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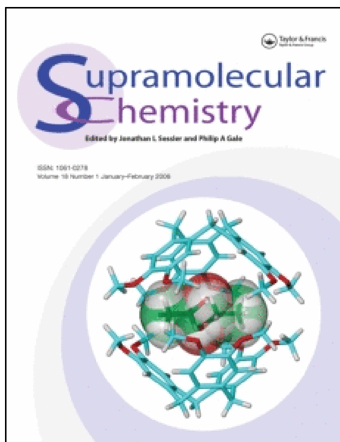

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NMR studies of conformations and molecular recognition of pyridinio-appended and nicotinamide-appended β-cyclodextrin

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NMR studies of conformations and molecular recognition of pyridinio-appended and nicotinamide-appended β-cyclodextrin

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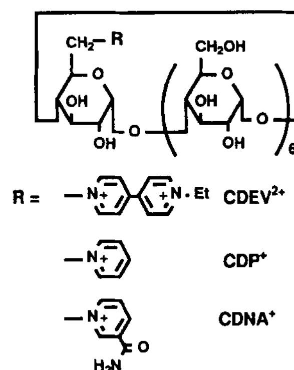
The conformations of pyridinio-appended β-cyclodextrin (CDP^+) and nicotinamide-appended β-cyclodextrin ($CDNA^+$) were studied by NMR spectroscopy. The orientations of the pyridine residue of CDP^+ and the nicotinamide residue of $CDNA^+$ were determined by using a combination of NMR spectroscopic techniques. NMR spectra indicate that the shapes of the cavities of CDP^+ and $CDNA^+$ were changed after forming complexes. This change depended on the shape of the guest. $CDNA^+$ could separate the ^1H resonances at the C_β position of racemic tryptophan into two sets of resonances for each enantiomer.



INTRODUCTION

Cyclodextrin (CD) has a hydrophobic cavity and can form inclusion complexes with many kinds of guests.[1,2] The hydrophobic cavity of CD can be used to control reactions through micro-solvent and steric effects. CD can also be used as a molecular-recognizing host. In order to improve these properties, many modified CDs were synthesized. In many cases orientations of substituents had not been determined. It is necessary to understand the conformation of a modified CD, especially the orientations of the substituents, in order to understand its properties. We recently demonstrated that the combination of one-dimensional (1D) and two-dimensional (2D) NMR was a powerful tool for investigating the conformations of modified CD,[3] and we determined the orientation of the viologen residue of viologen-appended β-cyclodextrin ($CDEV^{2+}$) as a new photosensitized reduction system.[4]

We have been studying nicotinamide-appended β-cyclodextrin ($CDNA^+$) as a NADH model.[5,6] The reduced form of $CDNA^+$ was expected to be used as an asymmetric reductant. In this paper we describe the conformations of $CDNA^+$ and pyridinio-appended β-cyclodextrin (CDP^+), their conformational changes by the addition of guests, and their abilities as molecular recognition hosts.

RESULTS AND DISCUSSION

Structure of CDP^+

Figure 1 shows the 1D ^1H-NMR spectra of $CDEV^{2+}$, CDP^+, and $CDNA^+$. The 1D ^1H-NMR spectrum of modified CD is too complicated to make the assignment of its resonances. The addition of 2D NMR spectra can give much more information. We have already reported the method of assignment and determined the structure of $CDEV^{2+}$.[4] The outline is shown in Figure 2. The structure of CDP^+ was determined in the same way. At first, resonances were grouped into each set of resonances for protons belonging to the same pyranose unit by use of an HOHAHA spectrum. The HOHAHA spectrum is useful for the extraction of the set of resonances for the protons belonging to the same pyranose unit from

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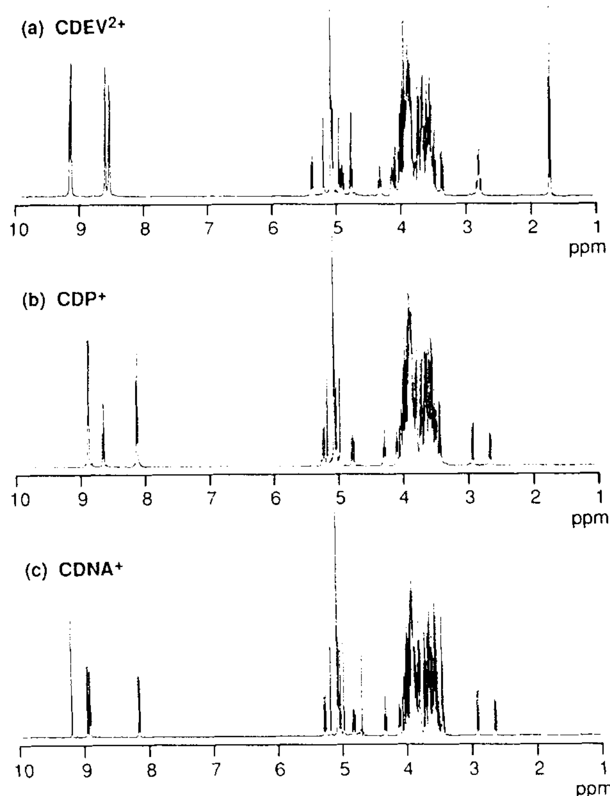

Figure 1 500 MHz ^{1}H-NMR spectra of $CDEV^{2+}$, CDP^+ and $CDNA^+$ in D_2O at 30°C.

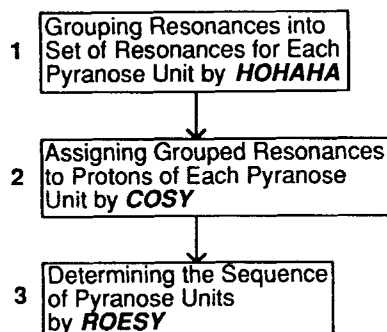

Figure 2 Method of assignment for the ^{1}H-NMR spectrum of modified CD.

an overlapping spectrum region. CDP^+ has resonances for anomeric protons at around 5 ppm. Four were resolved but the other three resonances were overlapping. Therefore the resonances belonging to four pyranose units could be grouped but the other resonances could not be grouped. Next, all grouped resonances were assigned to protons of each pyranose unit by use of the COSY spectrum. Finally, the sequence of pyranose units was determined by the use of the ROESY spectrum. The distance between H_1 of a pyranose unit

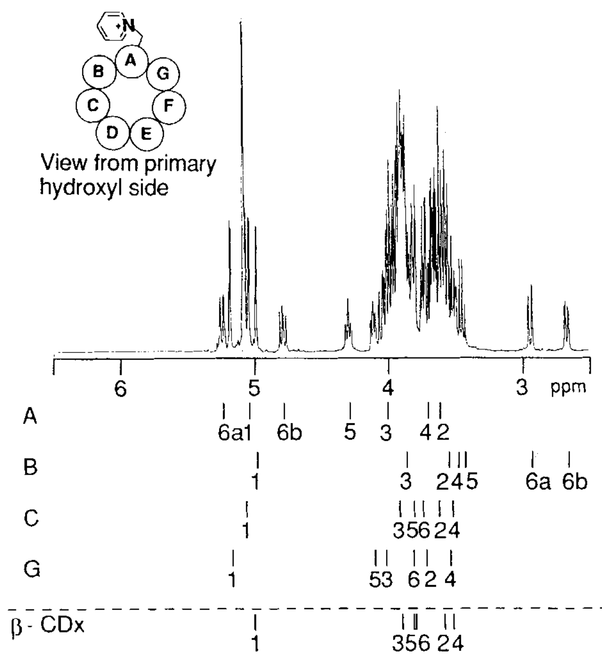

Figure 3 500 MHz ^{1}H-NMR of spectrum of $CDNA^+$ in D_2O at 30°C shown only in the region of the resonances for CD with assignments.

and H_4 of the adjacent pyranose unit is short enough to give an NOE. This NOE was observed by ROESY. ROESY is superior to NOESY to observe NOEs in the case of medium-sized molecules such as CD[7].

The glucose unit modified with a pyridine moiety at the C_6 position was defined as the glucopyranose unit **A** (**G-A**). **G-A** has largely downfield shifted resonances for H_{6a} and H_{6b} due to the modification with the pyridine moiety at the C_6 position. The other glucopyranose units were labelled from **G-B** to **G-G** as shown in Figure 3.* The glucopyranose unit of which the H_4 resonance had the negative crosspeak with the H_1 resonance of **G-A** in the ROESY spectrum was identified as **G-B**. In a similar way, **G-C** and **G-G** could be identified.

The result of the assignment is shown in Figure 3. **G-B** is an unmodified glucopyranose unit but its resonances for H_{6a} and H_{6b} are abnormally shifted upfield (around 2.7 ppm). This shift is probably attributed to the anisotropic ring current effect from the pyridine residue. On the other hand, resonances for H_5 and H_3 of **G-A** and **G-G** are shifted downfield. These shifts are probably due to the electrostatic effect of the pyridinium cation and/or the anisotropic ring current effect from the pyridine residue. These indicate that the pyridine residue faces toward **G-B**.

The conformation around the C_5–C_6 bond was

*Some investigators label glucopyranose units in a clockwise direction as viewed from the primary hydroxyl side.

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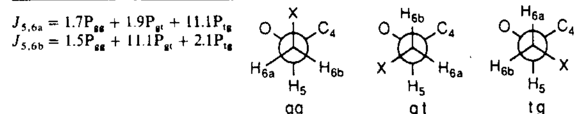
analysed. The three staggered rotamers can be considered as the stable rotamers around the C_5–C_6 bond. The resonances arising from these rotamers cannot be observed separately and only one set of resonances was observed. This may be because the interconversion rate between them was fast on the NMR timescale. In general, only when the interconversion rate between two states is slower than $2^{-1/2}\pi\,\Delta\nu$, can their resonances be observed separately. $\Delta\nu$ is the difference in the chemical shifts (Hz = s^{-1}). Therefore we analysed the resonances as time-averaged. A measured coupling constant, $J_{5,6a}$ or $J_{5,6b}$, is the averaged value of component coupling constants in the three rotamers, gg, gt, and tg, weighted by their fractional populations.[8] On the basis of the observed

Table 1 Calculated fractional populations of rotamers of C_5–C_6 bonds and observed coupling constants ($J_{5,6a}$, $J_{5,6b}$) of **G-A** and **G-B**

		Pgg	*Pgt*	*Ptg*	$J_{5,6a}$	$J_{5,6b}$
$CDEV^{2+}$	**G-A**	0.14	0.86	≈0	1.8	9.8
	G-B	0.58	0.42	≈0	1.8	5.5
CDP^{+}	**G-A**	0.16	0.84	≈0	1.8	9.6
	G-B	0.76	0.23	0.01	1.8	3.7
$CDNA^{+}$	**G-A**	0.12	0.84	0.04	2.2	9.6
	G-B	0.78	0.21	0.01	1.9	3.5

$J_{5,6a} = 1.7P_{gg} + 1.9P_{gt} + 11.1P_{tg}$
$J_{5,6b} = 1.5P_{gg} + 11.1P_{gt} + 2.1P_{tg}$



coupling constants, the populations of rotamers around the C_5–C_6 bond can be analysed. The coupling constants ($J_{5,6a}$ and $J_{5,6b}$) of **G-A** and **G-B** could be determined because their H_6 resonances were separated from the others. The coupling constants of these resonances were estimated with a spin-simulation program on a Varian VXR-500S system as the ABX spin system (Table 1). Both in **G-A** and **G-B**, the rotamer tg scarcely existed because of steric and/or stereo-electronic interactions. It is well known that the rotamer tg of the D-glucopyranose monomer is little present because of unfavourable parallel 1,3-interactions between C_4–O and C_6–O.[8] In **G-A**, the rotamer gt was the major component. This means the pyridine residue faced **G-B**.

From the above considerations and examination of CPK molecular models, the 3D structure of CDP^{+} was estimated as shown in Figure 4. The angle between the long axis of the pyridine moiety and the plane through the primary hydroxy groups of each glucose residue is approximately 30°. The pyridine residue deviates from the centre of the cavity and faces **G-B**. The primary hydroxy side of the cavity has room to include a guest despite modification at the C_6 position.

The structure of CDP^{+} is quite similar to that of $CDEV^{2+}$ (Fig 4), but the shape of the H_6 resonances in **G-B** of the former differs from that in the latter (Fig 5). In **G-B** of $CDEV^{2+}$, the fractional populations of gg and gt are almost the same (Table 1). In **G-B** of CDP^{+}, the rotamer gg is the major component. This difference causes the difference in the shape of

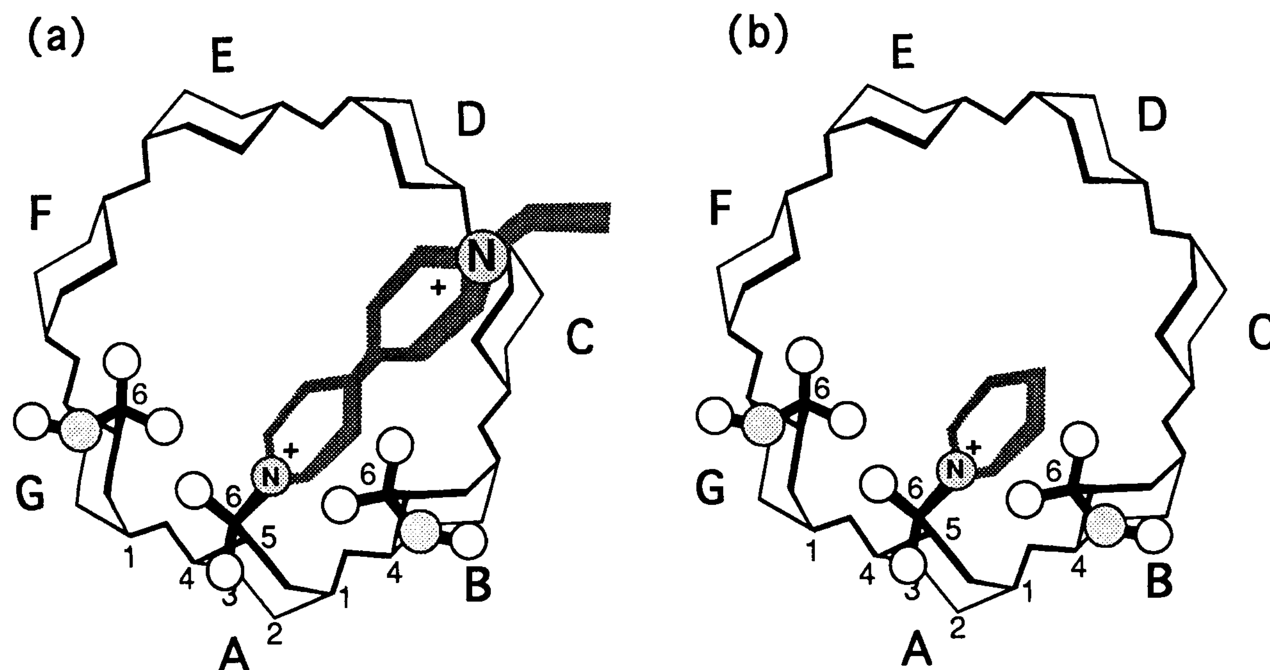


Figure 4 The estimated structures of (*a*) $CDEV^{2+}$ and (*b*) CDP^{+}.

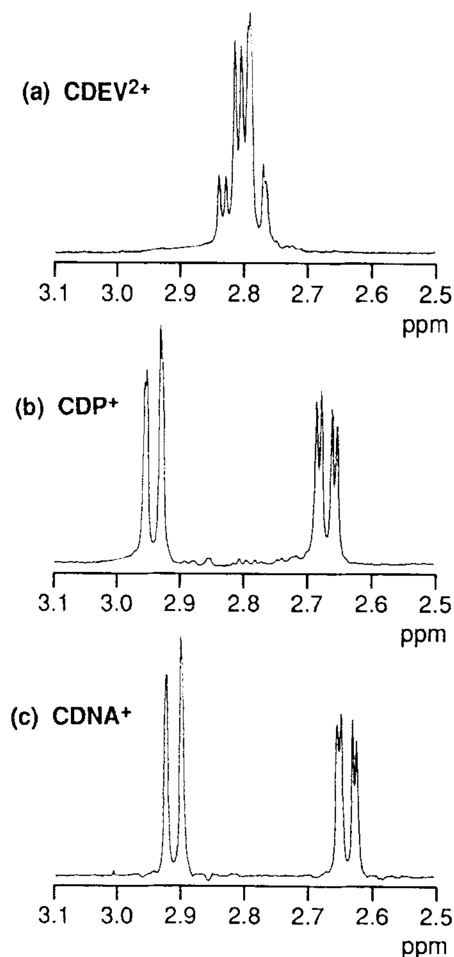


Figure 5 500 MHz ^{1}H-NMR spectra of (*a*) $CDEV^{2+}$, (*b*) CDP^+ and (*c*) $CDNA^+$ in the region of the protons at C_6 in glucopyranose units B in D_2O at 30°C.

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the resonances of H_6 in **G-B**. The hydroxide at C_6 in **G-B** of $CDEV^{2+}$ is located between two pyridinium cations. The pyridinium cation in CDP^+ is located on one side of the hydroxide at the C_6 position of **G-B**. This difference creates the difference of mobility of the hydroxide and its fractional population.

Structure of CDNA⁺

1D and 2D NMR spectra suggest that the pyridine ring of $CDNA^+$ is located in a position similar to that in CDP^+. The pyridine moiety of $CDNA^+$ has an amide group and there are two possible conformations with respect to this group (Fig 6). The ROESY spectrum suggests that both conformations are present. Resonances belonging to each conformation cannot be found separately in the 1D NMR spectrum, and only an averaged resonance is recorded. This phenomenon indicates that reversible changes of conformation are fast on the NMR timescale. It can be presumed that the conformation shown in Fig 6(*a*) is the major one on the basis of the NOE value.

Conformational changes of CDP^+ and $CDNA^+$ by the addition of guests

The conformational changes of CDP^+ and $CDNA^+$ after producing the inclusion complexes were studied. The extent of distortion of a modified CD cavity can be evaluated by making an inclusion complex with 1-adamantane carboxylic acid (ACA).[4] Figures 7 and 8 show the spectra of CDP^+ and $CDNA^+$ in the absence and presence of ACA. After forming the

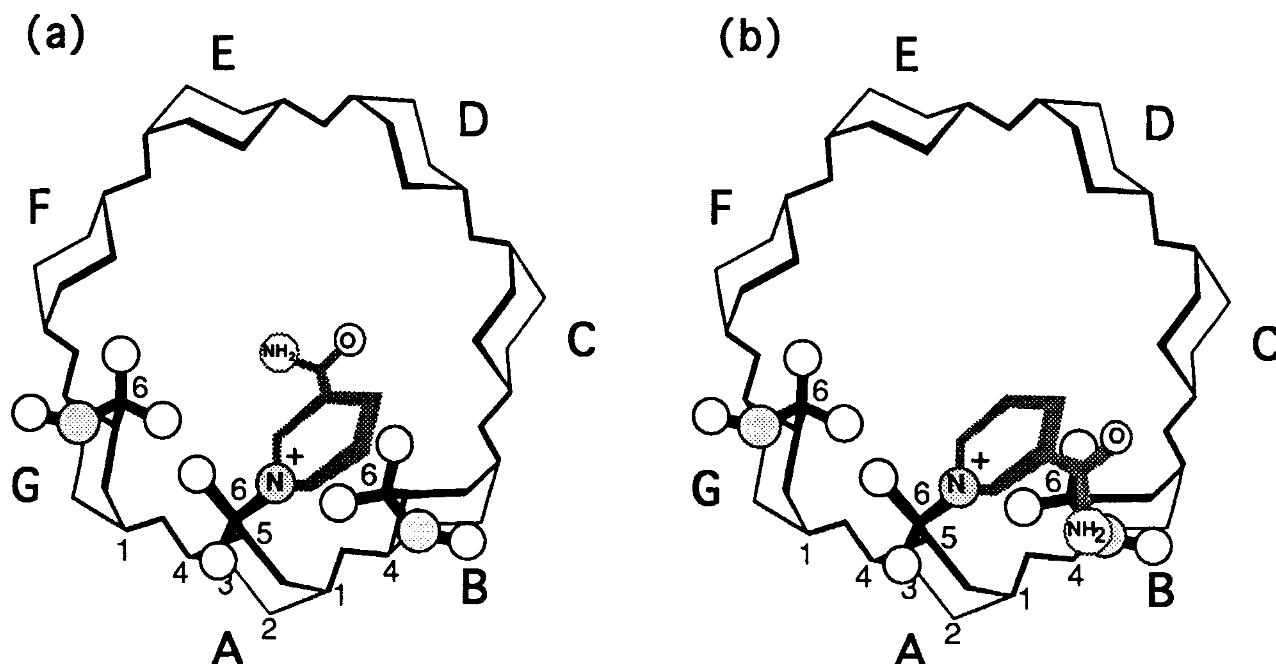

Figure 6 Two possible conformations of $CDNA^+$.

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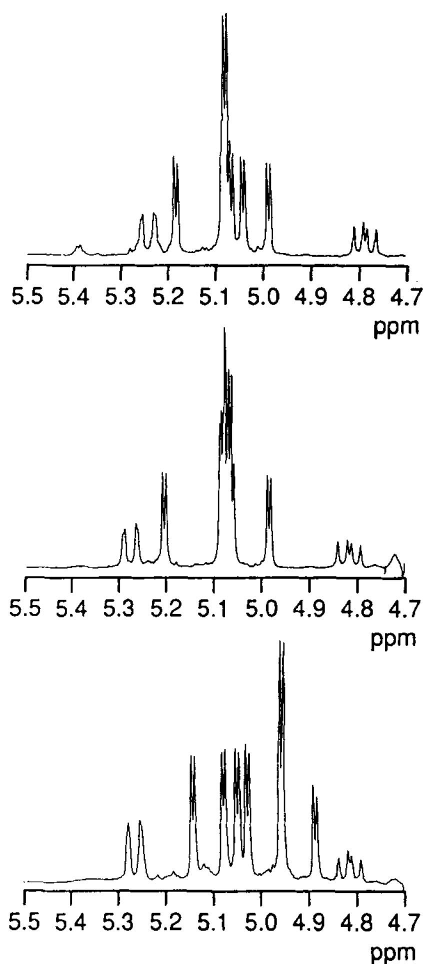


Figure 7 Anomeric proton resonance regions of 500 MHz ^{1}H-NMR spectra of CDP^+ (4×10^{-3} M) in D_2O at 30°C. Top: CDP^+ only; middle: in the presence of ACA (4×10^{-3} M); bottom: in the presence of AQS (4×10^{-3} M).

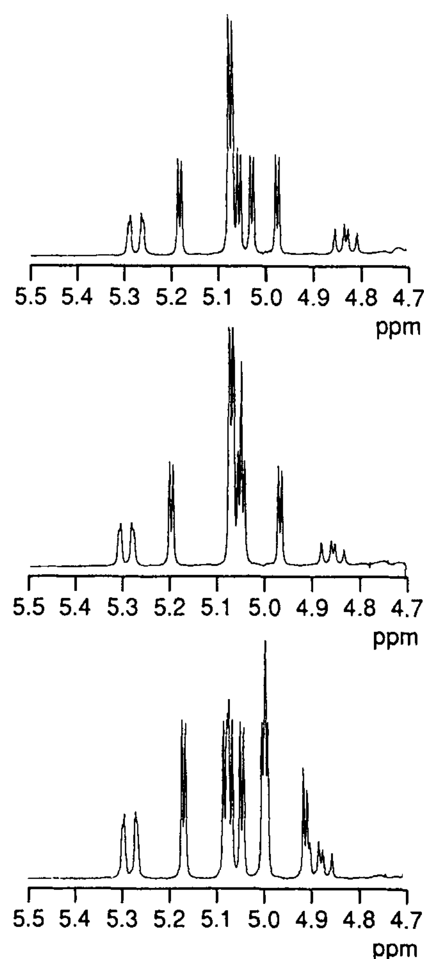
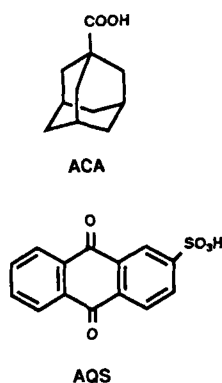


Figure 8 Anomeric proton resonance regions of 500 MHz ^{1}H-NMR spectra of $CDNA^+$ (4×10^{-3} M) in D_2O at 30°C. Top: $CDNA^+$ only; middle: in the presence of ACA (4×10^{-3} M); bottom: in the presence of AQS (4×10^{-3} M).

inclusion complex with ACA the extent of scatter in both the chemical shift of the proton at C_1 of each glucose unit and the dihedral angle between H_1 and H_2 of each glucose unit was small (ACA is a spherical molecule which does not exert any ring current effect). These changes indicated that the cavities of CDP^+ and $CDNA^+$ were distorted before making the



inclusion complex. On the other hand, after forming the inclusion complex with anthraquinone 2-sulphonic acid (AQS) (a planar molecule), the extent of scatter in the chemical shift of H_1 in each glucose unit was large (Figs 7 and 8). If an aromatic guest molecule rotates relatively freely (on the NMR timescale) in the cavity of CD, the magnitude of the anisotropic shielding effect from the aromatic ring to each glucose unit is observed as the same time-averaged value, and the distortion of the cavity cannot be observed by NMR. The degeneracy of the resonance of H_1 was removed after addition of AQS. This suggests that AQS does not rotate freely. This restricted rotation may cause the distortion of the cavity and the unequivalent shielding effect of AQS on each glucose unit. Since the H_1 protons orient outside the cavity, the distortion of the cavity is the main reason for removing the degeneracy of the resonance of H_1. The unequivalent shielding effect of AQS on each glucose unit, if present, is not large.

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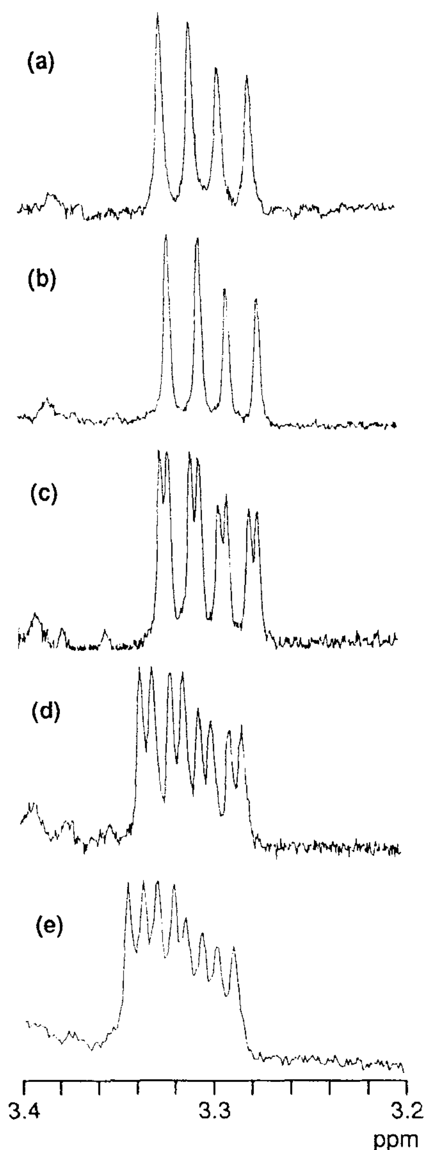
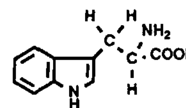


Figure 9 500 MHz ^{1}H-NMR spectra of tryptophan in the region of protons in C_β in D_2O in the presence of $CDNA^+$. (*a*) D-tryptophan at 30°C; (*b*) L-tryptophan at 30°C; (*c*) DL-tryptophan at 30°C; (*d*) DL-tryptophan at 15°C; (*e*) DL-tryptophan at 5°C.

Enantioselective induced shift

The induced shift of the ^{1}H resonance of racemic tryptophan by the addition of $CDNA^+$ was studied. The concentration of DL-tryptophan was slightly lower than that of $CDNA^+$ (2×10^{-3} mol dm^{-3} in D_2O). At 30°C, the resonances for protons at C_β of racemic tryptophan were separated into two sets for each enantiomer in the presence of $CDNA^+$, whereas the enantioselective induced shift was not observed in the presence of CDP^+ (Fig 9). On decreasing the temperature, this difference of induced shifts between enantiomers became larger. At 5°C, the resonances were also separated into two sets in the presence of CDP^+. $CDNA^+$ is superior to CDP^+ for enantioselective induced shift.



CONCLUSION

The conformations of CDP^+ and $CDNA^+$ were studied by using NMR techniques. The long axis of the pyridine moiety is directed nearly perpendicular to the *z*-axis of CDP^+ but deviates from the centre of the cavity. The pyridine moiety faces the glucose unit B. The shape of the resonance of H_6 in **G-B** of CDP^+ was different from that of $CDEV^{2+}$. This difference was caused by the change in fractional population of rotamers of the C_5–C_6 bond of **G-B**. The pyridine ring of $CDNA^+$ is located in a position similar to that in CDP^+. The amide group of $CDNA^+$is mainly situated in the entrance of the cavity. NMR spectra indicate that the shapes of the cavities of CDP^+ and $CDNA^+$ changed after formation of the complex. This change depended on the shape of the guest. Resonances for protons at C_β of racemic tryptophan were separated into two sets for each enantiomer in the presence of $CDNA^+$, even at 30°C.

EXPERIMENTAL SECTION

$CDEV^{2+}$, $CDNA^+$, and CDP^+ were synthesized by the methods previously reported.[5,9] ACA and AQS were purchased from Tokyo Kasei Kogyo, and used without further purification. Deuterium oxide, with an isotopic purity of 99.95%, was purchased from Merck.

NMR spectra were obtained by using a Varian VXR500S spectrometer operating at 499.483 MHz.

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