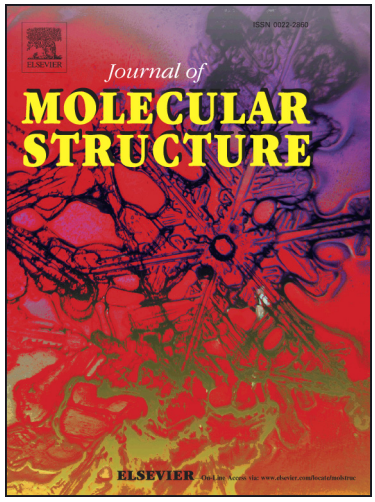

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Formation of 1:1 and 2:1 host-guest inclusion complexes of αcyclodextrin with cycloalkanols: A ^{1}H and ^{13}C NMR spectroscopic study

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Abstract

Binding constants (K_a's) for the formation of inclusion complexes of αcyclodextrin (αCD) with cycloalkanols (*c*-C*n*OH; *n*=4 ~ 8) were determined by means of ^{1}H and ^{13}C NMR titration, under two different conditions: i) only 1:1 host-guest inclusion complexes are formed when the guest is in excess; ii) the formation of 2:1 inclusion complexes occurs only after that of 1:1 inclusion complexes, when the host is in excess. The results of this work showed that αCD can include *c*-C4OH or *c*-C5OH only when the molar ratio is 1:1; larger ring-sized cycloalkanols such as *c*-C6OH, *c*-C7OH or *c*-C8OH can be included only when the molar ratio is 2:1. These

findings, together with those obtained for the four derivatives of αCD, *per*-6-*O*-methyl-αCD, *per*-2-*O*-methyl-αCD, *per*-3-*O*-methyl-αCD, and *per*-2,6-di-*O*-methyl-αCD, suggested that αCD forms 2:1 inclusion complexes with *c*-C6OH, *c*-C7OH or *c*-C8OH in a tail-to-tail manner, in which the secondary hydroxy sides of the two CD molecules face each other. Two-dimensional ROESY measurements confirmed our results.

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 [1]. 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*-αD-glucopyranosyl-βCD (G1-βCD) 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 [2]. For a kinetic study on the inclusion systems of α or G1-βCD 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 [3]. In systems in which a dimerized αCD (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 [4]. ^{13}C NMR data obtained for the formation of inclusion complexes of αCD 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 [5]. 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 αCD, but not with β or γCD. 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 [6, 7] and indirect visible-light absorbance titration were already reported [8]. 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 αCD 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 [9]. 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 αCD 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 [5]. 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 αCD, such as *per*-6-*O*-methyl-αCD, *per*-2-*O*-methyl-αCD, *per*-3-*O*-methyl-αCD, and *per*-2,6-di-*O*-methyl-αCD.

Experimental

Sample preparation

αCDs 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-αCD, *per*-2-*O*-methyl-αCD, and *per*-3-*O*-methyl-αCD compounds were synthesized as described in previous studies [10-13]; *per*-2,6-*O*-dimethyl-αCD 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 αCD 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 αCD 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.

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

The concentration of αCD 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 αCD 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 αCD 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 [14]. Similar experiments were performed for the four αCD derivatives, e.g., *per*-6-*O*-methyl-αCD, *per*-2-*O*-methyl-αCD, *per*-3-*O*-methyl-αCD, and *per*-2,6-di-*O*-methyl-α CD, using *c*-C6OH or *c*-C7OH as guests.

^{13}C NMR titration experiments with αCD in excess (Experiment 2)

The concentration of cycloalkanol was fixed at 5 mmol dm^{-3}, while that of αCD 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 αCD 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 [15]. Similar experiments were performed for the four αCD derivatives, *per*-6-*O*-methyl-αCD, *per*-2-*O*-methyl-αCD, *per*-3-*O*-methyl-αCD, and *per*-2,6-di-*O*-methyl-αCD, using *c*-C6OH or *c*-C7OH as guests.

^{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, $\delta$was determined using that of the HDO (4.650 ppm) signal as an internal reference. $\Delta\delta$ for the H3 of αCD, which gives a signal at 3.853 ppm in the absence of cycloalkanols, was calculated. For ^{13}C NMR spectra, the δvalue 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 [14], i.e., the obtained δvalue is reliable. δvalues for the ^{13}C NMR signals of the guest in the absence of αCD 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 αCD and those of cycloalkanols is shown in Figure 1.

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.

Results and Discussion

Determination of K_a's

The ^{1}H NMR signal of H3 in αCD shifted as a consequence of the increase of the cycloalkanols concentration (see Figure 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 2. The K_a value for the formation of the 1:1 inclusion complexes was determined using a method previously described [14]. The ^{13}C NMR signals of the carbon atoms of cycloalkanols shifted with an increase of the αCD concentration (Figure 3). The calculated $\Delta\delta$ was plotted against the concentration of αCD to produce the ^{13}C NMR titration plots for four carbon atoms of *c*-C7OH is also shown in Figure 3. For each ^{13}C NMR titration 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 1, 2, and 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 1 and 2). Thus, our data strongly suggested that *c*-C4OH exclusively forms 1:1 inclusion complexes with αCD 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 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 1 and 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 αCD with cyclopentane, which suggested the formation of a 2:1 system [16]. 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 αCD, the counter side of the included guest is exposed to the aqueous solution. This allows the access of another αCD 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 αCD molecule. As a result, only a 1:1 inclusion complex can be formed with *c*-C5OH and αCD.

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 αCD; the precipitates were then collected and dried up to be dissolved again in D_2O in the absence of αCD; ^{1}H NMR spectra were recorded to establish whether the precipitate contained αCD and cycloalkanols. The area intensity of the ^{1}H NMR signal of H1 atom of αCD 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 αCD is 2:1, indicating that using an excess of αCD 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) [15]. The K_a value for the formation of the 1:1 inclusion complex, determined by ^{1}H 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 αCD.

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 αCD 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 αCD derivatives, in which one of the three hydroxy groups is substituted with a methoxy group in every glucopyranosyl unit; four αCD derivatives were employed, namely *per*-6-*O*-methyl-αCD, *per*-2-*O*-methyl-αCD, *per*-3-*O*-methyl-αCD, and *per*-2,6-di-*O*-methyl-αCD. In particular, *per*-6-*O*-methyl-αCD lacks a hydroxy group in the primary rim of the αCD in every glucopyranosyl unit; *per*-2-*O*-methyl-αCD and *per*-3-*O*-methyl-αCD lack a hydroxy group on C2 and C3, respectively, in the secondary hydroxy side of the αCD in every glucopyranosyl unit; *per*-2,6-di-*O*-methyl-αCD lacks two hydroxy groups attached on carbons C6 and C2, in both of the primary and secondary hydroxy side of the αCD in every glucopyranosyl unit. If the two αCD 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 αCD molecules interact in a tail-to-tail manner (e.g., the secondary hydroxy sides face each other), *per*-2-*O*-methyl-αCD, *per*-3-*O*-methyl-α CD, and *per*-2,6-di-*O*-methyl-αCD 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 αCD derivatives. The K_a's along with the correlation coefficients are listed in Table 4, 5, and 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.

In the presence of an excess of the host, *c*-C6OH and *per*-6-*O*-methyl-αCD produced a precipitate similar to that of the native αCD. The K_a's were estimated with ^{1}H NMR titration to be 77 $mol^{-1}dm^3$ (Table 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 *c*-C6OH with *per*-6-*O*-methyl-αCD were calculated to be 81 ±3 and 85 ±6 $mol^{-1}dm^3$, respectively (Table 5). Similarly, the combination of *c*-C7OH and *per*-6-*O*-methyl-αCD 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-αCD is not

inhibited by the methoxy groups at the primary hydroxy side (Figure 4)

Interestingly, neither *c*-C6OH nor *c*-C7OH formed white precipitates with *per*-2-*O*-methyl-αCD, *per*-3-*O*-methyl-αCD , or *per*-2,6-di-*O*-methyl-αCD. The calculated K_a's for these systems were found to be very similar to one another, as shown in Tables 4 and 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-αCD, *per*-3-*O*-methyl-αCD, and *per*-2,6-di-*O*-methyl-αCD, respectively. The K_a's for *c*-C7OH with *per*-2-*O*-methyl-αCD, *per*-3-methyl-αCD, and *per*-2,6-di-*O*-methyl-αCD were estimated as 96 ±12, 32 ±3, and 156 ±13 $mol^{-1}dm^3$, respectively (Table 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 4). Considering these findings together with those obtained for the native αCD with cycloalkanols, we claim that *c*-C4OH or *c*-C5OH can exclusively form 1:1 inclusion complexes with αCD, while larger ring-sized cycloalkanols, such as *c*-C6OH, *c*-C7OH or *c*-C8OH, form 2:1 inclusion complexes. The two αCD 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 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 αCD was increased from 20 to 50 mmol dm^{-3} (Figure 5a, b). This result suggested that the *c*-C5OH molecule is included deeply enough in the internal cavity of an αCD 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 αCD 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 5c, d). This can be well explained by the formation of a 2:1 inclusion complex in a tail-to-tail manner (Figure 4a). When α CD and *c*-C7OH have the same concentrations, the *c*-C7OH molecule cab be included in an αCD molecule deeply enough to produce cross peaks with H3 and H5 in a two-dimensional ROESY spectrum. In contrast, when the concentrations of αCD 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 αCD 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 αCD in a tail-to-tail manner.

Conclusion

The K_a's for the formation of 1:1 inclusion complexes of αCD with cycloalkanols were determined by means of ^{1}H and ^{13}C NMR titration experiments. The analysis of the K_a's showed that αCD 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 αCD 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 αCD molecules of 2:1 inclusion complexes interact with each other in a tail-to-tail manner via hydrogen bonds between the secondary hydroxy groups,

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Table 1. K_a's ($mol^{-1}dm^3$) for the formation of the 1:1 inclusion complexes with αCD and cycloalkanols in D_2O at 298 K; these were determined by ^1H 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 2. K_a's ($mol^{-1}dm^3$) for the formation of the 1:1 inclusion complex with αCD 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. K_a's ($mol^{-1}dm^3$) for the formation of both 1:1 and 2:1 inclusion complexes with αCD 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.

Table 4. K_a's ($mol^{-1}dm^3$) for the formation of the 1:1 inclusion complexes with αCD derivatives and cycloalkanols in D_2O at 298 K. These were determined by ^1H 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*
6-*O*-methyl-αCD	77	0.9987	87	0.9989
2-*O*-methyl-αCD	80	0.9994	88	0.9993
3-*O*-methyl-αCD	29	0.9984	48	0.9977
2,6-di-*O*-methyl-αCD	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 5. K_a's ($mol^{-1}dm^3$) for the formation of both 1:1 and 2:1 inclusion complexes with 6-*O*-methyl-αCD 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}.

Table 6. K_a's ($mol^{-1}dm^3$) for the formation of the 1:1 inclusion complex with αCD 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*
2-*O*-methyl-αCD[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	
3-*O*-methyl-αCD[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	
2,6-di-*O*-methyl-αCD[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.

Figure captions

Figure 1. Structures for the native αCD, 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.

Figure 2. Left: The ^{1}H NMR spectra in the region of H3 signals of αCD (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 αCD, plotted against the concentration of *c*-C7OH. The solid line shows the calculated value.

Figure 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 αCD 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 αCD. The carbon atom numbers correspond to those indicated in Figure 1.

Figure 4. a) Schematic drawing of the 2:1 inclusion complex of 6-*O*-methyl-αCD 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 αCD derivative, 2-*O*-methyl-αCD, 3-*O*-methyl-αCD, or 2,6-di-*O*-methyl-αCD. 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.

Figure 5. Two-dimensional ROESY spectra for *c*-C5OH or *c*-C7OH in the presence of αCD; [αCD]:[*c*-C5OH] = a) 20:20, b) 50:20, and [αCD]:[*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 αCD are displayed on the vertical axis.

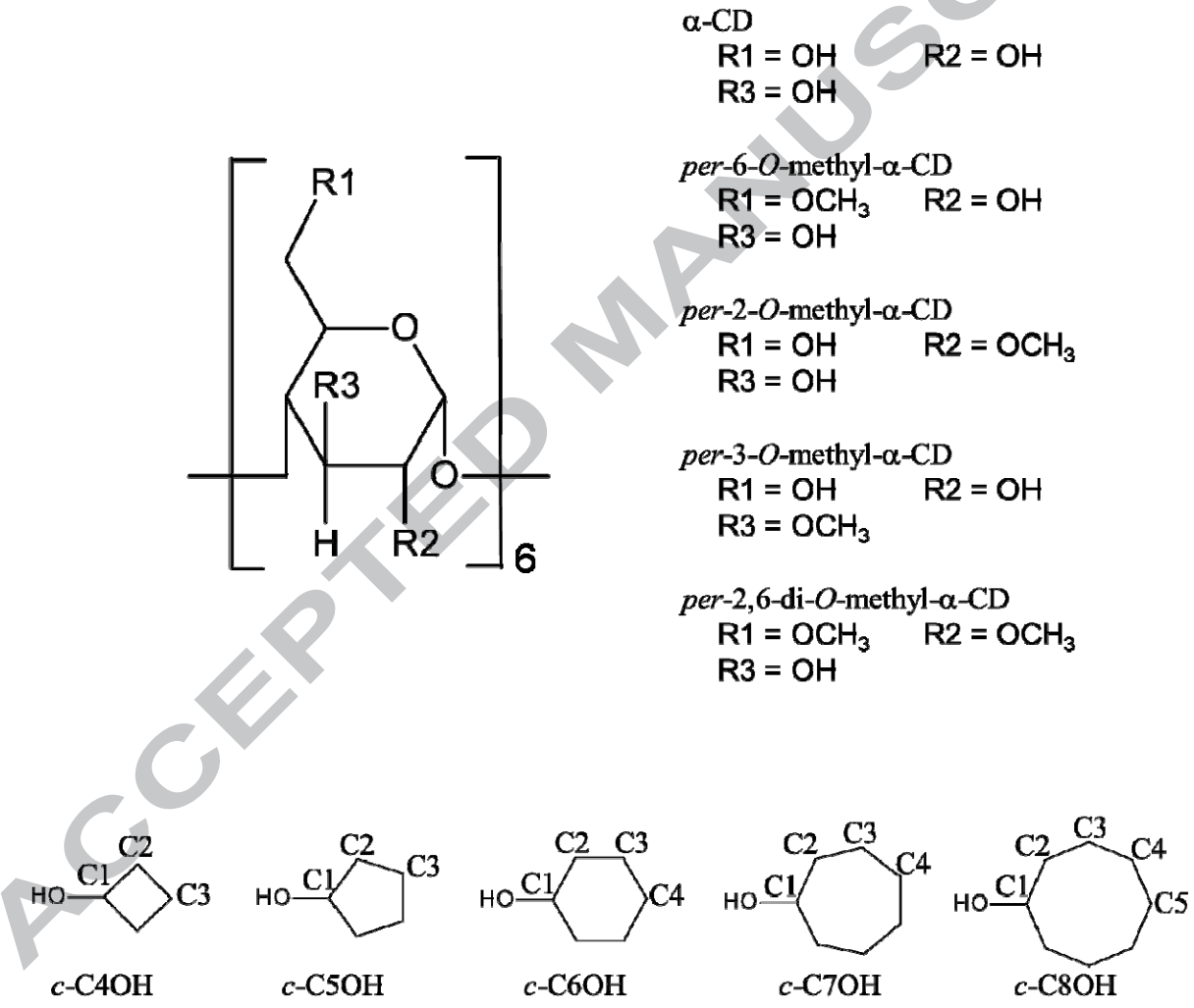


Figure 1. Akita et al.

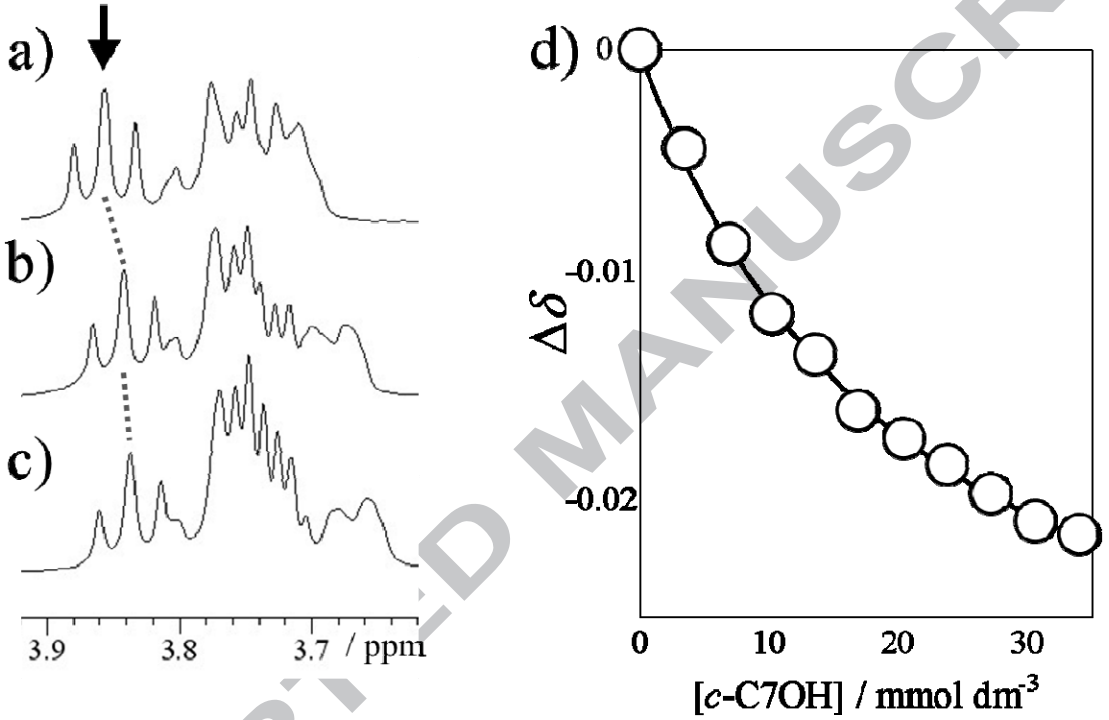


Figure 2. Akita et al.

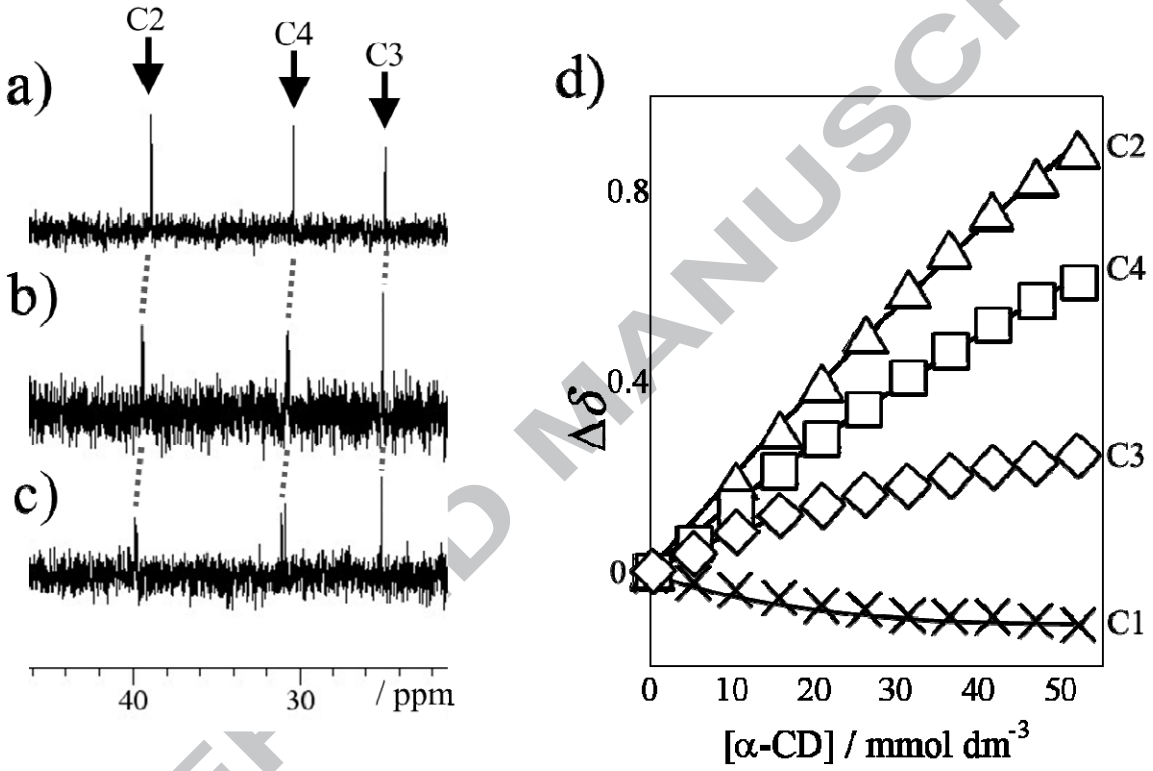


Figure 3. Akita et al.

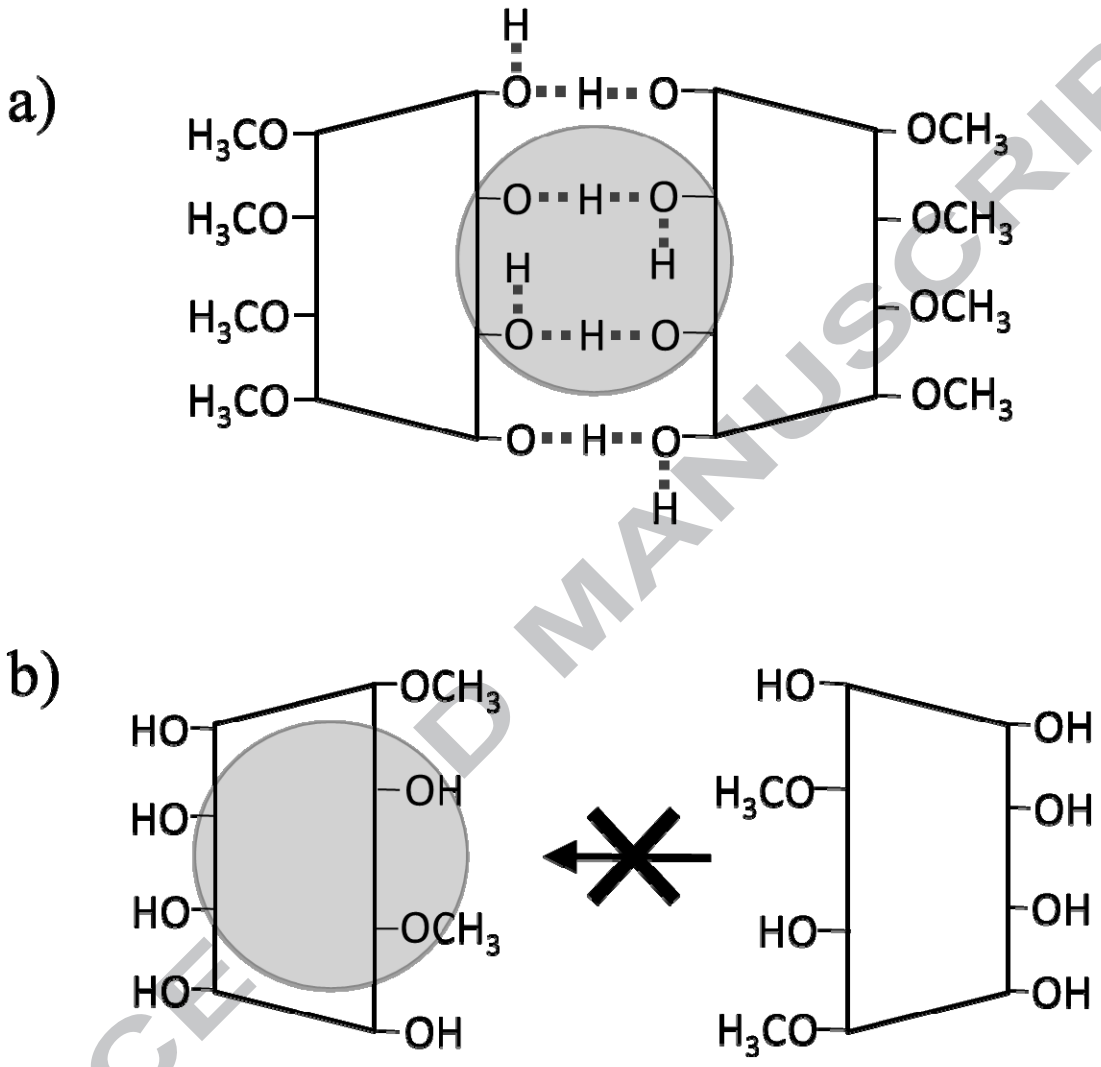


Figure 4. Akita et al.

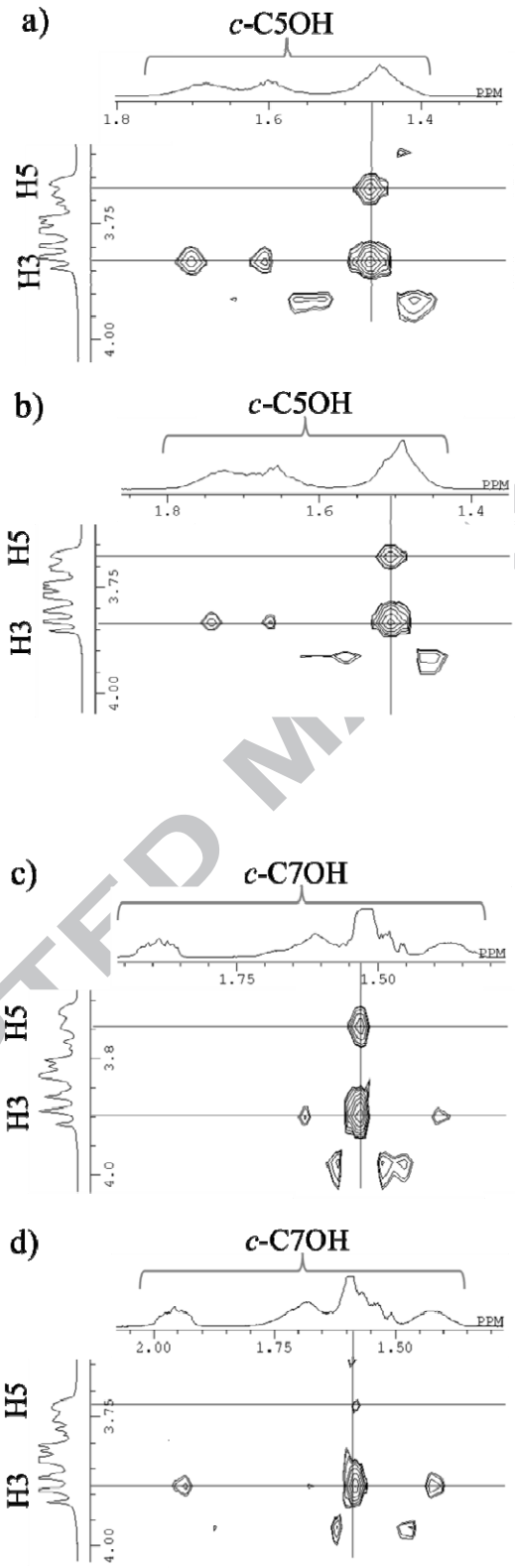
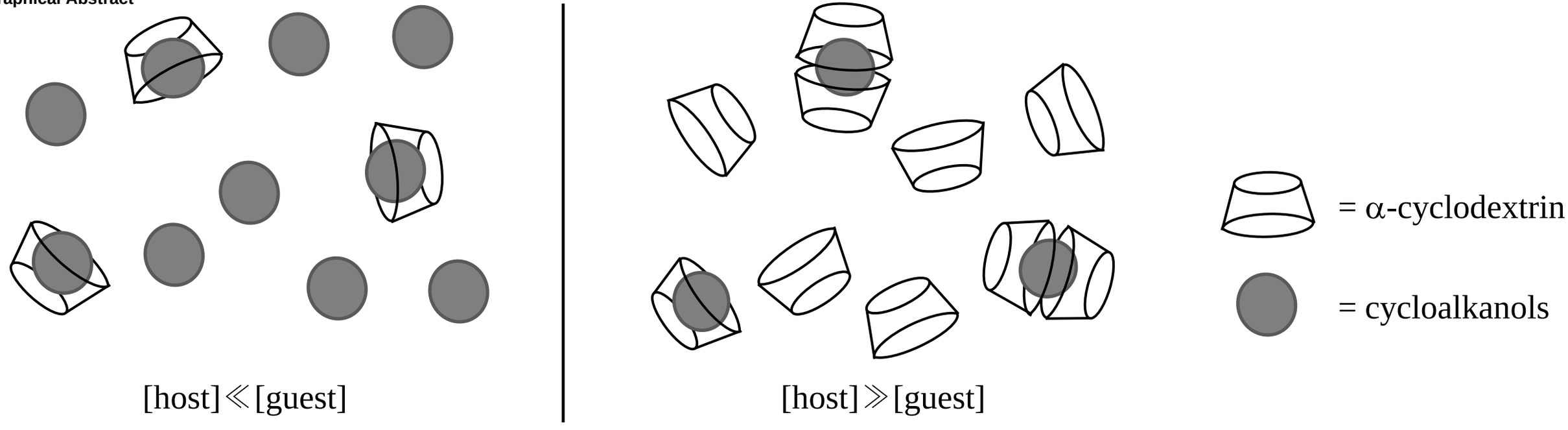


Figure 5. Akita et al.

Graphical Abstract



Cyclohexanol, cycloheptanol, and cyclooctanol form 1:1 and 2:1 (host : guest) inclusion complexes with α-CD under the existence of excessive amount of α-CD. The smaller ring-sized cycloalkanols, cyclobutanol and cyclopentanol, form only 1:1 inclusion complex with α-CD. These facts were revealed by ^{1}H and ^{13}C NMR titration methods. The molecular orientation of the 2:1 inclusion complex was estimated to be formed by the tail-to-tail manner.

Highlights

- Cycloalkanols smaller than *c*-C5OH form only 1:1 inclusion complex with αCD.
- Cycloalkanols larger than *c*-C6OH form 2:1 inclusion complex with αCD.
- Binding constants were determined by means of ^{1}H and ^{13}C NMR titration.
- The 2:1 inclusion complex is stabilized by hydrogen bonding between two αCDs.
- Two αCDs with a guest formed the 2:1 inclusion complex in a tail-to-tail manner.