The United Graduate School of Agricultural Sciences,

Tottori University

NMR Spectroscopy on Inclusion Equilibria ; Forming of 2:1 (host : guest) Cyclodextrin Inclusion Complexes

(NMR 分光法による包接平衡の研究；

2:1(ホスト:ゲスト)型シクロデキストリン包接錯体の形成)

THESIS BY

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As Partial Fulfillment of the Requirement

for the Award of the Degree of Doctor of Phirosophy

At

Department of Bioresources Science

The United Graduate School of Agricultural Sciences,

Tottori University

September　2014

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2014

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Chapter Ⅰ

General Introduction

Ⅰ - i　Cyclodextrin

Cyclodextrins (CDs, hosts) are cyclic oligomer consisting of some α-D-glucopyranose units linked by α-1,4-glycosidic bonds. Typical CDs, α-, β-, and γ-CD have six, seven, and eight units, respectively. The shape of CDs is hollow truncated cone, although the cavity size is different each other. Solubility of CDs is relatively high, because of that they have a number of hydroxy groups on their narrower (primary rim) and wider (secondary rim) edges. On the other hand, the cavity is hydrophobic in nature due to the presence of apolar hydrogens (H3 and H5), together with the glucoside oxygens. Figure 1-1 and Table 1-1 show the shapes and some physicochemical properties of CDs [1, 2].

The most important characteristic of CDs is the complexation with a variety of guest molecules or ions by inclusion them in the cavity (Figure 1-2) [3-6]. The stabilities of such complexes are affected with the size, hydrophobicity, and structure of the guests. When the cavity size of CDs well matches with a guest, CDs efficiently include the guest, forming a stable complex. Furthermore, the rate of an organic reaction and stereochemistry are significantly changed by the inclusion complexation of CD with a guest [7]. These modes of action are very similar to those of enzymes and biological receptors. Now, the abilities of CDs, molecular cupsulation and stereochemical recognition, have been utilized and investigated in various field, e.g., food science, chemistry, pharmacy, and so on [8-11].

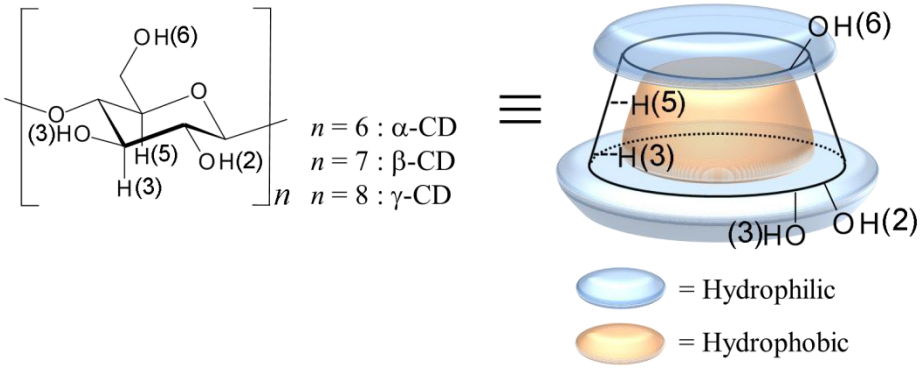

Figure 1-1. The structures of CDs. The number in parenthesises represent the carbon number attached to the atoms or functional groups.

Table 1-1. Physicochemical properties of CDs.

	α-CD	β-CD	γ-CD
Molecular weight / g mol^{-1}	972	1135	1297
Aqueous solubility (at 298 K) / % (w/v)	14.5	1.85	23.2
Cavity diameter / nm	0.47~0.53	0.60~0.65	0.75~0.83
Outer periphery diameter / nm	1.46	1.56	1.75
Height of torus / nm	0.79	0.79	0.79

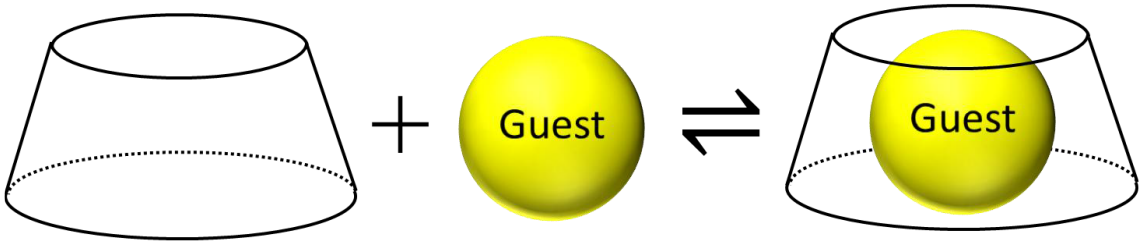

Figure 1-2. The inclusion equilibrium of CD and guest with 1:1 stoichiometry.

Ⅰ-ⅱ Binding forces contributing to formation of inclusion complex

The stability of a CD complex is usually designated by binding constant (K_a), which corresponds to the equilibrium constant for the complexation of CD with a guest. A large value of K_a indicates strong interaction between a host and a guest. The intensity of K_a value depends on the molecular size, structure, and physicochemical properties of a guest, so that CD has the ability of molecular recognition. Other molecules, such as crown ether, cryptand, cyclophan, calixarene and cucurbituril, also have this ability [12]. Because CD is neutral molecule, the driving forces for the formation of CD inclusion complexes in aqueous solutions are mainly following interactions, 1) Hydrophobic interactions, 2) van der Waals interactions, and 3) Hydrogen bonding.

1. Hydrophobic interactions

Complexation reaction of CD usually occurs in aqueous solutions. When a hydrophobic guest is dissolved in water, the network of hydrogen bonding of each H_2O around the guest is enhanced to form 'iceberg structure'. When the hydrophobic guest is included by the hydrophobic CD cavity, the iceberg structure disrupted and the trapped H_2O released to a bulk solution. The net entropy of H_2O greatly increases through this process. In other ward, the hydrophobic interaction between CD and guest is involved in increasing of the degree of freedom of H2O, as a result, entropy change is favorable positive ($\Delta S^\circ > 0$), and enthalpy change is slightly positive. ($\Delta H^\circ \geqq 0$) [13-15].

2. van der Waals interactions

This interaction involves two different terms. One is interaction arising from the polarity of molecules such as dipole-dipole and dipole induced dipole interaction. Another is the London dispersion force that works between apolar molecules. The former interactions are very weak in water with a large permittivity, because the strength of such interactions is inversely proportional to the permittivity of solvent. However, in CD cavity, the interactions become fairly strong, since the CD cavity has significantly small permittivity. The latter works between molecules locating at very short distance to be in contact with each other. It becomes very large when the steric structure of the guest molecule well fits to the CD cavity. The van der Waals interaction are accompanied by changes in thermodynamic parameters such as $\Delta H^\circ < 0$ and $\Delta S^\circ < 0$, which are opposite to the hydrophobic interactions. When the contribution of van der Waals interactions for complexation is smaller than the contribution of hydrophobic interactions, the thermodynamic changes accompanied by complexation are $\Delta H^\circ \geqq 0$ and $\Delta S^\circ > 0$. In contrast, when the van der Waals interactions are stronger than the hydrophobic interactions, thermodynamics changes are $\Delta H^\circ < 0$ and $\Delta S^\circ < 0$. The importance of these forces in the CD complexation has been emphasized through thermodynamic and theoretical investigations [16-20].

3. Hydrogen bonding

This is also one of the primary binding forces for CD complexation, because CD molecule has many primary and secondary hydroxy groups that can

form hydrogen bonding with guest molecules [21]. Sometimes, hydrogen bonding is formed between the hydroxyl groups of two CDs, when they face each other in liner with head-to-head (facing of primary rims) or tail-to-tail (facing of secondary rims) manner [22]. Several X-ray crystallographic investigations have suggested the interactions [23-27].

The interactions described above act simultaneously and cooperatively, on the complexation of normal CDs and their derivatives with guests. Some CD derivatives, having positive or negative charge on their substituted group, e.g., amino, carboxyl, guanidino group, have an extra force, electrostatic interaction, to stabilize the inclusion complex with the guest which has contrary charge [28]. Thus, CD is often substituted to improve their inclusion abilities, solubility, function of molecular recognition, and attach new function such as penetration of cell membrane, targeting on particular cell to drug delivery, and so on [29-33]. In this thesis, 'β-CD derivative with high-solubility' and 'α-CD derivative whose some hydroxyl group in replaced to methoxy group' are used.

Ⅰ-ⅲ NMR Techniques for the studies of cyclodextrin inclusion complexes

As mentioned in previous section, K_a is an important indicator to demonstrate that how strong the complexation of CD with the guest is. To determine the values, titration methods by calorimetry, UV-Vis spectroscopy, fluorescence spectroscopy, and NMR spectroscopy, can be used [29, 34, 35]. In particular, NMR spectroscopy has some advantage that be discussed in this section [36, 37]

Ⅰ-ⅲ-ⅰ One dimensional NMR

Generally, ^{1}H NMR spectra give the information of chemical shifts (δ's), spin-spin coupling constant and peak area. A class of proton, the environment of a proton and the number of protons in a molecule are estimated from the information, respectively. ^{13}C NMR spectra are also useful to understand the molecular structure. ^{13}C NMR has the advantage that the obtained signals are sharper and clearer than that of ^{1}H NMR, although it needs long time to obtain the spectrum due to rare existence of ^{13}C.

Ⅰ-ⅲ-ⅱ Two dimensional NMR (ROESY)

The information of the two-dimensional rotating-frame nuclear Overhauser effect spectroscopy (ROESY) spectra is the ^{1}H nuclei spatially in the vicinity of each other. It is useful to estimate the molecular orientation of inclusion complex of a CD with a guest. CDs have two ^{1}Hs in their cavity (H3 and H5) as mentioned in section I - i. If the cross peaks between the ^{1}Hs of CD

and that of guest are observed, it indicates that the guest (or part of the guest) locates in the CD cavity [38, 39].

Ⅰ-ⅲ-ⅲ Determination of K_a

The δ's of ^{1}Hs and ^{13}Cs in both a free CD and a free guest are changed in general with the formation of inclusion complex. Individual K_a values from the δ's change (Δδ's) for all the observed signals can be determined by analyzing relationship between Δδ and concentration of host or guest [37].

Notice that there are two ways for NMR titration. One is that the concentration of CD is low and constant, where that of guest is excess. Another is that the concentration of guest is low and constant, where that of CD is excess (Figure 1-4). In the former method, it can be supposed that only 1:1 inclusion complex is formed. However, the latter method has the possibility that two or more CDs interact with one guest molecule. To obtain correct K_a, correct assumption must be applied to the system.

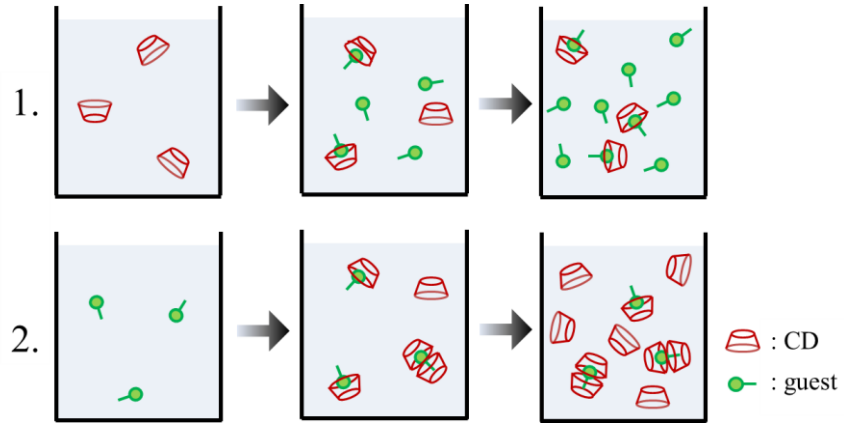

Figure 1-4. Difference of stoichiometry between two titration methods.
1. [host]≪[guest], leading the formation of only 1:1 inclusion complex
2. [host]≫[guest], having possibility of the formation of 2:1 inclusion complex

Ⅰ-ⅲ-ⅳ The relation of Δδ and K_a ; for 1:1 and 2:1 inclusion complex

If only 1:1 inclusion complex is formeed in the system, the equilibrium is;

CD + G ⇌ CDG

where, the concentration of CD is as c, of G (guest) is as g, and of CDG (1:1 inclusion complex) is as x. The initial concentration of CD is as c_0, of G is as g_0. K_a is expressed as;

$$K_a = \frac{x}{cg} = \frac{x}{(c_0-x)(g_0-x)} \qquad (1)$$

On the other hand,

$$\delta = \frac{c}{c_0}\delta_0 + \frac{x}{c_0}\delta_1$$

$$\Delta\delta = \delta - \delta_0 = \frac{c}{c_0}\delta_0 + \frac{x}{c_0}\delta_1 - \delta_0 = \frac{x}{c_0}\Delta\delta_\infty \qquad (\Delta\delta_\infty = \delta_1-\delta_0) \quad (2)$$

where, δ_0 is the chemical sift of free CD, δ_1 is the chemical sift of CD forming the complex with guest.

Now, the relation between K_a and Δδ, equation 3, can be led from equation 1 and 2;

$$\Delta\delta = \frac{\Delta\delta_\infty}{2c_0}\left[c_0 + g_0 + \frac{1}{K_a} - \left\{\left(c_0 + g_0 + \frac{1}{K_a}\right)^2 - 4c_0g_0\right\}^{1/2}\right] \quad (3)$$

The NMR titration curve is analyzed by the curve-fitting method with equation 3 to obtain K_a for the formation 1:1 inclusion complex.

In contrast, If 1:1 inclusion complex is formed, followed by that of 2:1 in the system, the two step equilibria are;

CD + G ⇌ CDG

$CD + CDG \rightleftharpoons CD_2G$

where, the concentration of CD is as c, of G (guest) is as g, of CDG is as x, and of CD_2G is as y. The initial concentration of CD is as c_0, of G is as g_0. Notice that, now,

$c_0 = c + x + 2y$

$g_0 = g + x + y$

and,

$K_1 = \dfrac{x}{cg}$

$K_2 = \dfrac{y}{cx}$

then, the equation 4 can be led;

$K_1K_2c^3 + (K_1 + 2K_1K_2s_0 - K_1K_2c_0)c^2 + (1 + K_1s_0 - K_1c_0)c - c_0 = 0$ (4)

On the other hand,

$\delta = \dfrac{g}{g_0}\delta_0 + \dfrac{x}{g_0}\delta_1 + \dfrac{y}{g_0}\delta_2$

where, δ_0 is the chemical sift of free guest, δ_1 is the chemical sift of guest forming the 1:1 complex with CD, δ_2 is the chemical sift of guest forming the 2:1 complex with two CDs. Then;

$\Delta\delta = \delta - \delta_0 = \Delta\delta_1 \dfrac{x}{g_0} + \Delta\delta_2 \dfrac{y}{g_0}$ ($\Delta\delta_1 = \delta_1 - \delta_0$, $\Delta\delta_2 = \delta_2 - \delta_0$) (5)

By applying the appropriate values to K_1, K_2, and $\Delta\delta1$ $\Delta\delta2$ as initial values, the computer analysis to obtain K_1, K_2 is completed. Namely, this analysis for 2:1 inclusion complex is four parameter fits, and more complicated than that for 1:1, two parameter fits. However, the K_1 value is already known from the titration under the [host]≪[guest] condition as mentioned previously section. Then the

K_1 on equation 5 can be regarded as constant value. Finally, the four parameter fits can be treated as three parameter fits that is simpler.

As mentioned above, NMR spectroscopy gives abundant information about CDs and CD complexes in aqueous solution. In this thesis, the presence of some CD complexes with the stoichiometry, 2:1, are revealed by using this technique.

Ⅰ-ⅳ Objective of this thesis

Many studies about K_a for formation of variety CD complexes have been reported. The knowledge of K_a, stoichiometry and molecular orientation of CD complexes is important to understand the mechanisms of the inclusion phenomena and develop new structure, such as polimer, rotaxane, and micelle, taking advantage of inclusion reaction [40, 41]. Determination of the properties needs close attention because that they are sensitive to some conditions, pH, solvent, temperature, etc. Especially, the stoichiometry is affected by the molecular ratio of host versus guest (for example, as mentioned in figure 1-4). However, in some reports, only 1:1 inclusion complex are discussed with the experiment under the [host]≪[guest] condition. Then, it was proposed that certain K_a, stoichiometry and molecular orientation of CD complexes should be obtained from two experimental conditions, [host]≪[guest] and [host]≫[guest]. In the studies of this thesis, it was aimed to investigate a few interesting inclusion phenomena involved in formation of 2:1 (host : guest) inclusion complex of CD by means of NMR spectroscopy. This thesis consists of two parts ;

1) **A ^{1}H NMR Titration Study on the Binding Constants for D- and L-Tryptophan Inclusion Complexes with 6-*O*-α-D-Glucosyl-β-cyclodextrin. Formation of 1:1 and 2:1 (Host : Guest) Complexes**

2) **Formation of 1:1 and 2:1 host-guest inclusion complexes of α-cyclodextrin with cycloalkanols: A ^{1}H and ^{13}C NMR spectroscopic study**

In Chapter Ⅱ, the K_a for formation of the complex of 6-*O*-α-D-Glucosyl-β-cyclodextrin (G1-β-CD) with D- or L-tryptophan is evaluated by ^{1}H NMR titration under both of the conditions, [host]≪[guest] and [host]≫[guest]. Normally, in the β-CD system, it is hard to make the condition, [host]≫[guest], because of poor solubility of β-CD. The G1-β-CD can make it easy, due to that it has high-solubility although its ability of complexation with any guest is almost equal to normal β-CD. Then, the effect of formation of 2:1 inclusion complex to chiral recognition is discussed.

In Chapter Ⅲ, also the focus of discussion is 2:1 inclusion complex. In the system of α-CD and cycloalkanols, the white precipitate is observed by addition of excess α-CD to some larger cycloalkanols, cyclohexanol, cycloheptanol, and cyclooctanol. But it is not in the case of smaller cycloalkanols, cyclobutanol and cyclopentanol. The formation of precipitate suggests the decrease of solubility. It is expected that it's due to the formation of hydrogen bonding between two α-CD molecules. To establish the presence of the force, the four α-CD derivatives are used.

Chapter Ⅱ

A ^{1}H NMR Titration Study on the Binding Constants for D- and L-Tryptophan Inclusion Complexes with 6-*O*-α-D-Glucosyl-β-cyclodextrin. Formation of 1:1 and 2:1 (Host : Guest) Complexes

Ⅱ-ⅰ Introduction

The addition of cyclodextrins (CDs) to aqueous protein solutions causes various changes in the properties of proteins [42, 43]. For example, we reported that thermal stabilities of proteins are lowered by the addition of CDs to aqueous protein solutions [44-46]. The refolding reactions of thermally denatured proteins are partially hindered by CDs [45, 46]. We also found that the H-D exchange rate for the peptide bonds of lysozyme is accelerated by CDs in an aqueous solution [47]. These peculiar effects of CDs on the conformation of proteins are concerned with the inclusion of the side chains of amino acid residues by CDs. Among CDs, 6-*O*-α-D-glucosyl-β-cyclodextrin (G1-β-CD) caused larger changes than the other CDs such as α- and γ-CD. G1-β-CD was used in the place of parent β-CD, since the former is more water-soluble than the latter. The fact that G1-β-CD gives the most effective influence on the conformational changes of proteins suggests that the size of hydrophobic cavity of G1-β-CD fits the side chains of aromatic amino acids, such as tryptophan, phenylalanine, and tyrosine [48]. Inclusion of the indole moiety of tryptophan by G1-β-CD was confirmed by our ^{1}H NMR study on the system of lysozyme [49]. However, it was unknown how strong G1-β-CD interacts with tryptophan, though interactions between parent β-CD and tryptophan have been evaluated by means of potentiometric and spectrophotometric techniques at various pH's [50-54]. In the present study, we tried to determine the binding constants for complexation of G1-β-CD with D- and L-tryptophan in D_2O at 298 K by means of ^{1}H NMR titration technique. The method is favorable for the accurate

determination of binding constants of CD inclusion complexes, since many NMR signals used for numerical analysis are simultaneously available [55]. To begin with the investigation, the deuterium ion exponent (pD) of the D_2O solution was adjusted to be 11.0, at which it is anticipated that the binding constants are significantly larger and more reliable than those at neutral or acidic pD [50-54]. Furthermore, the spatial arrangement of the inclusion complexes was examined by two-dimensional NMR measurement (Rotating-frame nuclear Overhauser Effect SpectroscopY, ROESY) to support the NMR titration measurements.

Ⅱ-ⅱ Experimental

Ⅱ-ⅱ-ⅰ Sample preparation

The G1-β-CD was supplied from Ensuiko Sugar Refining Co., Ltd., and was used after purification by Sephadex and ODS column chromatography followed by cleaning up with activated charcoal. The D- and L-tryptophan were purchased from Peptide Institute Inc. and Wako chemical Co., respectively, and were used without further purification. All other chemical reagents used for this study were special grade. A 0.05 mol dm^{-3} Na_2HPO_3 solution in D_2O and a 0.1 mol dm^{-3} NaOH solution in D_2O were mixed by 50:2 (v:v) ratio to prepare a buffer solution of pD 11.0. The pD value was obtained by the direct read of pH by a HORIBA portable pH meter and added by 0.4. For the ^1H NMR titration of tryptophan with G1-β-CD, we prepared eleven solutions of tryptophan (5.9 mmol dm^{-3} for D-isomer and 5.4 mmol dm^{-3} for L-isomer) with different

concentrations from 0 to 61 mmol dm^{-3} of G1-β-CD in a D_2O solution at pD 11.0. For the ^1H NMR titration of G1-β-CD with tryptophan, we prepared eleven solutions of G1-β-CD (2.0 mmol dm^{-3}) with different concentrations from 0 to 25 mmol dm^{-3} of D- or L-tryptophan in a D_2O solution at pD 11.0. These solutions were admitted into NMR sample tubes with 5.0 mm diameter and vibrated for a minute by use of a test tube mixer (Shibata Scientific Technology LTD., TTM-1).

Ⅱ-ⅱ-ⅱ ^1H NMR spectral measurements

The one-dimensional (1D) ^1H NMR and two-dimensional (2D) ROESY spectral measurements were performed with a 400 MHz JEOL JNM-A400 FT-NMR spectrophotometer at 298 K. The 1D spectra were obtained with 8 scans. The 2D ROESY spectra were acquired with the pulse program of "roesy" attached to the spectrophotometer, and mixing time was set to be 250 ms, relaxation delay, 0.9359 s, number of scans, 1024, spectral width, 8,000 Hz, and numbers of points, 512 for t_2, and 256 points for t_1, followed by zero-filling. The chemical shifts were determined with the HDO (δ 4.650) signal as an internal reference. Chemical shifts (δ) for ^1H NMR signals of tryptophan in D_2O in the absence of G1-β-CD: $^1H(2)$, 7.169 (s); $^1H(4)$, 7.597 (d); $^1H(5)$, 7.139 (t); $^1H(6)$, 7.055 (t); $^1H(7)$, 7.396 (d); ^1H(*gauche*), 3.323 (q); ^1H(*trans*), 3.128 (q) at pD 11.0. Chemical shifts (δ) for ^1H NMR signal of the $^1H(3)$ of G1-β-CD in D_2O in the absence of tryptophan: 3.840 (t) at pD 11.0.

Ⅱ-ⅲ Results and Discussion

Eleven solutions of D- or L-tryptophan with different concentrations of G1-β-CD were prepared in a buffer D_2O solution at pD 11.0, as described in Experimental. The ^1H NMR spectra of the samples showed that the signals of ^1H bound to α-carbon of tryptophan overlap with the signals of G1-β-CD ^1H's, and changes (Δδ) in chemical shift (δ) of the α-carbon ^1H with increasing concentration of G1-β-CD could not be followed. However, signals due to the other ^1H's of D-tryptophan, including *gauche*- and *trans*-^1H's bound to β-carbon and the indole ^1H's, were separated from the G1-β-CD signals. Figure 2-1 shows Δδ for D-tryptophan with elevating concentration (c_{host}) of G1-β-CD at pD 11.0 and 298 K (The numbering for ^1H's of tryptophan is depicted rihgt the figure). The relationships between Δδ's and c_{host} were analyzed by the

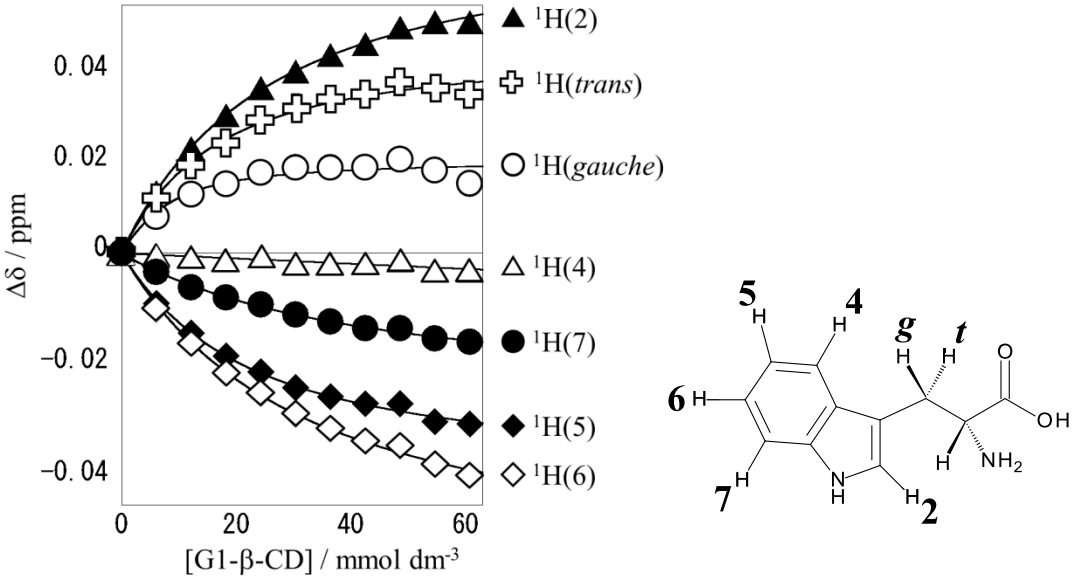

Figure 2-1. The plot of Δδ's for ^1H signals of D-tryptophan (5.9 mmol dm^{-3}) with increasing concentration of G1-β-CD at pD 11.0 and 298 K. The solid line curves show fitting curves on an assumption of simple 1:1 complexation.

curve-fitting method [37], upon an assumption of simple 1:1 complexation of G1-β-CD with D-tryptophan. The calculated curves (solid line) were fairly well-fitted to observed data, and individual binding constants (K_1), together with the difference ($\Delta\delta_c$) in δ between the fully complexed and free guest, are summarized in Table 2-1. The Δδ for the ^{1}H(4) was too small to determine K_1 accurately. Obviously, the K_1 value obtained from the ^{1}H(*gauche*) was much larger than those from the ^{1}H's of the indole moiety. The K_1 value for the ^{1}H(*trans*) was also significantly large. This discrepancy is mainly brought about by the fact that the Δδ's for the ^{1}H(*gauche*) and ^{1}H(*trans*) increased with the addition of G1-β-CD till c_{host} = 40 mmol dm^{-3} and then decreased in the region of $c_{host} \geq$ 50 mmol dm^{-3}. Repeated measurements for the system gave similar results. This fact indicates that the assumption of simple 1:1 complexation is wrong for this host-guest system. It is possible that not only 1:1 but also 2:1 (host : guest) complexation occur. The possibility of existing 2:1 equilibrium as well as 1:1 one for host and guest chemistry is pointed out, not only for cyclodextrin but also for other inclusion complexes, e.g. inclusion of

Table 2-1. The individual binding constants (K_1), the changes ($\Delta\delta_c$) in δ between the complexed and free guest, and correlation coefficsients (r) determined by the curve-fitting analysis of relationships between Δδ and c_{host} upon an assumption of simple 1:1 complexation of G1-β-CD with D-tryptophan in D_2O at pD 11.0 and 298 K

	^{1}H(2)	^{1}H(5)	^{1}H(6)	^{1}H(7)	^{1}H(*gauche*)	^{1}H*(trans)*
K_1/mol^{-1}dm^3	47	56	37	27	217	81
$\Delta\delta_c$	0.072	-0.040	-0.057	-0.026	0.021	0.046
r	0.9995	0.9983	0.9989	0.9978	0.9698	0.9955

aromatic compounds included by cucurbit[8]uril studied by U.V.-Vis. Spectroscopy [56].

In order to determine the K_1 value for the system directly, we examined an effect of the addition of D-tryptophan on the Δδ of the G1-β-CD ^{1}H's, where the concentration of G1-β-CD was so lower than that of D-tryptophan that the 2:1 complexation is negligible. The signals of the ^{1}H(3), ^{1}H(5), and ^{1}H(6) of G1-β-CD significantly shifted to the high-field direction with the addition of D-tryptophan (Figure 2-2). The ^{1}H(3) signals were well-defined, whereas those for the ^{1}H(5) and ^{1}H(6) were ill-defined, and we carried out the curve-fitting analysis of the relationship between Δδ for ^{1}H(3) and the concentration (c_{guest}) of

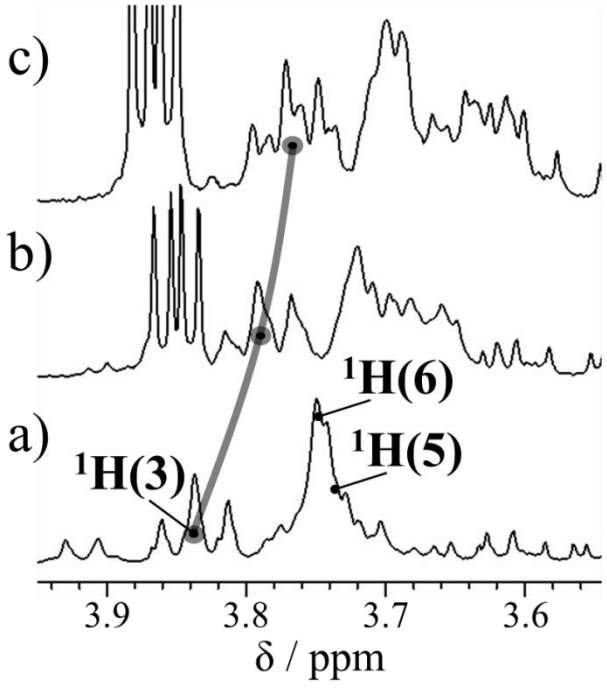

Figure 2-2. The chemical shift change of ^{1}H(3) signal of G1-β-CD (2.1 mmol dm^{-3}) with increasing concentration of D-tryptophan at pD 11.0 and 298 K. [D-tryptophan] = 0.0 (a), 15.9 (b), and 31.7 (c) mmol dm^{-3}.

D-tryptophan upon an assumption of simple 1:1 complexation to give the K_1 value of 59 $mol^{-1}dm^3$. This value is somewhat smaller than that (88 $mol^{-1}dm^3$) obtained for a parent β-CD-D-tryptophan system by Sebestyen *et al*. in an aqueous solution at pH 10.5 [51]. Then, using the K_1 value of 59 $mol^{-1}dm^3$, we analyzed the relationships between Δδ and c_{host} shown in Figure 1 on the assumption that 1:1 complexation is followed by 2:1 complexation by means of the method reported by Funasaki *et al.* [57] to obtain the binding constant (K_2) for 2:1 complexation. The K_2 values obtained from Δδ's for five ^{1}H's of ^{1}H(2), ^{1}H(5), ^{1}H(6), ^{1}H(*gauche*), and ^{1}H(*trans*) were fairly agree with one another, and the average value was 42 ± 3 $mol^{-1}dm^3$.

Very similar behavior was observed for a G1-β-CD-L-tryptophan system in D_2O at pD 11.0 and 298 K. Individual K_1 values, together with $\Delta\delta_c$ obtained on an assumption of simple 1:1 complexation, are summarized in Table 2-2. Again, the K_1 values for the ^{1}H(*gauche*) and ^{1}H(*trans*) were much larger than those obtained for the indole ^{1}H's, suggesting that 1:1 complexation is followed by 2:1 complexation. Then, we examined an effect of the addition of

Table 2-2 The individual binding constants (K_1), the changes ($\Delta\delta_c$) in δ between the complexed and free guest, and correlation coefficients (r) determined by the curve-fitting analysis of relationships between Δδ and c_{host} upon an assumption of simple 1:1 complexation of G1-β-CD with L-tryptophan in D_2O at pD 11.0 and 298 K

	^{1}H(2)	^{1}H(4)	^{1}H(5)	^{1}H(6)	^{1}H(7)	^{1}H(*gauche*)	^{1}H(*trans*)
K_1/$mol^{-1}dm^3$	46	7	59	35	25	192	706
$\Delta\delta_c$	0.081	-0.026	-0.038	-0.059	-0.024	0.027	0.014
r	0.9994	0.9780	0.9994	0.9993	0.9939	0.9874	0.8931

L-tryptophan on the Δδ for the ^{1}H(3) of G1-β-CD, where the concentration of G1-β-CD was lower than that of L-tryptophan. The curve-fitting analysis of the relationship between Δδ for the ^{1}H(3) of G1-β-CD and c_{guest} of L-tryptophan upon an assumption of simple 1:1 complexation gave the K_1 value to be 54 mol^{-1}dm^3. This value is somewhat smaller than that (85 mol^{-1}dm^3) obtained for a parent β-CD-L-tryptophan system in an aqueous solution at pH 10.5 [51]. Then, using the K_1 value of 54 mol^{-1}dm^3, we analyzed the relationships between Δδ and c_{host} on the assumption of 1:1 and 2:1 complexation to obtain the K_2 value. The K_2 values obtained from Δδ's for four ^{1}H's of ^{1}H(2), ^{1}H(5), ^{1}H(*gauche*), and ^{1}H(*trans*) were fairly agree with one another, and the average value was 12 ± 3 mol^{-1}dm^3. This value is significantly smaller than that (42 ± 3 mol^{-1}dm^3) for the G1-β-CD-D-tryptophan system, though the K_1 values for D- and L-tryptophan were virtually equal to each other. This result indicates that the first G1-β-CD molecule includes the indole moiety, which is distant from the chiral center of the guest, and the second G1-β-CD molecule includes the chiral center. The binding constants for D- and L-tryptophan are summarized as Table 2-3.

Table 2-3. The binding constants for 1:1 inclusion complex (K_1), and for 2:1 (host : guest) inclusion complex (K_2) of G1-β-CD with D- and L-Tryptophan, at pD 11.0, 298 K.

	D-tryptophan	L-tryptophan
K_1/mol^{-1}dm^3	59	54
K_2/mol^{-1}dm^3	42	12

Finally, we measured two-dimensional ROESY spectra of tryptophan with exceeded concentration of G1-β-CD. Figure 2-3 shows the ROESY spectra of D-tryptophan in the presence of an excess amount of G1-β-CD at pD 11.0. Cross-peaks were observed between the ^{1}H(5) signal of G1-β-CD and the ^{1}H(4), ^{1}H(5), ^{1}H(6), and ^{1}H(7) signals due to the indole ring of tryptophan and between the ^{1}H(3) signal of G1-β-CD and ^{1}H(2) and ^{1}H(4) signals due to the indole ring (Figure 2-3a). Cross-peaks were also observed between the ^{1}H(3) and ^{1}H(5) signals of G1-β-CD and the ^{1}H(*gauche*) and ^{1}H(*trans*) signals due to β-carbon of tryptophan. The ROESY spectra observed for L-isomer were essentially similar to those for D-counterpart. Based on these results, we postulated the structure of 2:1 complex of G1-β-CD with tryptophan as illustrated in Figure 2-3b. In this structure, the first G1-β-CD molecule deeply includes the indole moiety of tryptophan within the interior cavity from the secondary side of G1-β-CD, and the second G1-β-CD molecule includes the chiral center of tryptophan from the secondary side of G1-β-CD.

Ⅱ-ⅳ Conclusion

^{1}H NMR titration data were analyzed on an assumption that G1-β-CD includes D- and L- tryptophan to form 1:1 and 2:1 (host : guest) inclusion complexes at pD 11.0. The K_1 values calculated for the formation of 1:1 inclusion complex were almost the same for two stereoisomers. The K_2 values calculated for the formation of 2:1 inclusion complex for D-isomer was greater

than the L-counterpart. The difference in the magnitude of K_2 was explained by a postulate that the inclusion of the first G1-β-CD molecule occurs at a distant position from the chiral center of tryptophan, whereas inclusion of the second G1-β-CD molecule occurs in the vicinity of the chiral center. The results of ROESY measurement supported the postulate.

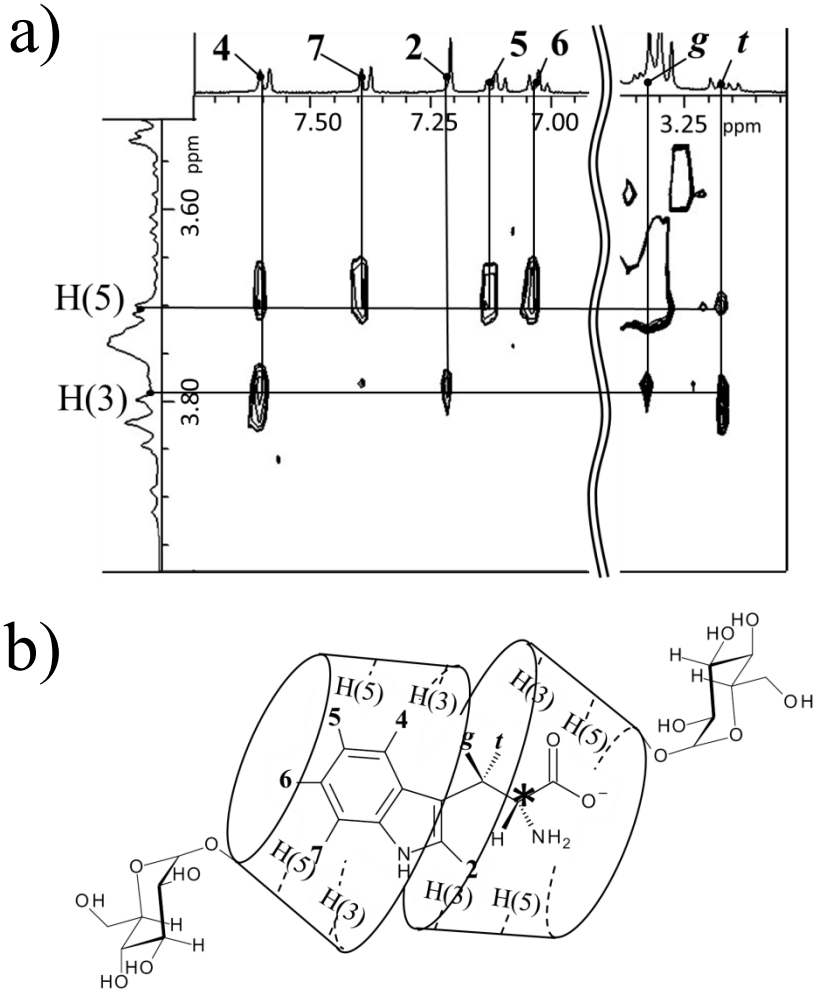

Figure 2-3. Two-dimensional ROESY spectra of D-tryptophan (5.9 mmol dm^{-3}) in the presence of G1-β-CD (30.4 mmol dm^{-3}) at pD 11.0 and 298 K (a), and the supposed molecular structure of 2:1 inclusion complex for D-tryptophan with G1-β-CD (b). The asterisk shows the chiral center of D-tryptophan. More information on the experimental conditions are shown in the chapter of experimental.

Chapter Ⅲ

Formation of 1:1 and 2:1 host-guest inclusion complexes of α-cyclodextrin with cycloalkanols: A ^{1}H and ^{13}C NMR spectroscopic study

Ⅲ-ⅰ Introduction

The stoichiometry of complexation of cyclodextrins (CDs) employed as hosts strongly depends on specific chemical properties, including the shape and hydrophobicity of the guest as well as the size of the internal cavity and/or the substituents of the host [57]. At concentrations of CDs significantly higher than those of the guests, and for guests larger than the cavities of CDs, the guest may be included by several CD molecules at multiple points. Our recent study on the formation of inclusion complexes with 6-*O*-α-D-glucopyranosyl-β-CD (G1-β-CD) and D- or L-tryptophan clearly showed that the binding constants (K_a's) for the 1:1 and 2:1 host-guest inclusion complexes could be obtained by NMR titration [58]. For a kinetic study on the inclusion systems of α- or G1-β-CD with dimethyl(nitrophenyl) phosphates or thiophosphates, the assumption that a 2:1 inclusion complex occurs was needed for the curve-fitting analysis of the system [59]. In systems in which a dimerized α-CD (which has two independent hydrophobic cavities per molecule) was used as a host, with an exceeding concentration of the guest, the dimer included two alkanol molecules at a time in different cavities, forming 1:2 inclusion complexes [60]. ^{13}C NMR data obtained for the formation of inclusion complexes of α-CD with 1-alkanols or 1-alkanoates, which are characterized by relatively long chains, suggested that the formation of 2:1 inclusion complexes occurred together with that of 1:1 inclusion complexes [61]. These results indicated that the existence of ternary complexes should be taken into consideration when investigating the interaction of a host with a relatively large guest.

We have recently demonstrated that some cycloalkanols, namely

cyclohexanol, cycloheptanol, and cyclooctanol, form precipitates in aqueous solutions with α-CD, but not with β- or γ-CD. Analysis of the precipitates revealed the stoichiometry of these complexes to be 2:1; that is, 2:1 inclusion complexes could possibly be formed. K_a's for the complexation of cycloalkanols with CDs determined by isothermal calorimetry [62, 63] and indirect visible-light absorbance titration were already reported [64]. In our opinion, however, the authors of these studies did not investigate in detail the possibility of the formation of 2:1 inclusion complexes. To the best of our knowledge, K_a's for the 2:1 complexation of α-CD with cycloalkanols have not yet been reported; this relevant point needs to be investigated with the use of appropriate techniques.

NMR spectroscopy is a powerful technique to determine K_a's of inclusion complexes of CD with various guests [65]. In a typical NMR titration experiment, the concentration of one of two components of the complex (either the host or the guest) is fixed, while the changes of the concentration of the other component are monitored. The chemical shift (δ) of an NMR signal of the component with the constant concentration increases gradually while the inclusion complex is formed by the addition of its counterpart. The chemical shift change ($\Delta\delta$) of the NMR signal plotted against the molar concentration of its counterpart produces the NMR titration plot. If the stoichiometry of the inclusion complex is known, then K_a's can be estimated from the most fitted curve of the NMR plot. Because of the number of atoms in the host-guest systems, plural NMR signals should be used. This approach allows an independent and simultaneous estimation of a number of K_a's from one single NMR titration experiment. The closer to one another the obtained K_a's are, the better the

curve-fitting analysis is for the assumed stoichiometry. If the existence of 2:1 inclusion complexes is assumed, the K_a value for 1:1 inclusion complexes should be determined at those conditions for which only 1:1 inclusion complexes are formed. This can be accomplished with an NMR titration experiment carried out at a low and constant concentration of the host, while the guest is in excess. Conversely, the K_a's for 2:1 inclusion complexes can be determined by performing NMR titration experiments at a low and constant concentration of the guest, while the host is in excesses. If the obtained K_a's are not similar to one another, participation of more than two host molecules to the formation of the complex has to be taken into consideration.

In the present study, we determined the K_a's of inclusion complexes of α-CD with cycloalkanols by means of ^{1}H and ^{13}C NMR titration experiments. In particular, the K_a's for the formation of 1:1 inclusion complexes were determined by ^{1}H NMR titration, as this approach is faster than that based on ^{13}C NMR; K_a's for 2:1 inclusion complexes were obtained with ^{13}C NMR titration, because ^{13}C NMR spectra can provide sharply separated signals for these systems, due to the presence of carbon atoms of cycloalkanols [61]. In addition, the molecular orientation of the two host molecules in 2:1 inclusion complexes was estimated by two-dimensional NMR measurements. Moreover, the importance of secondary hydroxy groups for the formation of 2:1 inclusion complexes is discussed based on the results of the titration experiments using derivatives of α-CD, such as *per*-6-*O*-methyl-α-CD, *per*-2-*O*-methyl-α-CD, *per*-3-*O*-methyl-α-CD, and *per*-2,6-di-*O*-methyl-α-CD.

Ⅲ-ⅱ Experimental

Ⅲ-ⅱ-ⅰ Sample preparation

α-CDs were obtained from Ensuiko Sugar Refining Co., Ltd., and were used after being cleaned with activated charcoal followed by freeze-drying. The *per*-6-*O*-methyl-α-CD, *per*-2-*O*-methyl-α-CD, and *per*-3-*O*-methyl-α-CD compounds were synthesized as described in previous studies [66-69]; *per*-2,6-*O*-dimethyl-α-CD was purchased from Wako Chemical Co. Cyclobutanol (*c*-C4OH), cyclopentanol (*c*-C5OH), cyclohexanol (*c*-C6OH), cycloheptanol (*c*-C7OH) and cyclooctanol (*c*-C8OH) were purchased from Wako Chemical Co., and were used after purification by distillation. All other chemical reagents used for this study were of reagent grade.

D_2O solutions of α-CD and/or its derivatives as well as cycloalkanols were prepared and used for ^{1}H and ^{13}C NMR measurements. The molar concentration of CDs was kept constant and that of cycloalkanols was changed to produce NMR titration plots (Experiment 1). K_a's for the formation of 1:1 inclusion complexes determined in this way were then used to obtain those of 2:1 inclusion complexes. For this purpose, the molar concentration of a cycloalkanol was kept constant and that of the α-CD was changed (Experiment 2). The NMR titration plots were then curve-fitted with the assumption of the formation of both 1:1 and 2:1 inclusion complexes.

Ⅲ-ⅱ-ⅱ ^{1}H NMR titration experiments with cycloalkanols in excess (Experiment 1)

The concentration of α-CD was fixed at 2 mmol dm^{-3}; the concentration

of the cycloalkanol was varied from 0 to 40 mmol dm^{-3} and from 0 to 30 mmol dm^{-3} for *c*-C4OH, *c*-C5OH or *c*-C6OH, and for *c*-C7OH and *c*-C8OH, respectively. The exact concentration of α-CD was 2.103, 2.097, 2.103, 2.171 and 2.309 mmol dm^{-3} for *c*-C4OH, *c*-C5OH, *c*-C6OH *c*-C7OH and *c*-C8OH, respectively. Tetramethylammmonium chloride (TMA) at 10.1 mmol dm^{-3} was added to be used as an internal reference for ^{13}C NMR signals. These solutions were inserted into NMR-sample tubes with a diameter of 5.0 mm and shaken for a minute with a test tube mixer (Shibata Scientific Technology Ltd., TTM-1). The ^{1}H NMR spectra were recorded as described in the section entitled "^{1}H and ^{13}C NMR spectral measurements". The chemical shift change ($\Delta\delta$) of the ^{1}H NMR signal of H3 of α-CD was plotted against the concentration of cycloalkanols. To estimate the K_a's, these plots were curve-fitted with the assumption that the formation of 1:1 inclusion complexes occurred [70]. Similar experiments were performed for the four α-CD derivatives, e.g., *per*-6-*O*-methyl-α-CD, *per*-2-*O*-methyl-α-CD, *per*-3-*O*-methyl-α-CD, and *per*-2,6-di-*O*-methyl-α-CD, using *c*-C6OH or *c*-C7OH as guests.

Ⅲ-ⅱ-ⅲ ^{13}C NMR titration experiments with α-CD in excess (Experiment 2)

The concentration of cycloalkanol was fixed at 5 mmol dm^{-3}, while that of α-CD was varied from 0 to 50 mmol dm^{-3}. The exact concentrations of *c*-C4OH, *c*-C5OH, *c*-C6OH *c*-C7OH and *c*-C8OH were 5.354, 6.270, 6.190, 5.360 and 5.429 mmol dm^{-3}, respectively. TMA (10.1 mmol dm^{-3}) was added as an internal reference for ^{13}C NMR. D_2O solutions of *c*-C6OH, *c*-C7OH or

c-C8OH with α-CD in excess produced white precipitates at room temperature; these were dissolved at 323 K and re-cooled to room temperature. The solutions were then inserted into NMR-sample tubes with a diameter of 5.0 mm, and mixed for 1 min with a test tube mixer. In order to avoid the formation of white precipitates, the NMR tubes were heated at 323 K before the ^{13}C NMR measurements. The $\Delta\delta$ of the ^{13}C NMR signals of the guest was plotted against the concentration of the host. These plots were curve-fitted with the assumption that either only the formation of 1:1 or of both 1:1 and 2:1 inclusion complexes occurred [37]. Similar experiments were performed for the four α-CD derivatives, *per*-6-*O*-methyl-α-CD, *per*-2-*O*-methyl-α-CD, *per*-3-*O*-methyl-α-CD, and *per*-2,6-di-*O*-methyl-α-CD, using *c*-C6OH or *c*-C7OH as guests.

Ⅲ-ⅱ-ⅳ ^{1}H and ^{13}C NMR measurements

One-dimensional ^{1}H and ^{13}C NMR measurements were performed with a 400 MHz JEOL JNM-A400 FT-NMR spectrophotometer at 298 K. To produce ^{1}H NMR spectra, eight scans were recorded and averaged within several minutes; 1370 scans were recorded and averaged to produce the ^{13}C NMR spectra in about 50 min. This record time was chosen to avoid reformation (estimated to occur in 1 h) of white precipitates in the solutions of *c*-C6OH, *c*-C7OH or *c*-C8OH. For ^{1}H NMR spectra, δ was determined using that of the HDO (4.650 ppm) signal as an internal reference. $\Delta\delta$ for the H3 of α-CD, which gives a signal at 3.853 ppm in the absence of cycloalkanols, was calculated. For ^{13}C NMR spectra, the δ value was determined using the ^{13}C NMR signal of TMA (57.952 ppm) as an internal reference. It was proven that the interaction of CD with TMA is very

weak [70], i.e., the obtained δ value is reliable. δ values for the ^{13}C NMR signals of the guest in the absence of α-CD were determined as follows: *c*-C4OH, C1 68.89, C2 35.55, C3 14.42 ppm; *c*-C5OH, C1 76.18, C2 37.45, C3 25.86 ppm; *c*-C6OH, C1 73.04, C2 36.98, C3 26.52, C4 27.65 ppm; *c*-C7OH, C1 75.55, C2 38.95, C3 24.90, C4 30.42 ppm; *c*-C8OH, C1 74.99, C2 35.97, C3 24.96, C4 29.59, C5 27.45 ppm. Numbering for the hydrogen and carbon atoms of α-CD and those of cycloalkanols is shown in Figure 3-1.

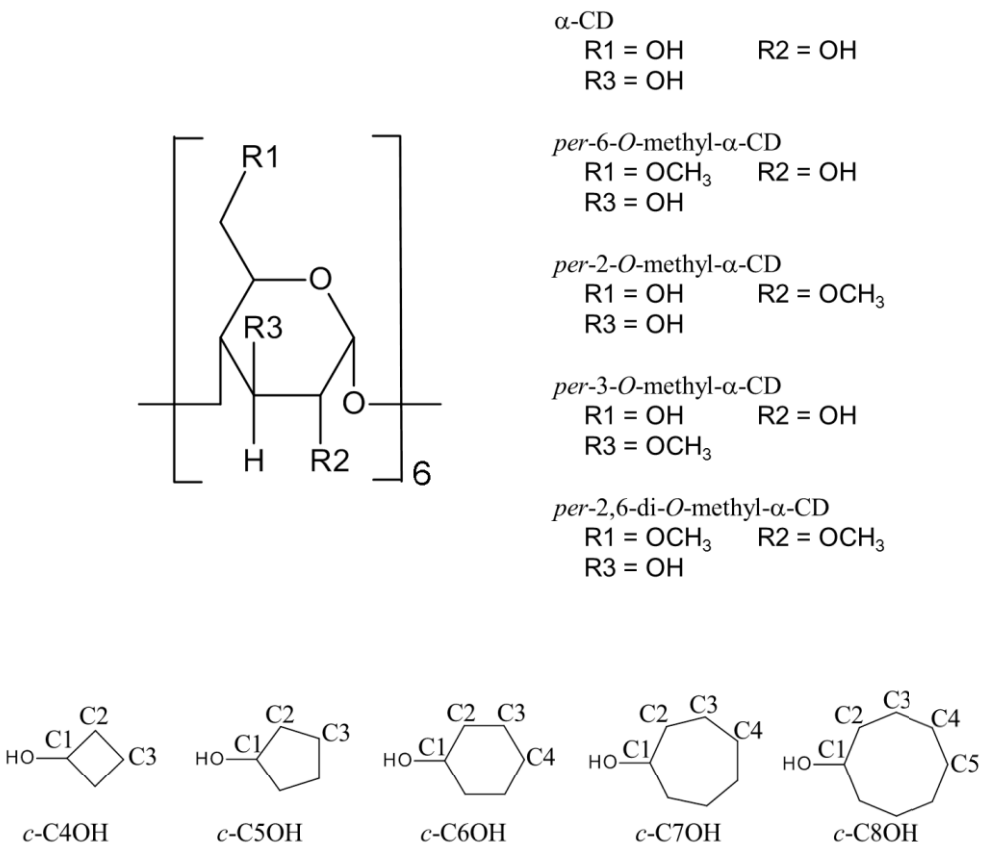

Figure 3-1. Structures for the native α-CD, its four derivatives (top) and the five cycloalkanols (bottom). For the sake of clarity, all hydrogen atoms are omitted, except for H3, which was used for the ^{1}H NMR titration. The numbering of the carbon atoms used for the ^{13}C NMR titration is also shown.

Ⅲ-ⅱ-ⅴ ROESY spectral measurements

The two-dimensional rotating-frame nuclear Overhauser effect spectroscopy (ROESY) was employed. ROESY spectra were acquired with the pulse routine named "roesy" in the spectrophotometer software. Relevant parameters were set as follows: mixing time as 250 ms, relaxation delay as 0.9359 s, number of scans as 1024, spectral width as 8,000 Hz, and numbers of points as 512 for t_2 and as 256 points for t_1, This was followed by zero-filling. The ROESY measurements were performed at two different concentrations of α-CD, e.g., 20 and 50 mmol dm^{-3}; the concentration of cycloalkanols was fixed at 20 mmol dm^{-3}. Thus, the relative host-guest concentrations obtained were 1:1 and 2.5:1. Sample solutions were heated up to 323 K and re-cooled to room temperature before measurements to avoid the formation of white precipitates.

Ⅲ-ⅲ Results and Discussion

Ⅲ-ⅲ-ⅰ Determination of K_a's

The ^{1}H NMR signal of H3 in α-CD shifted as a consequence of the increase of the cycloalkanols concentration (see Figure 3-2). The calculated $\Delta\delta$ was plotted against the molar concentration of *c*-C7OH to produce the ^{1}H NMR titration plot as shown in Figure 3-2. The K_a value for the formation of the 1:1 inclusion complexes was determined using a method previously described [70]. The ^{13}C NMR signals of the carbon atoms of cycloalkanols shifted with an increase of the α-CD concentration (Figure 3-3). The calculated $\Delta\delta$ was plotted against the concentration of α-CD to produce the ^{13}C NMR titration plots for four carbon atoms of *c*-C7OH is also shown in Figure 3-3. For each ^{13}C NMR titration

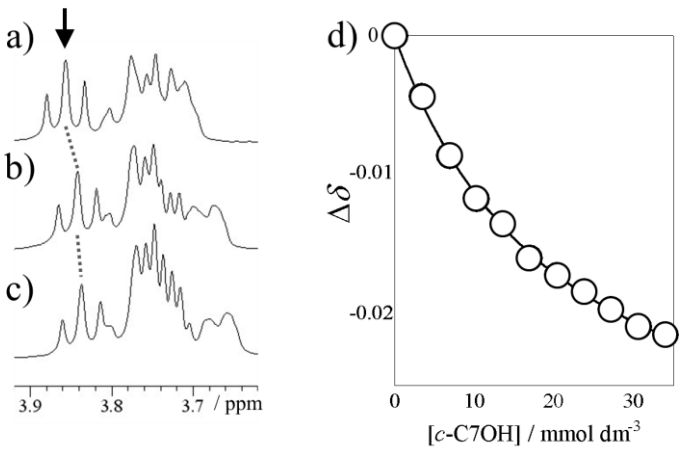

Figure 3-2. Left: The ^{1}H NMR spectra in the region of H3 signals of α-CD (2 mmol dm^{-3}) in D_2O at 298 K; the molar concentration of *c*-C7OH is 0, 15, and 30 mmol dm^{-3} in a), b), and c), respectively. The arrow shows the position of H3 signal. Right: The $\Delta\delta$ of the ^{1}H NMR signal for H3 of α-CD, plotted against the concentration of *c*-C7OH. The solid line shows the calculated value.

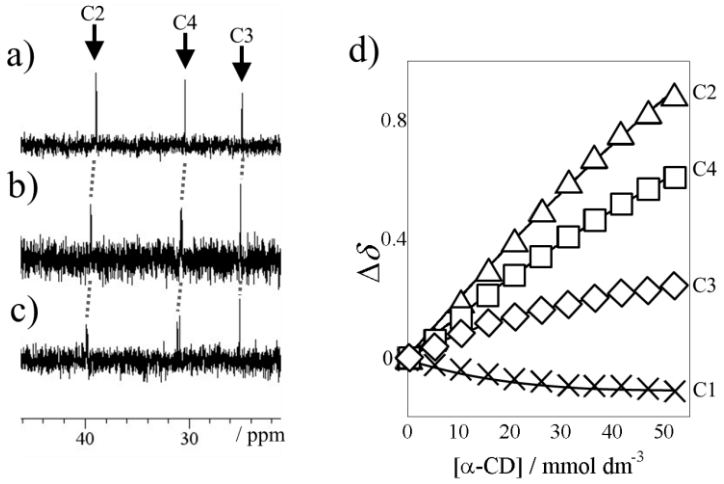

Figure 3-3. Left: The ^{13}C NMR spectra in the region of ^{13}C NMR signals of C2, C3 and C4 carbon atoms of *c*-C7OH (5 mmol dm^{-3}) in D_2O at 298 K; the molar concentration of α-CD is 0, 15, and 30 mmol dm^{-3} in a), b), and c), respectively. The arrows show the positions of ^{13}C NMR signals. The ^{13}C NMR signal for C1, which appears around 76 ppm, is not shown. Right: The $\Delta\delta$ of the ^{13}C NMR signals for C1, C2, C3 and C4 carbon atoms of *c*-C7OH plotted against the concentration of α-CD. The carbon atom numbers correspond to those indicated in Figure 3-1.

plot of the carbons of cycloalkanols, we initially curve-fitted the data under the assumption that only 1:1 inclusion complexes were formed. The K_a's determined by ^{1}H NMR titration (Experiment 1) and ^{13}C NMR titration (Experiment 2) together with the correlation coefficients are listed in Tables 3-1, 3-2, and 3-3. The average values and standard deviations are also given for those determined with ^{13}C NMR titration.

The calculated K_a value for *c*-C4OH was determined to be 35 $mol^{-1}dm^3$ (Experiment 1), and those of C1, C2 and C3 were determined to be 36, 34, and 35 $mol^{-1}dm^3$ (Experiment 2), respectively (Tables 3-1 and 3-2). Thus, our data strongly suggested that *c*-C4OH exclusively forms 1:1 inclusion complexes with α-CD in D_2O. The correlation coefficient (near to 1) confirmed this point. However, a worse correlation was found for C1 as compared to C2 and C3; this may be due to the chemical shift change in the ^{13}C NMR signal of C1, which is significantly smaller than that of C2 or C3. The calculated K_a's for *c*-C5OH were

Table 3-1. K_a's ($mol^{-1}dm^3$) for the formation of the 1:1 inclusion complexes with α-CD and cycloalkanols in D_2O at 298 K; these were determined by ^{1}H NMR titration, with an excess concentration of the guest and under the assumption that only the formation of 1:1 inclusion complexes occurred; *r* is the correlation coefficient.

	Guest				
	c-C4OH[a]	*c*-C5OH[a]	*c*-C6OH[a]	*c*-C7OH[b]	*c*-C8OH[b]
K_a	35	34	53	54	44
r	0.9994	0.9997	0.9991	0.9994	0.9991

a : The concentrations of host and guest are 2 and 0-40 mmol dm^{-3}.

b : The concentrations of host and guest are 2 and 0-30 mmol dm^{-3}.

Table 3-2. K_a's ($mol^{-1}dm^3$) for the formation of the 1:1 inclusion complex with α-CD and cycloalkanols in D_2O at 298 K. These were determined by ^{13}C NMR titration method, with an excess concentration of the host and under the assumption that only the formation of 1:1 inclusion complexes occurred; *r* is correlation coefficient.

Numbering of carbons	guest[a]			
	c-C4OH		*c*-C5OH	
	K_a	*r*	K_a	*r*
1	36	0.9687	39	0.9998
2	34	0.9997	35	0.9998
3	35	0.9997	35	0.9997
Ave. ± SD	35 ± 1		36 ± 2	

a : The concentrations of host and guest are 0-50 and 5 mmol dm^{-3}.

Table 3-3. K_a's ($mol^{-1}dm^3$) for the formation of both 1:1 and 2:1 inclusion complexes with α-CD and cycloalkanols in D_2O at 298 K. These were determined by ^{13}C NMR titration method, with an excess concentration of the host and under the assumption that the formation of both 1:1 and 2:1 inclusion complexes occurred; *r* is correlation coefficient.

Numbering of carbons	guest[a]								
	c-C6OH			*c*-C7OH			*c*-C8OH		
	K_1	K_2	*r*	K_1	K_2	*r*	K_1	K_2	*r*
1	51	34	0.9991	55	39	0.9978	-[b]	-[b]	-[b]
2	58	32	0.9995	55	39	0.9995	38	50	0.9994
3	53	36	0.9995	56	40	0.9987	38	49	0.9997
4	57	35	0.9995	57	39	0.9992	39	50	0.9997
5							38	50	0.9999
Ave. ± SD	55 ± 3	34 ± 2		56 ± 1	39 ± 1		38 ± 1	50 ± 1	

a : The concentrations of host and guest are 0-50 and 5 mmol dm^{-3}.

b : Data are not shown because of the weak the intensity of the signals.

determined to be 34 $mol^{-1}dm^3$; those of C1, C2 and C3 were determined to be 39, 35 and 35 $mol^{-1}dm^3$, respectively (Tables 3-1 and 3-2). These data indicated that when *c*-C5OH is employed, only 1:1 inclusion complexes are formed. However, these findings are inconsistent with the results of previous calorimetric experiments on inclusion complexes of α-CD with cyclopentane, which suggested the formation of a 2:1 system [71]. The discrepancy between our results and those previously reported may be attributed to the presence in our systems of the hydroxy group. In particular, when a cyclopentane molecule is included in the hydrophobic internal cavity of α-CD, the counter side of the included guest is exposed to the aqueous solution. This allows the access of another α-CD molecule to include the exposed moiety of the guest, which is still hydrophobic. In contrast, in the case of *c*-C5OH, the hydroxy group is exposed to the aqueous medium, and the exposed moiety is not hydrophobic enough to be included by another α-CD molecule. As a result, only a 1:1 inclusion complex can be formed with *c*-C5OH and α-CD.

Assuming the exclusive formation of 1:1 inclusion complexes, and considering experimental conditions in which the host was in excess, *c*-C6OH was found to produce scattered K_a's. These were determined with ^{13}C NMR titration of C1, C2, C3 and C4 to be 77, 26, 57 and 27, respectively (data not shown), the average and the standard deviation being 47 and 24 $mol^{-1}dm^3$, respectively. Although the correlation coefficients for C1, C2, C3 and C4 were found to be satisfactory (≥0.9973), the large standard deviation suggested that inclusion complexes other than 1:1 are formed. Similarly, *c*-C7OH and *c*-C8OH produced scattered K_a's.

Considering the large standard deviation of the K_a's for the complexation of *c*-C6OH, *c*-C7OH and *c*-C8OH, the assumption of an exclusive formation of 1:1 inclusion complexes may be not appropriated. Thus, white precipitates were prepared by adding these three cycloalkanols to D_2O with an excess of α-CD; the precipitates were then collected and dried up to be dissolved again in D_2O in the absence of α-CD; ^1H NMR spectra were recorded to establish whether the precipitate contained α-CD and cycloalkanols. The area intensity of the ^1H NMR signal of H1 atom of α-CD was compared to the total area intensity of all hydrogen atoms of a cycloalkanol molecule, except for that attached to C1 carbon of the cycloalkanol. The results showed that the molar ratio of the white precipitates for *c*-C6OH, *c*-C7OH or *c*-C8OH with α-CD is 2:1, indicating that using an excess of α-CD results in the formation of 2:1 inclusion complexes.

Therefore, we re-fitted the ^{13}C NMR titration plots with the new assumption that *c*-C6OH, *c*-C7OH or *c*-C8OH may also form 2:1 inclusion complexes; in this new set of calculations, K_a of 1:1 and of 2:1 inclusion complexes were defined as K_1 and K_2, respectively; by employing a curve-fitting procedure, K_1 and K_2 values were determined for *c*-C6OH, *c*-C7OH or *c*-C8OH (Table 3-3) [37]. The K_a value for the formation of the 1:1 inclusion complex, determined by ^1H NMR titration (Experiment 1) with the guest in excess, was used as the initial value for the curve-fitting calculation. As shown in Table 3, the standard deviations for K_1 and K_2 are smaller than those obtained with the previous approach, indicating that *c*-C6OH, *c*-C7OH or *c*-C8OH can also form 2:1 inclusion complexes in presence of an excess of α-CD.

Ⅲ-ⅲ-ⅱ Molecular orientation of the two α-CD molecules in 2:1 inclusion complexes

The formation of white precipitates in 2:1 inclusion complexes of *c*-C6OH, *c*-C7OH or *c*-C8OH with α-CD suggested that a strong hydrogen bonding occurs between the hydroxy groups of two different α-CD molecules to decrease the water solubility of the inclusion complex. Based on this finding, we explored the possibility that *c*-C6OH, *c*-C7OH or *c*-C8OH may form white precipitates with α-CD derivatives, in which one of the three hydroxy groups is substituted with a methoxy group in every glucopyranosyl unit; four α-CD derivatives were employed, namely *per*-6-*O*-methyl-α-CD, *per*-2-*O*-methyl-α-CD, *per*-3-*O*-methyl-α-CD, and *per*-2,6-di-*O*-methyl-α-CD. In particular, *per*-6-*O*-methyl-α-CD lacks a hydroxy group in the primary rim of the α-CD in every glucopyranosyl unit; *per*-2-*O*-methyl-α-CD and *per*-3-*O*-methyl-α-CD lack a hydroxy group on C2 and C3, respectively, in the secondary hydroxy side of the α-CD in every glucopyranosyl unit; *per*-2,6-di-*O*-methyl-α-CD lacks two hydroxy groups attached on carbons C6 and C2, in both of the primary and secondary hydroxy side of the α-CD in every glucopyranosyl unit. If the two α-CD molecules interact with each other in a head-to-head manner (e.g., the primary hydroxy sides face each other), the 2:1 inclusion complex cannot be formed, due to the a lack of hydroxy groups on the primary hydroxy side. This in turn suggests that the calculated K_a's, determined under the assumption of an exclusive formation of 1:1 inclusion complex, should not scatter. Conversely, if the two α-CD molecules interact in a tail-to-tail manner (e.g., the secondary hydroxy sides face each other),

per-2-*O*-methyl-α-CD, *per*-3-*O*-methyl-α-CD, and *per*-2,6-di-*O*-methyl-α-CD should not form 2:1 inclusion complexes. In such a scenario, K_a's, determined under the assumption of an exclusive formation of a 1:1 inclusion complex, are expected to be very similar to one another.

To explore this hypothesis, ^{1}H and ^{13}C NMR titration experiments were performed for the four α-CD derivatives. The K_a's along with the correlation coefficients are listed in Table 3-4, 3-5, and 3-6. Only *c*-C6OH and *c*-C7OH were investigated, as the results of these experiments are, in our opinion, sufficient to establish which one between the primary and the secondary hydroxy group is the most important for complexation.

Table 3-4. K_a's ($mol^{-1}dm^3$) for the formation of the 1:1 inclusion complexes with α-CD derivatives and cycloalkanols in D_2O at 298 K. These were determined by ^{1}H NMR titration method, with an excess concentration of the guest and under the assumption that only the formation of 1:1 inclusion complexes occurred; *r* is correlation coefficient.

host	Guest			
	c-C6OH[a]		*c*-C7OH[b]	
	K_a	*r*	K_a	*r*
per-6-*O*-methyl-α-CD	77	0.9987	87	0.9989
per-2-*O*-methyl-α-CD	80	0.9994	88	0.9993
per-3-*O*-methyl-α-CD	29	0.9984	48	0.9977
per-2,6-di-*O*-methyl-α-CD	136	0.9997	125	0.9995

a : The concentrations of host and guest are 2 and 0-40 mmol dm^{-3}.

b : The concentrations of host and guest are 2 and 0-30 mmol dm^{-3}.

Table 3-5. K_a's ($mol^{-1}dm^3$) for the formation of both 1:1 and 2:1 inclusion complexes with *per*-6-*O*-methyl-α-CD and cycloalkanols in D_2O at 298 K. These were determined by ^{13}C NMR titration method, with an excess concentration of the host under the assumption that the formation of both 1:1 and 2:1 inclusion complexes occurred; *r* is correlation coefficient.

Numbering of carbons	guest					
	c-C6OH[a]			*c*-C7OH[b]		
	K_1	K_2	*r*	K_1	K_2	*r*
1	82	84	0.9971	116	125	0.9945
2	83	81	0.9992	96	143	0.9982
3	84	81	0.9996	90	110	0.9984
4	77	93	0.9972	85	142	0.9976
Ave. ± SD	81 ± 3	85 ± 6		97 ± 14	130 ± 16	

a : The concentrations of host and guest are 0-30 and 3 mmol dm^{-3}.

b : The concentrations of host and guest are 0-15 and 5 mmol dm^{-3}.

In the presence of an excess of the host, *c*-C6OH and *per*-6-*O*-methyl-α-CD produced a precipitate similar to that of the native α-CD. The K_a's were estimated with ^1H NMR titration to be 77 $mol^{-1}dm^3$ (Table 3-4); K_a's determined using ^{13}C NMR titration, under the assumption of an exclusive formation of 1:1 inclusion complexes, were estimated to be 75, 37, 57, and 38 $mol^{-1}dm^3$ for C1, C2, C3, and C4, respectively (data not shown), the average and the standard deviation being 52 and 18 $mol^{-1}dm^3$, respectively. These data confirmed that in these conditions inclusion complexes other than 1:1 are formed. Thus, the ^{13}C NMR titration plots were fitted again, assuming the formation of

both 1:1 and 2:1 inclusion complexes. The averaged values of K_1 and of K_2 for

Table 3-6. K_a's ($mol^{-1}dm^3$) for the formation of the 1:1 inclusion complex with α-CD derivatives and cycloalkanols in D_2O at 298 K. These were determined by ^{13}C NMR titration method, with an excess concentration of the host and under the assumption that only the formation of 1:1 inclusion complexes occurred; *r* is correlation coefficient.

Host	Numbering of carbons	Guest			
		c-C6OH		*c*-C7OH	
		K_a	*r*	K_a	*r*
per-2-*O*-methyl-α-CD[a]	1	62	0.9983	114	0.9990
	2	74	0.9998	88	0.9999
	3	71	0.9997	89	0.999
	4	67	0.9996	93	0.9999
	Ave. ± SD	69 ± 5		96 ± 12	
per-3-*O*-methyl-α-CD[b]	1	60	0.9995	35	0.9996
	2	42	0.9998	28	0.9996
	3	53	0.9993	34	0.9998
	4	39	0.9997	30	0.9998
	Ave. ± SD	49 ± 10		32 ± 3	
per-2,6-di-*O*-methyl-α-CD[c]	1	- [d]	- [d]	174	0.9992
	2	127	0.9997	155	0.9998
	3	128	0.9997	143	0.9993
	4	122	0.9997	151	0.9991
	Ave. ± SD	125 ± 3		156 ± 13	

a : The concentrations of host and guest are 0-40 and 5 mmol dm^{-3}, respectively.

b : The concentrations of host and guest are 0-30 and 3 mmol dm^{-3} when *c*-C6OH was used; 0-40 and 5 mmol dm^{-3} when *c*-C7OH was used.

c : The concentrations of host and guest are 0-80 and 5 mmol dm^{-3} when *c*-C6OH was used; 0-40 and 5 mmol dm^{-3} when *c*-C7OH was used.

d : These data were not used for calculations because the $\Delta\delta$ of the signal was too small.

c-C6OH with *per*-6-*O*-methyl-α-CD were calculated to be 81 ± 3 and 85 ± 6 $mol^{-1}dm^3$, respectively (Table 3-5). Similarly, the combination of *c*-C7OH and *per*-6-*O*-methyl-α-CD produced scattered K_a's, under the assumption of an exclusive formation of a 1:1 inclusion complex, the averaged K_a value and the standard deviation being 24 and 29 $mol^{-1}dm^3$, respectively. When the formation of 2:1 inclusion complexes was also taken into account, the averaged values of K_1 and K_2 were calculated as 97 ± 14 and 130 ± 16 $mol^{-1}dm^3$, respectively (Table 5). Thus, these results strongly indicated that the formation of 2:1 inclusion complexes of *c*-C6OH or *c*-C7OH with *per*-6-*O*-methyl-α-CD is not inhibited by the methoxy groups at the primary hydroxy side (Figure 3-4)

Interestingly, neither *c*-C6OH nor *c*-C7OH formed white precipitates with *per*-2-*O*-methyl-α-CD, *per*-3-*O*-methyl-α-CD, or *per*-2,6-di-*O*-methyl-α-CD. The calculated K_a's for these systems were found to be very similar to one another, as shown in Tables 3-4 and 3-6. In particular, the average K_a's and standard deviations determined by ^{13}C NMR titration were calculated to be 69 ± 5, 49 ± 10, and 125 ± 3 $mol^{-1}dm^3$ when *c*-C6OH was combined with *per*-2-*O*-methyl-α-CD, *per*-3-*O*-methyl-α-CD, and *per*-2,6-di-*O*-methyl-α-CD, respectively. The K_a's for *c*-C7OH with *per*-2-*O*-methyl-α-CD, *per*-3-methyl-α-CD, and *per*-2,6-di-*O*-methyl-α-CD were estimated as 96 ± 12, 32 ± 3, and 156 ± 13 $mol^{-1}dm^3$, respectively (Table 3-6). These data clearly showed that only 1:1 inclusion complexes are formed when these compounds are combined, and that *per*-*O*-methylation of the secondary hydroxy groups of the α-CD can prevent the formation of 2:1 inclusion complexes when *c*-C6OH or *c*-C7OH are used (Figure 3-4).

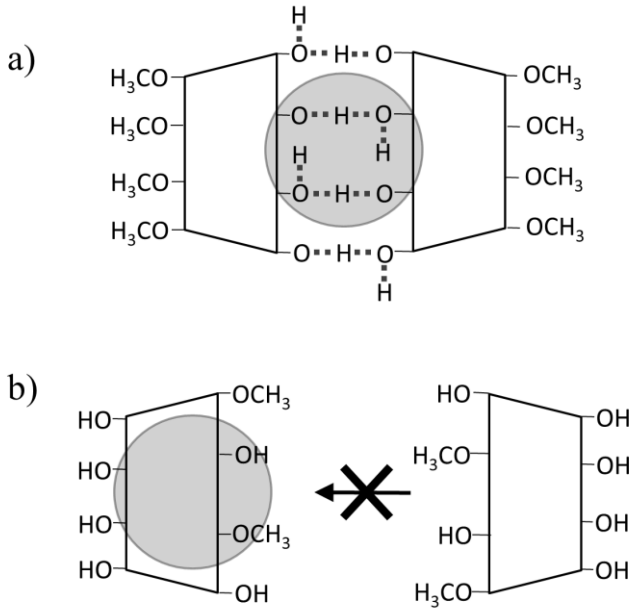

Figure 3-4. a) Schematic drawing of the 2:1 inclusion complex of 6-*O*-methyl-α-CD with a larger ring-sized cycloalkanol; the methoxy groups at the primary hydroxy side of the CDs are not incorporated in the formation of the 2:1 inclusion complex, while the secondary hydroxy groups of the CDs are. Hydrogen bonds are formed between the secondary hydroxy groups of the two α-CD derivatives, which interact in a tail-to-tail manner.
b) The stable 1:1 inclusion complex does not allow the access of another α-CD derivative, *per*-2-*O*-methyl-α-CD, *per*-3-*O*-methyl-α-CD, or *per*-2,6-di-*O*-methyl-α-CD. Because of the methoxy groups at the secondary hydroxy side, the hydrogen bonding between the secondary hydroxy groups of the two CDs are not strong enough to stabilize the 2:1 inclusion complex.

Considering these findings together with those obtained for the native α-CD with cycloalkanols, we claim that *c*-C4OH or *c*-C5OH can exclusively form 1:1 inclusion complexes with α-CD, while larger ring-sized cycloalkanols, such as *c*-C6OH, *c*-C7OH or *c*-C8OH, form 2:1 inclusion complexes. The two α-CD molecules of 2:1 inclusion complexes are thus expected to interact with each

other in a tail-to-tail manner, via the formation of hydrogen bonds between the secondary hydroxy groups.

To confirm these results, two-dimensional ROESY NMR spectra were collected. The cross peak intensity in two-dimensional ROESY spectra, between ^{1}H NMR signals of the hydrogen atoms, which belong to the internal cavity of the CD and to the guest, can provide crucial information about the orientation of the host and/or the guest in an inclusion complex. Thus, in this work, the cross peaks between ^{1}H NMR signals of H3 or H5 of α-CD and those of the cycloalkanols were examined. These hydrogen atoms are located in the internal cavity of the α-CD in the vicinity of the secondary (hydrogen H3) and primary (hydrogen H5) hydroxy sides. As shown in Figure 3-5, the intensity of the cross peaks between the ^{1}H NMR signals of H3 or H5 and that of *c*-C5OH hardly change when the concentration of α-CD was increased from 20 to 50 mmol dm^{-3} (Figure 3-5a, b). This result suggested that the *c*-C5OH molecule is included deeply enough in the internal cavity of an α-CD molecule to produce strong cross peaks between both these hydrogen atoms. The two-dimensional ROESY spectra for *c*-C4OH showed virtually the same results, however, the intensity of the cross peak between the ^{1}H NMR signals of H5 and that of *c*-C7OH significantly decreased when the α-CD concentration was increased from 20 to 50 mmol dm^{-3}. This finding indicated that the distance between H5 and *c*-C7OH increases, unlike that between H3 and *c*-C7OH (Figure 3-5c, d). This can be well explained by the formation of a 2:1 inclusion complex in a tail-to-tail manner (Figure 3-4a). When α-CD and *c*-C7OH have the same concentrations, the *c*-C7OH molecule cab be included in an α-CD molecule

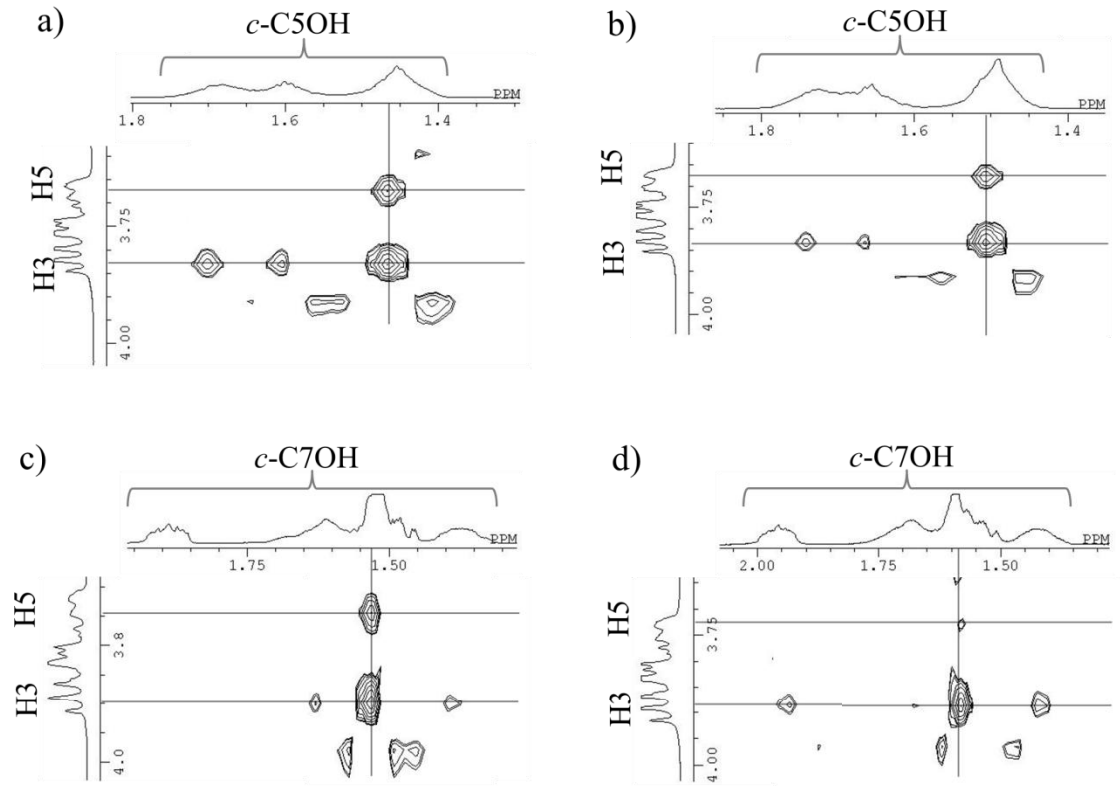

Figure 3-5. Two-dimensional ROESY spectra for *c*-C5OH or *c*-C7OH in the presence of α-CD; [α-CD]:[*c*-C5OH] = a) 20:20, b) 50:20, and [α-CD]:[*c*-C7OH] = c) 20:20, d) 50:20 mmol dm^{-3}. In the one-dimensional spectra, the region of ^{1}H NMR signals for cycloalkanols is displayed on the horizontal axis, those for α-CD are displayed on the vertical axis.

deeply enough to produce cross peaks with H3 and H5 in a two-dimensional ROESY spectrum. In contrast, when the concentrations of α-CD and *c*-C7OH are different enough (such as 50 and 20 mmol dm^{-3} respectively, in our study), the *c*-C7OH molecule moves to the middle of the two α-CD molecules, to form a 2:1 inclusion complex in a tail-to-tail manner, and consequently is stepped away from H5, although still crosses to H3. Based on the data reported in this contribution, we suggest that this rearrangement is the cause for the decrease in

the intensity of the cross peak between ^{1}H NMR signals of H5 and that of *c*-C7OH. The two-dimensional ROESY spectra of *c*-C6OH and *c*-C8OH produced virtually the same results of *c*-C7OH, which support our conclusion that *c*-C6OH, *c*-C7OH and *c*-C8OH form 2:1 inclusion complexes with α-CD in a tail-to-tail manner.

Ⅲ-ⅳ Conclusion

The K_a's for the formation of 1:1 inclusion complexes of α-CD with cycloalkanols were determined by means of ^{1}H and ^{13}C NMR titration experiments. The analysis of the K_a's showed that α-CD forms 1:1 inclusion complexes with *c*-C4OH or *c*-C5OH; in contrast, with larger ring-sized cycloalkanols, such as *c*-C6OH, *c*-C7OH or *c*-C8OH, our data suggested that α-CD form 2:1 inclusion complexes. The K_a's for the formation of both 1:1 and 2:1 inclusion complexes, K_1 and K_2, were also calculated, using the K_a obtained by ^{1}H NMR titration as the initial value. This was carried out under the assumption that the formation of 1:1 and 2:1 inclusion complexes occur simultaneously and with an excess concentration of the host. These data together with those obtained with ROESY measurements confirmed that the two α-CD molecules of 2:1 inclusion complexes interact with each other in a tail-to-tail manner via hydrogen bonds between the secondary hydroxy groups,

Chapter Ⅳ

General Conclusion

The present study deals with NMR spectroscopy on the complexation of cyclodextrin (CD) and its derivatives with some guest molecules in D_2O solution.

In a study described in Chapter Ⅱ, it was revealed that G1-β-CD forms 2:1 (host : guest) inclusion complex with D-, or, L-tryptophan at [host]≫[guest]. The equilibrium is two steps having two binding constants (K_a's,) namely K_1 and K_2. First inclusion, that its intensity is expressed as K_1, occurs on indole-ring, therefore, there is no difference in the K_1 between D- and L-tryptophan. The inclusion location is far from the chiral center of tryptophan. On the other hand, K_2 shows obvious difference between D- and L-tryptophan. It indicates that second 6-*O*-α-D-glucosyl-β-CD (G1-β-CD) includes the chiral center of tryptophan directly. Some efforts have been devoted to increase the chiral recognition ability of CDs by attachment of some functional group to CDs [31, 72]. However, this study shows that making [host]≫[guest] condition cause the effective chiral recognition, at least for tryptophan enantiomer.

In a study described in Chapter Ⅲ, it was revealed that α-CD forms 2:1 inclusion complex with lager cycloalkanols, cyclohexanol, cycloheptanol, and cyslooctanol, at [host] ≫ [guest], although that is not with smaller cycloalkanols, cyclobutanol and cyclopentanol. However, some α-CD derivatives lacking hydroxyl group on the secondary rim don't form the 2:1 inclusion complex with both lager and smaller guests. Moreover, when the 2:1 inclusion complex is formed, it becomes white precipitate that is hydrophobic. These suggests that the hydroxy groups on the secondary rim of first and second α-CD are face to face each other, and form hydrogen bonding, when the 2:1 inclusion complex is formed. Some reports refer to the importance of the effect

of this hydrogen boding's formation between two CDs [73, 74]. To understand the mechanism of the inclusion reaction is important to develop new systems.

Thus, the present studies clearly show that NMR spectroscopy is very useful for the research of CD complexes. Many studies have been reported about various CD complexes. However, some reports may overlook important stoichiometry, than 1:1. To obtain the certain K_a and stoichiometry of the inclusion reaction, both of the experiment under [host]≪[guest] and [host]≪[guest] should be done.

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Acknowledgements

I would like to express my deep gratitude to Professor Tatsuyuki Yamamoto, the Faculty of Life and Environmental Science of Shimane University, for his advice and encouragement as a major supervisor. This thesis would have not completed without his kind help.

I would like to express my sincerest and profound gratitude to Assistant Professor Keisuke Yoshikiyo, the Faculty of Life and Environmental Science of Shimane University, for his hearty help and encouragement throughout this study as a vice supervisor.

I am grateful to Associate Professor Tsuyoshi Ichiyanagi, the Faculty of Agriculture of Tottori University, for his relevant remark and guidance as a vice supervisor.

I am grateful to Professor Yoshihisa Matsui, a professor emeritus of, the Faculty of Life and Environmental Science of Shimane University, for his advice and unstinting help.

I'm also grateful to all members of our laboratory for their help.

June 11, 2014 Tomoki Akita

NMR Spectroscopy on Inclusion Equilibria ;

Forming of 2:1 (host : guest) Cyclodextrin Inclusion Complexes

Abstract

Cyclodextrins (CDs, hosts) is a cyclic oligomer consisting of some α-D-glucopyranose units linked by α-1,4-glycosidic bonds. The shape is truncated corn. Most important ability of CDs is formation of inclusion complex with variety guest without covalent bonds. The properties of complexes, binding constant (*K*a), stoichiometry, and molecular orientation, are sensitive to some conditions, pH, solvent, temperature, etc. Especially, the stoichiometry is affected by the molecular ratio of host versus guest. However, in some reports, only 1:1 inclusion complex are discussed with the experiment under the [host]≪[guest] condition. Then, it is proposed that certain K_a, stoichiometry, and molecular orientation of CD complexes, should be obtained from two experimental conditions, [host]≪[guest] and [host]≫[guest]. In the studies of this thesis, it was aimed to investigate a few interesting inclusion phenomena involved in formation of 2:1 (host : guest) inclusion complex of CD by means of NMR spectroscopy. NMR spectroscopy is a powerful method for understanding the properties of CD complexes. This thesis consists of two parts.

1) **A ^{1}H NMR Titration Study on the Binding Constants for D- and L-Tryptophan Inclusion Complexes with 6-*O*-α-D-Glucosyl-β-cyclodextrin.**

Formation of 1:1 and 2:1 (Host : Guest) Complexes

It was known that the properties of protein are changed by inclusion of the aromatic amino acid side chain, especially tryptophan, by β-CD. To understand the detail of the mechanism, the K_a for formation of the complex of 6-*O*-α-D-Glucosyl-β-cyclodextrin (G1-β-CD) with D- or L-tryptophan is evaluated by ^{1}H NMR titration under both of the conditions, [host] ≪ [guest] and [host] ≫ [guest]. Moreover, the molecular orientation of the complex is investigated by 2D NMR (ROESY). In the result, it was revealed that G1-β-CD forms 2:1 (host : guest) inclusion complex with D-, or, L-tryptophan at [host] ≫ [guest]. The equilibrium is two steps having two K_a's namely K_1 and K_2. First inclusion, that its intensity is expressed as K_1, occurs on indole-ring, therefore, there is no difference in the K_1 between D- and L-tryptophan. The inclusion location is far from the chiral center of tryptophan. On the other hand, K_2 shows obvious difference between D- and L-tryptophan. It indicates that second G1-β-CD includes the chiral center of tryptophan directly. The 2D NMR (ROESY) spectra supported this deduction.

2) **Formation of 1:1 and 2:1 host-guest inclusion complexes of α-cyclodextrin with cycloalkanols: A ^{1}H and ^{13}C NMR spectroscopic study**

In a previous study, it revealed that α-CD forms 2:1 inclusion complex with some 1-alkanol and 1-alcanoate ions with relatively long alkyl chains. It is interested in that the stoichiometry of the CD complex depends on the size and shape of guest molecule. Then, it is investigated by NMR titration method that whether the similar effect of the guest size is observed or not in the system of

α-CD and cycloalkanols. The result suggests that α-CD forms 2:1 inclusion complex with lager cycloalkanols, cyclohexanol, cycloheptanol, and cyslooctanol, at [host]≫[guest], although that is not with smaller cycloalkanols, cyclobutanol and cyclopentanol. When the 2:1 inclusion complex is formed, it becomes hydrophobic precipitate. It is expected that it's due to the formation of hydrogen bonding between two α-CD molecules. In actually, some α-CD derivatives whose hydroxy group on the secondary rim is repraced with methoxy group did not form the 2:1 inclusion complex with both lager and smaller guests. On the other hand, an α-CD derivative lacking hydroxy group on the primary rim formed 2:1 inclusion complex as same as normal α-CD. These suggest that the hydroxy groups on the secondary rim of first and second α-CD are face to face each other, and form hydrogen bonding, when the 2:1 inclusion complex is formed. 2D NMR spectra of the complexes of α-CD with cycloalkanols are obtained at both of the molecular ratios of host : guest, 1:1 and 2.5:1. The result supported the deduction of that α-CD forms the 2:1 inclusion complex with some of lager cycloalkanols by tail-to-tail manner producing hydrogen bonding between the secondary rims of two α-CD molecules.

As mentioned above, the present studies clearly show that NMR spectroscopy is very useful for the research of CD complexes. Many studies have been reported about various CD complexes. However, some reports may overlook important stoichiometry, than 1:1. To obtain the certain K_a and stoichiometry of the inclusion reaction, both of the experiment under [host]≪[guest] and [host]≪[guest] should be done.

NMR 分光法による包接平衡の研究；
2:1(ホスト:ゲスト)型シクロデキストリン包接錯体の形成

摘要

シクロデキストリン(CD)はα-D-グルコピラノース残基がα1→4 結合で環状に連なったオリゴ糖である。 その形状は中空の円錐台となっている。 CD の最も重要な機能は、共有結合を介さずに様々なゲスト分子と包接錯体を形成することである。 CD 錯体の、結合定数(K_a)や化学量論、分子配向といった性質は、pH や溶媒の種類、温度などの様々な条件の影響を受ける。 特に化学量論はホスト : ゲストの濃度比に依存して変化するが、いくつかの論文では[ホスト]≪[ゲスト]条件での実験から 1:1 型包接錯体のみの性質について言及されている。 これに対して、[ホスト]≪[ゲスト]、[ホスト]≫[ゲスト]の両条件での実験を行うことで、より確かな錯体の性質を知ることができると考えた。 本研究は、NMR 分光法から得られる多くの情報をもとに、新たに発見された 2:1 型包接錯体が関連する興味深い CD 包接現象について明らかにすることを目的とした。本論文は次の２章から成る。

1) ^{1}H NMR 滴定法による D-、L-トリプトファンと 6-*O*-α-D-グルコシル-β-シクロデキストリンとの結合定数の研究 ； 1:1 及び 2:1(ホスト:ゲスト)型錯体の形成

タンパクのトリプトファン残基がβ-CD に包接されることで、タンパクの性質の変化が引き起こされることが知られている。 その機構について詳細を知るため、D- あるいは L-トリプトファンと 6-*O*-α-D-グルコシル-β-CD (G1-β-CD)との包接錯体について、[ホスト]≪[ゲスト]及び[ホスト]≫[ゲスト]両条件下での ^{1}H NMR 滴定法によって結合定数を評価した。また、2D NMR (ROESY)法による分子配向の調査も行った。 その結果、[ホスト]≫[ゲスト]条件下において、G1-β-CD はトリプトファンと 2:1 (ホスト：ゲスト) 型包接錯体を形成することが明らかとなった。 その包接平衡は二段階となっており、その強度は２つの結合定数、K_1 及び K_2 で表される。 K_1 で表される一段階目の包接はトリプトファンのインドール環を包接するもので、K_1 にはトリプトファンの光学異性体間でほとんど差が無かった。 これは、トリプトファンのキラル中心から離れた位置での包接であることに起因すると考えられた。 一方で、K_2 には異性体間で有意な差が見られ、これは二段階目の包接がキラル中心の直接の包接であることを示唆していた。 この推論は 2D NMR (ROESY)法によっても支持された。

2) α-CD とシクロアルカノール類との 1:1 及び 1:2 型ホスト-ゲスト包接錯体形成 ; ^{1}H 及び ^{13}C NMR 分光法による研究

以前の研究で、比較的長鎖の 1-アルカノールと 1-アルカノエートイオンがゲスト分子の場合に、α-CD が 2:1 型包接錯体を形成することが明らかにされた。 ゲスト分子サイズに依存して包接錯体の化学量論が変化することは興味深く、環状のシクロアルカノールでも同様の傾向が見られるか、NMR 滴定法によって調査した。 その結果、α-CD は少なくとも

シクロヘキサノールからシクロオクタノールまでの比較的大きなシクロアルカノール類と 2:1 型包接錯体を形成することがわかった。　また、2:1 型包接錯体が形成されると水中で沈殿となることから、２分子の CD 環の水酸基間に水素結合が形成されることが親水性の低減の理由であると推測した。　実際に、ホストとして二級水酸基の半数をメトキシ化したα-CD 誘導体を用いると、2:1 型包接錯体の形成は見られなくなった。　一方で、一級水酸基のメトキシ化誘導体では、未修飾のα-CD と同様に、2:1 型包接錯体、沈殿の生成が確認された。　これらのことから、α-CD と比較的大きなシクロアルカノールとの 2:1 型包接錯体の形成において、２分子のα-CD の二級水酸基同士が向かい合って形成される水酸基同士の水素結合が重要な因子となっていることが示された。　また、α-CD とシクロアルカノール類との系において、ホスト：ゲスト濃度比が 1:1、あるいは 2.5:1 の場合のそれぞれで 2D NMR (ROESY)法を行った結果、上記、比較的大きなシクロアルカノール類との 2:1 型包接錯体の形成と、その分子配向が２分子のα-CD の二級水酸基同士が向かい合う tail-to-tail 型であることが支持された。

上記のように、本研究において、NMR 分光法が CD 錯体の研究において非常に有効な手法であることが示された。　現在、CD 錯体について様々な報告がされているが、それらは 1:1 型包接錯体以外の化学量論の錯体について見落としている可能性がある。　[ホスト]≪[ゲスト]及び[ホスト]≪[ゲスト]の両条件からの調査を行うことによって、この見落としを解消できるだろう。

List of Publications

１．**A ^{1}H NMR titration study on the binding constants for D- and L-tryptophan inclusion complexes with 6-*O*-α-D-glucosyl-β-cyclodextrin. Formation of 1:1 and 2:1 (host : guest) Complexes**

J. Mol. Struct., **1060** (2014) 138-141

―――――― Chapter Ⅱ

２．**Formation of 1:1 and 2:1 host-guest inclusion complexes of α-cyclodextrin with cycloalkanols: A ^{1}H and ^{13}C NMR spectroscopic study**

J. Mol. Struct., **1074** (2014) 43-50

―――――― Chapter Ⅲ