Conformational Enantiomerism of Bilirubin and Pamoic Acid Induced by Protonated Aminocyclodextrins

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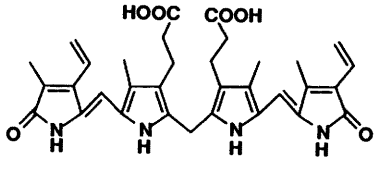
The conformational enantiomerism of (4*Z*,15*Z*)-bilirubin IXα (**1**) induced by protonated heptakis(6-amino-6-deoxy)-β-cyclodextrin (amino-β-CDx) has been studied in aqueous media by means of CD spectroscopy. A bisignate CD Cotton effect suggests that the dianion of **1** associated with protonated amino-β-CDx selectively takes a conformation with (*P*)-helicity. The results clearly indicate that the electrostatic binding between the CO_2^- groups of **1** and the NH_3^+ groups of amino-β-CDx and the simultaneous inclusion of a dipyrrinone moiety of **1** into the chiral CDx cavity are essential for the conformational enantiomerism of **1**. This has also been applied to the amino-γ-CDx-induced conformational enantiomerism of pamoic acid.

Cyclodextrins (CDxs) are known to be hosts which can recognize the chiralities of some organic guest compounds.[1] The first report on chiral recognition by CDx was presented by Cramer and Dietsche[2] who studied the partial optical resolution of mandelic acid derivatives by β-CDx. Similar optical resolution by β-CDx has been observed for chiral phosphinate[3] and sulfinyl compounds.[4] In the study on the sulfinyl compounds by β-CDx, Mikołajczyk and Drabowicz[4] demonstrated a mechanism for chiral recognition involving hydrogen bonding and steric hindrance between the host and guest molecules and a simultaneous inclusion of a phenyl ring of the guest into the β-CDx cavity. Such a mechanism can be classified into a 'three-point attachment model' for chiral recognition.[5] The problem in this mechanism is the intermolecular hydrogen bonding interaction in water. Although hydrogen bonding has been assumed as one of the binding forces for forming inclusion complexes of CDxs,[4b,6] no direct evidence has been presented. In general, intermolecular hydrogen bonding between small molecules is very unlikely to occur in water because of strong hydration at hydrogen bonding sites of solutes. However, recent studies demonstrate that the hydrogen bonding interaction is possible even in water when a hydrophobic environment is placed in the vicinity of the hydrogen bonding sites.[7] We have reported the formation of the hydrogen bonded complex of β-CDx and (4*Z*,15*Z*)-bilirubin IXα (**1**).[8] Although **1** itself is an achiral compound, it becomes



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optically active when the conformation of **1** is fixed by complexing with α-, β- and γ-CDxs.[9] We have studied the mechanism for this conformational enantiomerism.[8b] The results reveal that hydrogen bonding between the CO_2^- groups of **1** and the secondary OH groups of CDx is very important to demonstrate the enantioselective complexation of **1** with CDx and that the CDx cavity does not play an essential role (Fig. 1). The CDx cavity seems to contribute to this system by providing a hydrophobic environment in the vicinity of the secondary OH groups. The three-point attachment model may explain the conformational enantiomerism of **1**.

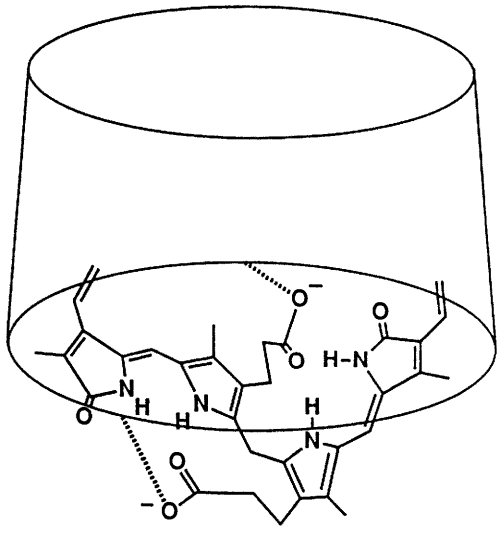
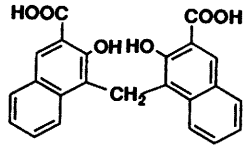


Fig. 1 Hydrogen-bonded complex of **1** and β-CDx assumed in the previous paper.[9b] The CO_2^- groups of **1** interact with the secondary OH groups of β-CDx through hydrogen bonding in water.

Similar conformational enantiomerism has been observed in the complexation of dinaphthylmethane derivatives such as pamoic acid [**2**, 4,4'-methylenebis(3-hydroxynaphthalene-



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2-carboxylic acid)] with γ-CDx.[10] γ-CDx induces the conformational enantiomerism of 1,1'-dinaphthylmethane which does not have any hydrogen bonding site, suggesting that a 'lock and key' mechanism is adequate to explain this conformational enantiomerism. The formation of the chiral pyrene excimer in the γ-CDx cavity is also classified into this category.[11] Although recent studies on the aminoacyl-tRNA synthetase demonstrate the molecular recognition by energy dissipation which surpasses the capabilities of the lock-and-key type recognition,[12] the lock-and-key concept presented by

Fischer[13] in 1894 is still applicable to molecular recognition by CDxs.[14] The chiral recognition of binaphthyl derivatives having axial chiralities by permethylated β-CDx[15] may also be explained by the lock-and-key mechanism.

The three-point attachment model is more general as the mechanism for chiral discrimination than the lock-and-key mechanism. Although hydrogen bonding can be used widely as the force for the point attachment in organic solvents,[16] the use of this interaction is strictly restricted in aqueous media. Thus, we tried to use an electrostatic interaction in place of hydrogen bonding in this study. Protonated heptakis(6-amino-6-deoxy)-β-CDx (amino-β-CDx) and octakis(6-amino-6-deoxy)-γ-CDx (amino-γ-CDx) were used as the cationic hosts which can electrostatically associate with the anions of **1** and **2**, respectively. The conclusion of the present study is very simple. Since the NH_3^+ groups are attached to the achiral carbons at the 6 positions of the glucopyranoses, incorporation of a part of the **1** or **2** molecule into the chiral CDx cavity is necessary to induce the conformational enantiomerism. In the present system, the electrostatic interaction between the host and the guest and the simultaneous inclusion of the guest into the asymmetric cavity of the host are essential for the conformational enantiomerism. A similar concept has also been used in chiral recognition by Lehn and his co-workers.[17] They carried out NMR and extraction studies on the enantioselective complexation of aromatic carboxylates with an optically active abiotic receptor containing a guanidinium binding site.

Results and discussion

Conformational enantiomerism of 1

The CD spectra of the dianion of **1** in water in the presence of β-CDx (pH 10.8) and amino-β-CDx (pH 8.1) are shown in Fig. 2. The intense bisignate CD was observed for the **1**–amino-β-CDx complex while the β-CDx complex shows a weak and oppositely signed bisignate CD. The signs of the CD induced by amino-β-CDx are positive and negative at longer and shorter wavelengths, respectively. Such a (+) to (−) bisignate CD spectrum indicates that the dipyrrinone moieties of **1** bound to amino-β-CDx selectively take a (*R*)-helix conformation [(*P*)-helicity].[18] The absolute values of the molar circular dichroism ($\Delta\varepsilon = [\theta]/3300$ dm^3 mol^{-1} cm^{-1}) for the amino-β-CDx complex ($\Delta\varepsilon$ = 46 at 465 nm and −35 at 411 nm) are about seven times larger than those for the β-CDx complex. The CD intensities of the **1**–amino-β-CDx complex are almost comparable to those of **1** bound to human serum albumin[19] and are much larger than those for **1** in sodium deoxycholate micelle[7b,20] and for the **1**–peptide complexes in water.[21] The CD intensities of the present complex are much smaller than those of the **1**–(*S*)-2-aminobutane[22] and **1**–quinine complexes[23] in organic solvents.

Of course, the CD intensity depends on the binding constant (*K*) of the **1**–CDx complex. Thus, the *K* values for the complexes of **1** were determined from the Benesi–Hildebrand plots[24] for the dependency of the CD intensity on [CDx]. A good linear relationship between $\Delta\varepsilon^{-1}$ and $[CDx]^{-1}$ (a correlation coefficient, r = 0.9992) provides 417 dm^3 mol^{-1} of the *K* value for the **1**–amino-β-CDx 1 : 1 complex at pH 7.2. This *K* value is much larger than that for the **1**–β-CDx complex at pH 10.8 (K = 23 dm^3 mol^{-1}). Since amino-β-CDx precipitates, the *K* value could not be determined at higher pH values. The larger *K* value for the amino-β-CDx complex suggests an electrostatic association between the host and the guest.

The pH titration curves obtained by monitoring the CD intensity of **1** in water in the presence of β-CDx and amino-β-CDx are shown in Fig. 3. The pK_a values of **1** in water have been estimated to be 8.12 and 8.44 using a rapid partition method.[25] The apparent pK_1 and pK_2 values of **1** are significantly higher

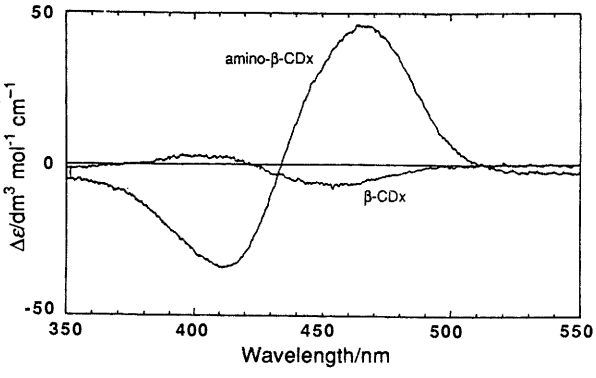

Fig. 2 CD spectra of **1** (2.5×10^{-5} mol dm^{-3}) in water containing amino-β-CDx (pH 8.1) and β-CDx (pH 10.8) at 25 °C. The concentration of CDxs was 0.01 mol dm^{-3}. NaOH was used to adjust pH. The measurements were carried out under a nitrogen atmosphere.

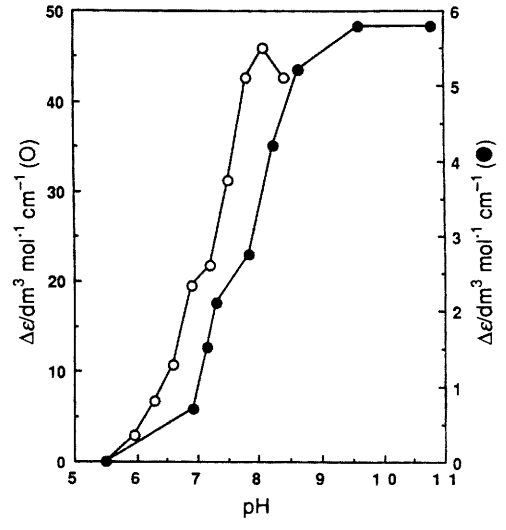

Fig. 3 Plots of $\Delta\varepsilon$ *vs.* pH for the **1**–amino-β-CDx (○) and **1**–β-CDx systems (●) at 25 °C. The concentrations of **1** and CDxs were 2.5×10^{-5} and 0.01 mol dm^{-3}, respectively. NaOH and HCl were used to adjust pH.

than those of common dicarboxylic acids. The acid–base equilibria of **1** have not been understood completely. In any event, the present results indicate that only the dissociated form of **1** becomes optically active upon complexation with amino-β-CDx or β-CDx. In the previous work, we concluded that **1** complexes with β-CDx through a hydrogen bonding interaction between the CO_2^- groups of **1** and the secondary OH groups of β-CDx (see Fig. 1).[8b] The oppositely signed CD with larger $\Delta\varepsilon$ as well as the larger *K* value suggest that the electrostatic interaction between the NH_3^+ groups of amino-β-CDx[26] and the CO_2^- groups of **1** is important for the amino-β-CDx-induced conformational enantiomerism of **1**.

Table 1 shows the effects of the aliphatic carboxylate anions on the CD intensity of **1** in water in the presence of amino-β-CDx and β-CDx. The signals of the amino-β-CDx-induced CD of **1** are weakened upon addition of the co-existing guest anions. This can be interpreted in terms of replacement of the dianion of **1** with the added carboxylate anion at the NH_3^+ side of amino-β-CDx. Meanwhile, the β-CDx-induced CD of **1** is not affected by added carboxylate anions. The aliphatic carboxylate anions do not interact strongly with β-CDx because of their highly amphiphilic nature. Interesting effects of the co-existing guest anion were found in the case of an alicyclic carboxylate, adamantane-1-carboxylate. The CD signals of **1** bound to

Table 1 Relative CD intensities ($\Delta\varepsilon_{rel}$) of **1** (2.5×10^{-5} mol dm^{-3}) in water containing amino-β-CDx and β-CDx (0.01 mol dm^{-3}) in the presence of aliphatic and alicyclic carboxylate anions at 25 °C

CDx[a]	Carboxylic acid/mol dm^{-3}	$\Delta\varepsilon_{rel}$[b]
Amino-β-CDx	None	1.0
Amino-β-CDx	Acetic acid (0.1)	0.58
Amino-β-CDx	Acetic acid (0.5)	0.27
Amino-β-CDx	Propanoic acid (0.1)	0.55
Amino-β-CDx	Propanoic acid (0.5)	0.09
Amino-β-CDx	Butanoic acid (0.05)	0.24
Amino-β-CDx	Butanoic acid (0.1)	0.21
Amino-β-CDx	Pentanoic acid (0.05)	0.21
Amino-β-CDx	Pentanoic acid (0.1)	0
Amino-β-CDx	Adamantane-1-carboxylic acid (0.001)	0.89
Amino-β-CDx	Adamantane-1-carboxylic acid (0.005)	0.46
Amino-β-CDx	Adamantane-1-carboxylic acid (0.01)	0
β-CDx	Acetic acid (0.5)	1.16
β-CDx	Propanoic acid (0.5)	1.01
β-CDx	Butanoic acid (0.5)	1.12
β-CDx	Pentanoic acid (0.5)	1.01
β-CDx	Adamantane-1-carboxylic acid (0.005)	2.00
β-CDx	Adamantane-1-carboxylic acid (0.01)	2.97

[a] The measurements were carried out in anaerobic water at pH 7.2 and 10.8 for amino-β-CDx and β-CDx, respectively. The pH values were adjusted by NaOH. [b] The CD intensities of **1** were measured at the band where the first Cotton effect was observed.

Table 2 Relative CD intensities ($\Delta\varepsilon_{rel}$) of **1** (2.5×10^{-5} mol dm^{-3}) in water containing amino-β-CDx and β-CDx (0.01 mol dm^{-3}) in the presence of aliphatic alcohols at 25 °C

CDx[a]	Alcohol/mol dm^{-3}	$\Delta\varepsilon_{rel}$[b]
Amino-β-CDx	None	1.0
Amino-β-CDx	Methanol (0.5)	0.85
Amino-β-CDx	Ethanol (0.5)	0.85
Amino-β-CDx	Propan-1-ol (0.5)	0.65
Amino-β-CDx	Butan-1-ol (0.5)	0.64
Amino-β-CDx	Pentan-1-ol (0.05)	0.59
Amino-β-CDx	Pentan-1-ol (0.1)	0.53
Amino-β-CDx	Cyclohexanol (0.005)	0.67
Amino-β-CDx	Cyclohexanol (0.01)	0.48
Amino-β-CDx	Cyclohexanol (0.02)	0.32
Amino-β-CDx	Cyclohexanol (0.03)	0.24
Amino-β-CDx	Cyclohexanol (0.05)	0.19
β-CDx	Methanol (0.01)	1.05
β-CDx	Ethanol (0.01)	1.05
β-CDx	Butan-1-ol (0.01)	1.28
β-CDx	Pentan-1-ol (0.01)	1.74
β-CDx	Hexan-1-ol (0.01)	2.24
β-CDx	Octan-1-ol (0.005)	2.34
β-CDx	Cyclohexanol (0.04)	2.49
β-CDx	Cyclooctanol (0.005)	3.15
β-CDx	Cyclooctanol (0.01)	3.97

[a] The measurements were carried out in anaerobic water at pH 7.2 and 10.8 for amino-β-CDx and β-CDx, respectively. The pH values were adjusted by NaOH. [b] The CD intensities of **1** were measured at the band where the first Cotton effect was observed.

amino-β-CDx are diminished upon addition of this bulky carboxylate while those bound to β-CDx are enhanced. Similar effects of the co-existing guests were observed when aliphatic alcohols[27] were used in place of the carboxylates (see Table 2). Addition of aliphatic alcohol also weakens the amino-β-CDx-induced CD of **1**. On the contrary, the β-CDx-induced CD is enhanced upon addition of alcohols as reported previously.[8b] Enhancement of the CD intensities in the case of the **1**–β-CDx system has been explained reasonably by assuming the complex to have a structure exhibited in Fig. 1.[8b] Namely, the co-existing guest such as aliphatic alcohol or adamantane-1-carboxylate[28] is included in the β-CDx cavity to increase the hydrophobicity at the rims of the β-CDx cavity. Such an inclusion complex

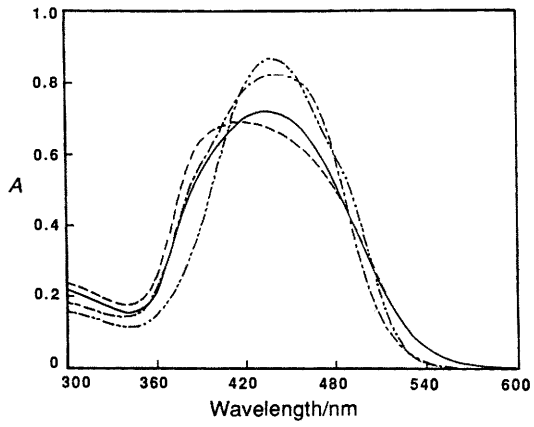

Fig. 4 UV–VIS absorption spectra of **1** in water at pH 7.2 in the absence (— · · —) and the presence of amino-α-CDx (– – – –), amino-β-CDx (— · —) and amino-γ-CDx (——) at 25 °C. The concentrations of **1** and CDxs were 2.5×10^{-5} and 0.01 mol dm^{-3}, respectively.

provides a more preferable environment for forming hydrogen bonds between the CO_2^- groups of **1** and the secondary OH groups of β-CDx. The inclusion of **1** into the β-CDx cavity is not necessary for the conformational enantiomerism of **1**. Meanwhile, the opposite effects of adamantane-1-carboxylate and the aliphatic alcohols on the amino-β-CDx-induced CD are observed. These findings clearly demonstrate that the inclusion of **1** into the CDx cavity is essential for the conformational enantiomerism of **1**. ^{1}H NMR spectroscopy reveals that cyclohexanol, for example, is completely included into the amino-β-CDx cavity. It can be concluded, therefore, that the co-existing guest expels **1** from the amino-β-CDx cavity. Since the NH_3^+ groups are attached to the achiral carbons at the 6-positions of the glucopyranose units, the inclusion of a part of the molecule **1** into the chiral CDx cavity has to take place for this conformational enantiomerism. The Corey–Pauling–Koltun (CPK) molecular model demonstrates that inclusion of both dipyrrinone moieties in a CDx cavity is sterically implausible.

An NMR study is one of the best ways to determine the structure of the inclusion complex in solution. Unfortunately, however, the NMR measurements were limited by precipitation of the **1**–amino-β-CDx complex at higher concentrations. So, we applied a chemical method to clarify the structure of the complex and the mechanism of the conformational enantiomerism. We used hexakis(6-amino-6-deoxy)-α-CDx (amino-α-CDx), amino-γ-CDx and heptakis(2,3-di-*O*-methyl-6-amino-6-deoxy)-β-CDx (aminoDMe-β-CDx) as the hosts, which have different characteristics from those of amino-β-CDx. None of these aminocyclodextrins induce CD of **1** at all, though **1** associates with these aminocyclodextrins. Fig. 4 shows the UV–VIS absorption spectra of **1** in water at pH 7.2 in the presence of aminocyclodextrins. Each spectrum of **1** in the presence of aminocyclodextrin is different from that of **1** in water without CDx, indicating that **1** interacts with aminocyclodextrins. The absorption spectrum of **1** in the presence of amino-α-CDx is significantly different from that of **1** in the presence of amino-β-CDx, as shown in Fig. 4. However, the absorption spectrum of **1** in the presence of both amino-β-CDx and cyclooctanol is quite similar to that of **1** in the presence of amino-α-CDx. Such spectroscopic behaviour can be explained by the formation of association complexes. The pigment **1** is electrostatically bound to amino-α-CDx or cyclooctanol-bearing amino-β-CDx at the outside of the CDx cavity. The absorption spectrum of **1** in water containing amino-γ-CDx is somewhat similar to that of **1** bound to amino-β-CDx. The pigment **1** may be included into the amino-γ-CDx cavity. It seems, however, that the size of the amino-γ-CDx cavity is too

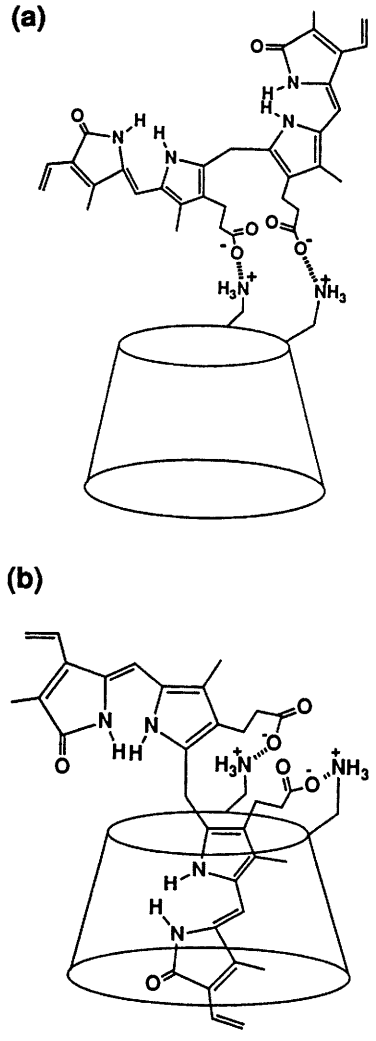

Fig. 5 Plausible structures of (*a*) 1-amino-α-CDx complex and (*b*) 1-amino-β-CDx complex

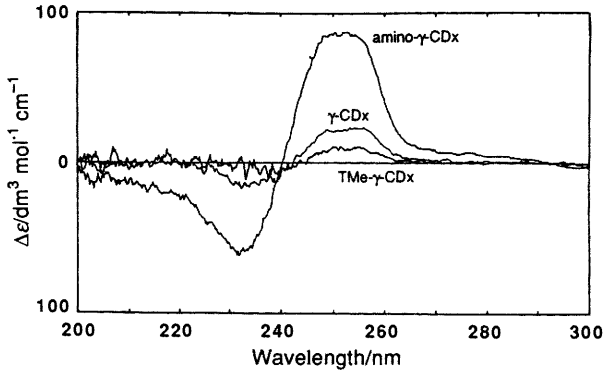

Fig. 6 CD spectra of **2** (2.0×10^{-5} mol dm^{-3}) in water at pH 5.5 in the presence of γ-CDx, TMe-γ-CDx and amino-γ-CDx (1.0×10^{-4} mol dm^{-3})

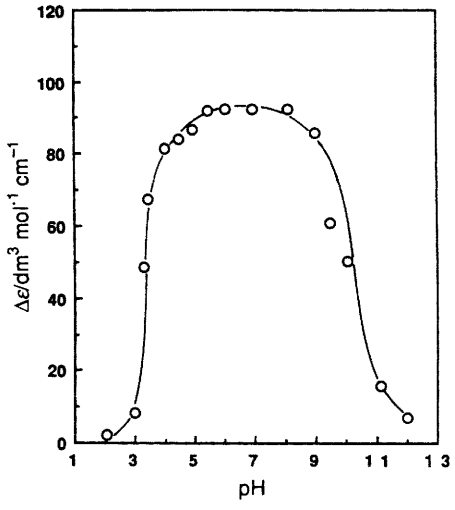

Fig. 7 Plot of Δε *vs.* pH for the **2**–amino-γ-CDx systems at 25 °C. The concentrations of **2** and amino-γ-CDx were 2.0×10^{-5} and 1.0×10^{-4} mol dm^{-3}, respectively.

large to fix the conformation of **1**. It is noteworthy that no CD is measured in the **1**–aminoDMe-β-CDx complex. The macrocyclic conformations of natural CDxs are stabilized by the intramolecular hydrogen bonds between the O(2) and O(3) hydroxy groups of adjacent glucopyranose units.[29] Since an intramolecular hydrogen bond is not formed, the cavity of aminoDMe-β-CDx should be more fluctuating than that of β-CDx or amino-β-CDx. The fluctuation of the CDx structure diminishes the asymmetric nature of the CDx cavity.

On the basis of these results, it can be concluded that **1** becomes optically active when two CO_2^- groups of **1** electrostatically interact with two NH_3^+ groups of amino-β-CDx and a part of the molecule **1** is included simultaneously and tightly into the asymmetric CDx cavity. Such a mode for the CDx-induced conformational enantiomerism has not been reported. All of the results suggest that the structure of the amino-β-CDx complex of **1** has (*P*)-helicity as shown in Fig. 5. The structure of the achiral 1-amino-α-CDx complex is also shown in Fig. 5.

Conformational enantiomerism of 2

In order to examine the generality of the new mode for CDx-induced conformational enantiomerism, the complexation of **2** with protonated aminocyclodextrins has also been studied. As in **1**, **2** has two chromophores connected to each other with an achiral methylene bridge. In the previous paper,[10] we reported that the conformation of **2** is fixed as (*P*)-helicity when it is included into the γ-CDx cavity. The 'lock-and-key' mechanism has been assumed as the mechanism for this conformational enantiomerism.

Fig. 6 shows the CD spectra of **2** in water at pH 5.5 in the presence of amino-γ-CDx, γ-CDx and octakis(2,3,6-tri-*O*-methyl)-γ-CDx (TMe-γ-CDx). Even at low CDx concentration (1×10^{-4} mol dm^{-3}), very intense (+) to (−) bisignate CD was measured in the aqueous amino-γ-CDx solution. The exciton-coupling theory suggests that **2** bound to amino-γ-CDx has (*P*)-helicity. The extremely intense CD suggests that the electrostatic interaction between the CO_2^- groups of **2** and the NH_3^+ groups of amino-γ-CDx is very important to induce the conformational enantiomerism of **2** by amino-γ-CDx. The pH–CD intensity profile is shown in Fig. 7. A bell-type profile is ascribed to the acid–base equilibria of both **2** and amino-γ-CDx. The intramolecular hydrogen bonds between the CO_2^- groups and the adjacent OH groups cause a lowering of the pK_1 ($pK_1 < 3$) of **2**.[10] The decrease in the CD intensity at lower pH (pH < 5) is due to the decrease of the amounts of dissociated **2**. The reduction in the CD intensity at higher pH (pH > 8) is ascribed to the deprotonation from the conjugate acid of amino-γ-CDx.

Neither amino-α- nor -β-CDxs induce bisignate CD. This means that the conformational enantiomerism is achieved by

the inclusion of **2** into the CDx cavity. The size of **2** is too large to be included in the cavities of α- and β-CDxs.[10]

Because of the precipitation of the **2**–amino-γ-CDx complex, ^{1}H NMR measurements could not be carried out at high concentrations of the host and the guest. So, we measured the ^{1}H NMR spectrum of the equimolar solution of **2** and amino-γ-CDx at a relatively low concentration (1×10^{-4} mol dm^{-3}) in D_2O at pD 5.5. Upon addition of **2**, all signals due to amino-γ-CDx are shifted to higher magnetic fields and broadened. The upfield shifts of all CDx signals are somewhat strange for the formation of a simple inclusion complex. In general, the inclusion of an aromatic compound into a CDx cavity causes upfield shifts of the protons at the 3 and 5 positions of the CDx. In such a case, the shifts of other protons are relatively small. Therefore, ^{1}H NMR spectroscopy of the present system suggests the formation of complexes having stoichiometries other than 1 : 1. All signals due to **2** are shifted to lower magnetic fields and are broadened upon addition of amino-γ-CDx. The protons at the 7, 6, 8, 5 and 4 positions of **2** in D_2O appear at 7.15, 7.25, 7.74, 7.94 and 8.27 ppm, respectively. Upon addition of amino-γ-CDx, these protons shift to 7.4, 7.5, 7.9, 8.2 and 8.4 ppm, respectively. Line broadening is not observed in the complexation of **2** with γ-CDx.[10] Complexes of **2**–amino-γ-CDx, having complex stoichiometries, should be formed in this system. Although the structure of the complex could not be determined in this study, we can conclude that the conformational enantiomerism of **2** by amino-γ-CDx is promoted through both electrostatic interaction between the host and the guest and the inclusion of the guest into the chiral host cavity.

The present paper reports a new mode for conformational enantiomerism induced by CDx. An anionic guest molecule is captured through an electrostatic interaction by a cationic host and an induced chirality of the guest is determined when a part of the guest molecule is included in an asymmetric cavity of the host. Such a mechanism is classified as three-point attachment.

The electrostatic interactions have recently been of interest in the field of supramolecular chemistry.[17,30] Echavarren *et al.*[17] prepared chiral receptors having a guanidine subunit and naphthalene moieties whose protonated forms recognize the chirality of *N*-acetyltryptophan. The association complex is formed through an electrostatic interaction between the protonated receptor and the *N*-acetyltryptophan anion. The steric hindrance due to the naphthalene moieties connected to the chiral centres of the receptor inhibits the formation of an enantiomer of the guest anion. The mode of conformational enantiomerism of **1** or **2** induced by protonated aminocyclodextrin is completely the same as that reported by Echavarren *et al.*

Aminocyclodextrins are very interesting hosts to study molecular recognition using electrostatic interactions. The first synthesis of amino-β-CDx was done by Tsujihara *et al.*[31] A modified method for preparing aminocyclodextrins has been presented.[32] Inclusion phenomena,[33] catalytic activities[34] and monolayer behaviour[35] of aminocyclodextrins have been studied so far. Quite recently, enantioselective binding of 2-phenylpropanoic acid and its dissociated anion with mono-amino-β-CDx has been reported without a description of the mechanism.[36] Eliseev and Schneider[37] reported molecular recognition of nucleotides, nucleosides and sugars by amino-cyclodextrins. The electrostatic binding between the NH_3^+ group of an aminocyclodextrin and the OPO_3^- group of a nucleotide and the inclusion of the saccharide residue through intermolecular hydrogen bonding have been assumed as the forces for molecular recognition. Since a hydrogen bond is hardly formed in water, the electrostatic interaction would be used widely as a force for molecular recognition in aqueous media.

Experimental

The heptahydrochloride salt of amino-β-CDx was prepared according to the procedures described in the literature.[32] The HCl salts of amino-α-CDx and amino-γ-CDx were also prepared by the same method.[32] Heptakis(2,3-di-*O*-methyl-6-amino-6-deoxy)-β-CDx (aminoDMe-β-CDx) was prepared by *O*-methylation of amino-β-CDx using the same method reported in the literature.[32] Octakis(2,3,6-tri-*O*-methyl)-γ-CDx (TMe-γ-CDx) was synthesized using a Hakomori method.[38] Other CDxs (Nacalai) were purchased and washed with THF using a Soxhlet extractor. The bile pigment **1** (Sigma) was dissolved in chloroform and washed with aq. $NaHCO_3$. The yellow–orange residue obtained after evaporation of chloroform was washed again with a small amount of methanol. Since **1** is easily oxidized under aerobic conditions, all sample solutions containing **1** were saturated with the N_2 gas. **2** (Aldrich) was recrystallized from ethanol.

UV–VIS absorption spectra were taken at 25 °C. ^{1}H NMR spectra (400 MHz) were measured on a JEOL GX-400 spectrometer at 23 °C. CD spectra were recorded using a Jasco J-500A spectropolarimeter, having a data processor, at 25 °C.

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