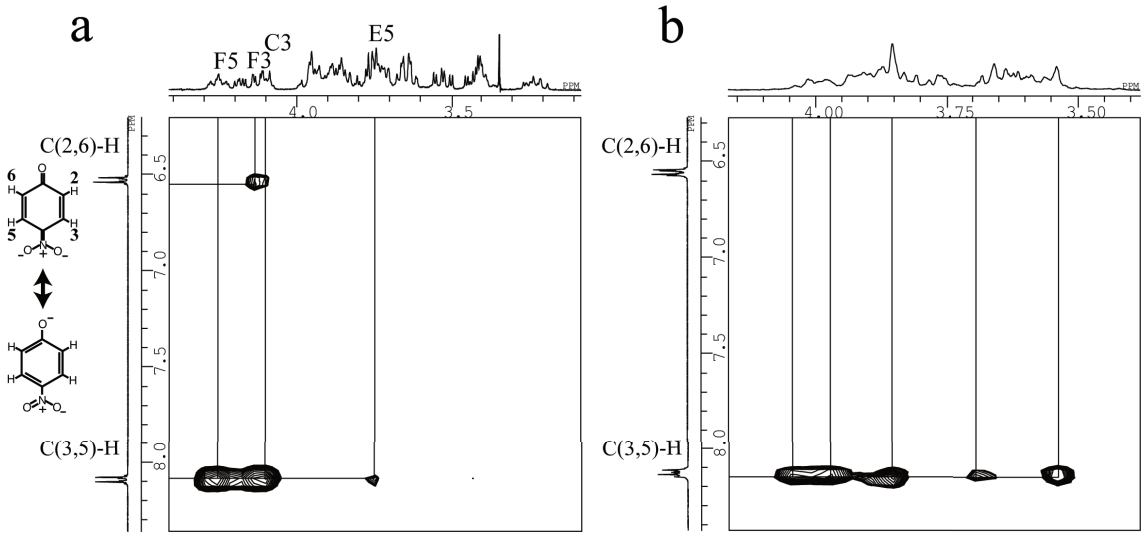


Figure 3-9. 2D ROESY spectrum of **1** (a) or **2** (b) with *p*-NP^- in D_2O containing 0.1 mol dm^{-3} $NaHCO_3/Na_2CO_3$ at 313 K, showing the region of aromatic protons of *p*-NP^- (F1) and aliphatic protons of the hosts (F2). The concentrations of the hosts and the guest are 10 and 50 mmol dm^{-3}, respectively.

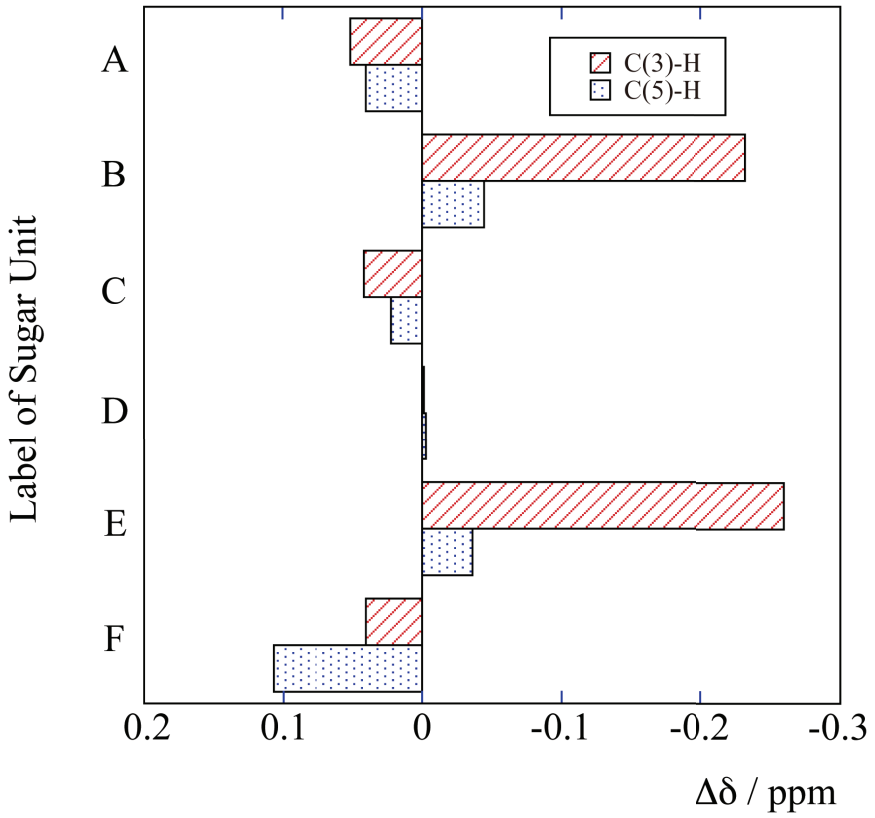


Figure 3-10. $\Delta\delta$ for C(3)- and C(5)-H's of each sugar units of **1** (10 mmol dm^{-3}) on the addition of 50 mmol dm^{-3} *p*-NP^- in D_2O containing 0.1 mol dm^{-3} $NaHCO_3/Na_2CO_3$ at 313 K.

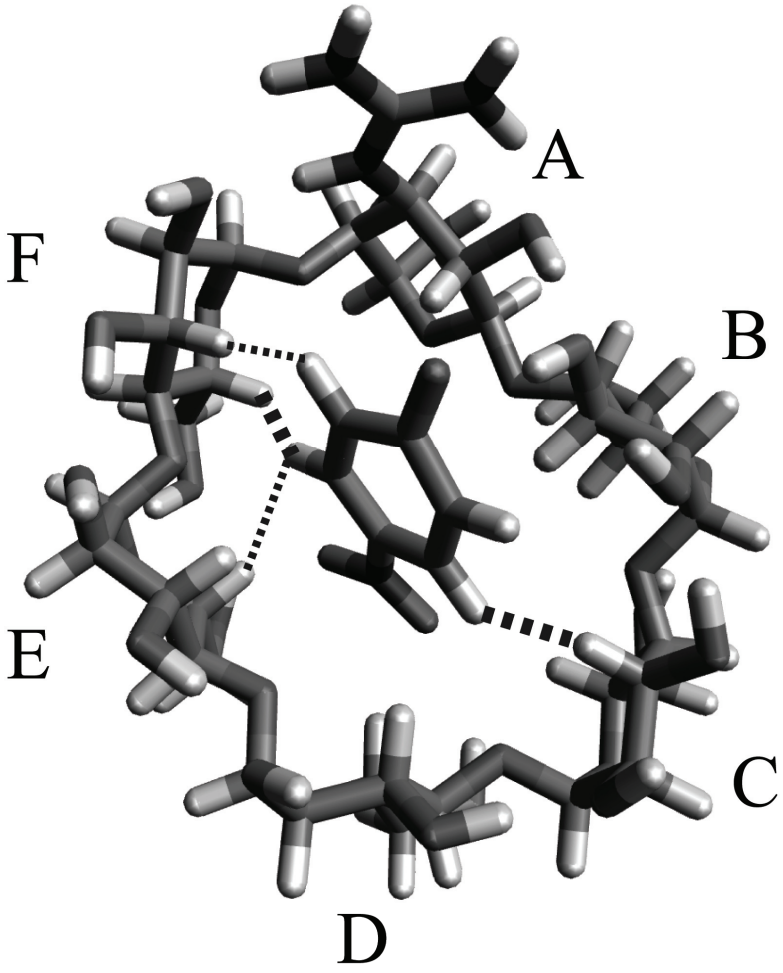


Figure 3-11. Estimated molecular orientation for the inclusion complex of *p*-NP^- with **1**, when viewed from the secondary hydroxy side. The broad or narrow dashed lines represent the strong or weak ROESY cross peaks, respectively.

3. 4 Summary

Thermodynamic parameters (ΔG, ΔH, and $T\Delta S$) for the complexation of mono(3-deoxy-3-guanidino-*altro*)-α-cyclodextrin (**1**) or mono(3-deoxy-3-guanidino)-α-CD (**2**) with *p*-nitrophenolate ion were determined by ^{1}H NMR titration experiments in D_2O solution containing 0.10 mol dm^3 Na_2CO_3-$NaHCO_3$ at 298, 308, 318, and 328 K, to investigate the effect of the macroring flexibility of **1** on the complexation with the planar guest. Both and values for the inclusion complexation of **1** with the guest (-39.6 kJ mol^{-1} and -23.3 kJ mol^{-1}, respectively) decreased more than those for **2** with the guest (-27.6 kJ mol^{-1} and -10.0 kJ mol^{-1}, respectively). This fact suggested that the degree of freedom of the guest and the flexibility of the macroring of **1** were decreased by the tight binding of the guest in the manner of induced fit. The existence of anisotropic ring current effect by the guest on the ^{1}H NMR signals for C(3)- and C(5)-H's of **1** confirmed that the molecular rotation of the guest is retarded in the complex of **1** with *p*-nitrophenolate ion. The molecular orientation of the guest in the inclusion complex with **1** was determined by means of ROESY (rotating-frame overhauser effect spectroscopy) experiment.

Chapter 4

Conclusion

In this study, three types of guanidino-appended α-CD were synthesized and their inclusion complexation properties were investigated by using UV-Vis and 1D and 2D ^{1}H and 1D ^{13}C NMR techniques.

In chapter 2, three structural isomers of monoguanidino-modified α-cyclodextrin (CD), i.e., mono(6-deoxy-6-guanidino)-α-CD, mono(3-deoxy-3-guanidino)-α-CD, and mono(3-deoxy-3-guanidino-*altro*)-α-CD, were synthesized to study equilibria for the formation of inclusion complexes with the *p*-NP^-. The K_a's for the equilibria, determined by UV-Vis titration at 298 K and pH 10.6, showed that mono(6-deoxy-6-guanidino)-α-CD with a positively charged guanidino group on the primary hydroxy side of α-CD binds the negatively charged *p*-NP^- more strongly than the native α-CD. On the contrary, other hosts, with the same functional group on the secondary hydroxy side, binds *p*-NP^- less strongly than the native α-CD. The K_a values for all the hosts decreased, while that for the native α-CD remained virtually constant, with increasing ionic strength of the solutions. The two-dimensional ^{1}H NMR spectra of rotating frame nuclear overhauser effect spectroscopy showed that *p*-NP^- included in the cavity of all three hosts, or α-CD directs the nitro group toward the primary hydroxy side of the host. These results were explained on the basis of electrostatic interaction, including charge-charge, charge-dipole, and dipole-dipole interaction, between the hosts and the guest.

In chapter 3, thermodynamic parameters for the complexation of mono(3-deoxy-3-guanidino-*altro*)-α-CD or mono(3-deoxy-3-guanidino)-α-CD with *p*-NP^- were determined by ^{1}H NMR titration experiments in D_2O solution containing

0.10 mol dm^3 Na_2CO_3-$NaHCO_3$ at 298, 308, 318, and 328 K, to investigate the effect of the macroring flexibility of the host with the deformed macroring on the complexation with the planar guest. Both and values for the inclusion complexation of the host having the deformed macroring with the guest (-39.6 kJ mol^{-1} and -23.3 kJ mol^{-1}, respectively) decreased more than those for the counterpart without the deformation of macroring (-27.6 kJ mol^{-1} and -10.0 kJ mol^{-1}, respectively). This fact suggested that the degree of freedom of the guest and the flexibility of the macroring of the host with macroring deformation were decreased by the tight binding of the guest in the manner of induced fit. The existence of anisotropic ring current effect by the guest on the ^1H NMR signals for C(3)- and C(5)-H's of the host confirmed that the molecular rotation of the guest is retarded in the complex of the host with *p*-NP^-. The molecular orientation of the guest in the inclusion complex with the host was determined by means of ROESY (rotating-frame overhauser effect spectroscopy) experiment.

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Thermodynamic Study on the Inclusion Equilibria of Guanidino modified Cyclodextrins with *p*-Nitrophenolate Ion

Abstract

Cyclodextrin (CD) is a cyclic oligomer composed of some α-D-glucopyranose units linked 1→4 as amylose. CDs are soluble to water, but the interior cavities of CDs are hydrophobic. The most important characteristic of CD is that this molecule forms inclusion complexes with specific guest molecules. Since native CDs have only hydroxy group as functional group, the ability of molecular recognition is limited. Chemical modifications or substitutions of some or all of the hydroxy groups would give CDs enhanced molecular recognition abilities.

Introduction of charged groups into CDs are of great interest, as the introductions give CDs new recognition sites. Guanidino group, which is known as a side chain of arginine, has a stable positive charge over a wide pH range. Some CD derivatives having guanidino groups at the primary hydroxy side are synthesized and their inclusion properties are reported. However, not much is reported on the substitution of guanidino group for the secondary hydroxy groups, or on the detailed investigation on the mechanism for the inclusion complexations of the derivatives having deformed cavities with guests.

In this study, three types of α-CD derivatives are synthesized to investigate the effect of the position of the substituents on the binding property of a negatively

charged guest molecule (*p*-nitrophenolate ion, *p*-NP^-) by means of UV-Vis spectroscopy. Structural and thermodynamic investigation on the inclusion complexation of one of the three hosts was also conducted to examine the effect of the macroring deformation on the molecular motion of the guest by means of ^1H NMR spectroscopy.

This thesis consists of the following two parts;

1. Inclusion complexation of three structural Isomers of mono(deoxyguanidino)-α-cyclodextrin with the *p*-Nitrophenolate Ion

The K_a values for the complexation of the three hosts with *p*-NP^- were determined by UV-Vis titration experiments in buffered solutions with different ionic strength to investigate the effect of electrostatic interaction on the inclusion complexation. The introduction of the guanidino group into the primary hydroxy side of α-CD reinforces the complexation with *p*-NP^-, whereas those on the secondary hydroxy side weaken. The cross peaks in ROESY spectra for three types of guanidino-modified CDs with *p*-NP^-, suggested that *p*-NP^- is included into the cavity of each host to direct the nitro group toward the primary hydroxy side of the host. The K_a values for the complexation of guanidino-modified CDs with *p*-NP^- decreased with increasing ionic strength of the buffer solutions to indicate that electrostatic interaction stabilizes the inclusion complex. The difference in the K_a values for the complexation of guanidino-modified CDs with *p*-NP^- were rationally explained in terms of the reinforced and the weakened dipole moments of the macroring of the hosts by the introduction of the positively charged

guanidino group into either the primary or the secondary hydroxy side of the native α-CD, respectively.

2. Thermodynamic and structural studies on the complexation of guanidino appended α-cyclodextrin derivatives with *p*-nitrophenolate ion

Thermodynamic parameters for the complexation of mono(3-deoxy-3-guanidino-*altro*)-α-CD or mono(3-deoxy-3-guanidino)-α-CD with *p*-NP^- were determined by ^1H NMR titration experiments to investigate the effect of the macroring flexibility of the host with the deformed macroring on the complexation with the planar guest. Both enthalpy and entropy changes for the inclusion complexation of the host having the deformed macroring with the guest decreased more than those for the counterpart without the deformation of macroring. This fact suggested that the degree of freedom of the guest and the flexibility of the macroring of the host with macroring deformation were decreased by the tight binding of the guest in the manner of induced fit.

1D and 2D NMR spectra for complexation of guanidine-appended CD having deformed macroring with *p*-NP^- indicate that *p*-NP^- is bound in the macroring of that CD having a certain orientation. This observation well explains the results of thermodynamic experiments, where the tight binding of the guest and the restriction of molecular motion of the complex were suggested from large negative values for both enthalpy and entropy changes.

As mentioned above, K_a values for inclusion complexation of positively charged guanidino-appended CD with negatively charged *p*-NP^- suggest that the introduction of a positively charged substituents to primary hydroxy side of CD strengthens the dipole moment of the CD and works favorably for the complexation with negatively charged guests. Further investigation on the inclusion equilibria for the complexation of guanidino-appended CD having deformed macroring with *p*-NP^- reveal that the molecular rotation of the *p*-NP^- is retarded in the deformed macroring.

A Thermodynamic Study on the Inclusion Equilibria of Guanidino modified Cyclodextrins with *p*-Nitrophenolate Ion

（グアニジノ基修飾シクロデキストリン誘導体と *p*-ニトロフェノレートイオンの包接平衡に関する熱力学的研究）

摘要

シクロデキストリン(CD) は，α-D-グルコピラノースが環状にα-1，4-グリコシド結合したオリゴ糖であり，円筒形の形状をしている。CD の空洞内部は疎水性を示し，空洞に適合する様々な分子やイオンを包接する特性を持つ。未修飾CD は，活性な官能基として水酸基のみを有するために中性の分子であり，その包接能には限界がある。そこで， CD の一級水酸基もしくは二級水酸基を様々な官能基で置換することにより，包接能を向上させる研究が多く報告されている。

電荷を有する官能基のCD への導入は，それと反対の電荷を持つゲスト分子との結合力が大幅に増加することから，しばしば包接能の大幅な向上を可能にする。アルギニンの側鎖であるグアニジノ基は，幅広いpH において正電荷を帯びる官能基であり，CD の一級水酸基をグアニジノ基で置換した誘導体が，これまでに報告されている。しかしながら，負電荷を帯びたゲスト分子との包接錯体形成に関する熱力学的研究や，CD の二級水酸基を同基で置換した誘導体の包接平衡に関する研究は，極めて少ない。

本研究は，グアニジノ基を有する３種のCD 誘導体を合成し，負電荷を持つ*p*-ニトロフェノレートイオン(*p*-NP^-) との包接平衡に及ぼす修飾位置の影響を，紫外可視分光

法により調べた。また，それらCD 誘導体の中で歪んだマクロ環を有する誘導体と*p*-NP$^-$との包接錯体に関して，包接平衡の熱力学的パラメータと，一次元及び二次元核磁気共鳴法(NMR 法) による研究結果から，包接錯体の構造を明らかにした。本論文は以下の２部の研究からなる。

第２章においては，グアニジノ基修飾CD による*p*-NP$^-$の包接において，置換基の修飾位置が及ぼすゲスト包接能への影響を調べるために，α-CD の一級もしくは二級水酸基の一つをグアニジノ基で置換したグアニジノ修飾α-CD 誘導体を３種類合成し，それらと負電荷を有する*p*-NP$^-$との結合定数(K_a) を様々なイオン強度の溶媒中で紫外可視分光法により測定した。その結果，グアニジノ基を一級水酸基側に持つ誘導体が最も強く*p*-NP$^-$を包接する事が分かった。一方で，グアニジノ基を二級水酸基側に持つ２種類の誘導体と*p*-NP$^-$との相互作用は，未修飾α-CD のそれよりも弱かった。３種類のCD 誘導体と*p*-NP$^-$とのK_a は，測定に用いた溶媒のイオン強度の低下とともに増加した。このことから，全ての誘導体において，包接錯体の安定化に静電的相互作用が強く寄与していることが示された。グアニジノ修飾CD 類と*p*-NP$^-$との2D ROESY スペクトル測定の結果から，３種類の誘導体すべてにおいて，*p*-NP$^-$はニトロ基をCD の一級水酸基側に向けて包接されていることが示唆された。

以上の結果から，一級水酸基側にグアニジノ基を持つ誘導体は，α-CD が本来持つ双極子モーメントをグアニジノ基により強化することで，負電荷を持つゲストをより強く包接し，二級側を修飾した２種の誘導体は，双極子モーメントを弱める位置に置換基が修飾されていることから， *p*-NP$^-$との相互作用が弱まったと考えられた。

第３章では，平面状のゲスト分子の包接に与えるCD マクロ環の歪みの影響を調べるために，α-CDを構成する６個のグルコースのうちの１個が，アルトロース型に変化した誘導体と*p*-NP$^-$ との包接平衡の熱力学的パラメータをNMR 法により求め, マクロ環に歪みのない対照化合物のそれと比較した。その結果，歪んだマクロ環を持つ誘導体の包接平衡のエントロピー変化，エンタルピー変化は，対照化合物のそれと比較して有意に減少することが分かった。この結果により，歪んだマクロ環の中で*p*-NP$^-$ の分子運動の自由度が減少することが示唆された。

1D 及び2D NMR 法を用いて包接錯体の構造を調べた結果, 歪んだマクロ環に包接された*p*-NP$^-$ は, ゲストに対して特定の配置で包接されていることが明らかになった。このことは，包接されたゲストの分子回転が空洞内で制限されていることを示しており，熱力学的パラメータの情報から得られた結果と一致した。

上記のように，本研究において，シクロデキストリンの水酸基の一つをグアニジノ基で置換した３種類の誘導体を合成し，それらと負電荷を持つ*p*-NP$^-$ との包接平衡のK_a を調べることで，CD の本来持つ双極子モーメントを強化する位置への荷電官能基の導入が，対電荷を持つゲストの包接に重要であることが明らかになった。また，歪んだマクロ環を持つCD 誘導体と*p*-NP$^-$ との包接平衡の研究から，歪んだマクロ環に包接された*p*-NP$^-$ の分子回転が抑制されることが明らかになった。

List of Publications

1) Keita Takezawa, Yoshihisa Matsui, Tatsuyuki Yamamoto, and Keisuke Yoshikiyo

Inclusion Complexation of Three Structural Isomers of Mono(deoxyguanidino)-α-cyclodextrin with the *p*-Nitrophenolate Ion

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- Chapter 2 -

2) Keita Takezawa, Tomoki Hirai, Tatsuyuki Yamamoto, and Keisuke Yoshikiyo

Thermodynamic and structural studies on the complexation of guanidino appended α-cyclodextrin derivatives with *p*-nitrophenolate ion

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- Chapter 3 -