Chiral recognition of α-amino acids by charged cyclodextrins through cooperative effects of Coulomb interaction and inclusion

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Chiral recognition of α-amino acid derivatives by charged β-cyclodextrins has been studied by means of ^{1}H NMR spectroscopy. Protonated heptakis(6-amino-6-deoxy)-β-cyclodextrin (per-NH_3^+-β-CD) forms complexes with the (*S*)-enantiomers of *N*-acetylated Trp, Phe, Leu and Val in their anionic forms more preferentially than the (*R*)-enantiomers, though the difference in the binding constants (*K*) between the enantiomers is small. Inclusion of the guest into the host cavity and intermolecular Coulomb interactions participate cooperatively in complexation. Monoaminated β-cyclodextrin (mono-NH_3^+-β-CD) also recognizes the chirality of the amino acids while native cyclodextrins such as α- and β-cyclodextrins do not. The *K* values for the per-NH_3^+-β-CD–guest anion complexes are much larger than those for the mono-NH_3^+-β-CD complexes.

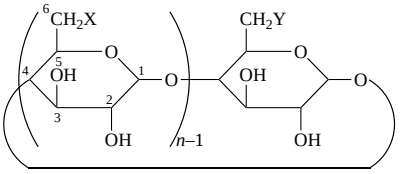
The large *K* value of the per-NH_3^+-β-CD–(*S*)-AcTrp complex is ascribed to a large positive ΔS value which might be due to extended dehydration of both host and guest during complexation. The enantioselective complexation was also found in the system composed of the α-amino acid methyl esters in the cationic forms and heptakis[6-(2-thioglycolic acid)-6-deoxy]-β-cyclodextrin in the anionic form where the (*R*)-enantiomers were preferable guests. The present study demonstrates the advantage of Coulomb interactions as an attractive force in recognition of central chirality in host–guest chemistry.

Recognition of central chirality by β-cyclodextrin (β-CD) was reported for the first time by Cramer and Dietsche.[1] Following their work, a number of studies on chiral recognition by native CDs and their derivatives have been reported.[2] Quite recently, Liu *et al*. reported the extraordinarily high enantioselectivities in the complexation of native amino acids with 6^A-(*m*-toluidinyl)-6^A-deoxy-β-CD[3] or 6^A-*O*-ethoxyhydroxyphosphoryl-β-CD.[4] For example, the difference in the free-energy changes for complexation between the enantiomers ($\Delta\Delta G$) of leucine (Leu) is 8.5 kJ mol^{-1}.[3] In most cases, however, enantioselectivities of native and/or modified CDs for guests having an asymmetric carbon are modest at best. An exceptional example other than the case reported by Liu *et al*.[3,4] is the partial optical resolution of sulfinyl compounds by β-CD.[5] In the case of isopropoxyl methyl sulfoxide, the optical purity of the resolved sulfoxide is 68%. Hydrogen bonding, inclusion and steric hindrance have been assumed to work cooperatively to resolve the enantiomers of the sulfoxides. Such a 'three-point attachment' mechanism is the general mechanism for chiral recognition in host–guest chemistry. Hydrogen-bonding and/or Coulomb interactions are involved in achieving chiral recognition through this mechanism. The hydrogen-bonding interaction, however, is of little importance in water because of strong hydration to hydrogen-bonding sites of both host and guest molecules. In the present study, we tried to use Coulomb interaction as an attractive force, which is useful even in water, to discriminate between the enantiomers of α-amino acid derivatives by CDs. Tables 1 and 2 summarize the structures and the abbreviations of the charged CDs and the amino acids, respectively, which appear herein.

Charged CDs have been the subject of several studies.[6] The first attempt at the use of the Coulomb interaction in chiral recognition was carried out by Tabushi *et al*., though the results were not dramatic.[7] Lincoln and co-workers[8] studied the chiral recognition of 2-phenylpropanoic acid by 6^A-amino-6^A-deoxy-β-CD (mono-NH_2-β-CD). They found that protonation of the amino group of mono-NH_2-β-CD reduces the ability to include the neutral guest. The reduction in the binding ability was ascribed to the destruction of the hydrophobic nature of the CD cavity by the hydrophilic NH_3^+ group.[9] Although the binding constant (*K*) for complexation of the 2-phenylpropanoate anion with mono-NH_3^+-β-CD is somewhat larger than those for the systems where no Coulombic binding exists, the $NH_3^+ \cdots CO_2^-$ interaction is not particularly effective in stabilizing the complex.[8] In the chiral recognition of 2-phenylpropanoic acid by monoaminated β-CD, the enantioselectivity $\Delta\Delta G$ is very small whether the host and guest have opposite charges or not. The enantiomers of some guests having an asymmetric carbon have been known to be separated by capillary zone electrophoresis (CZE) using CDs as chiral selectors even when $\Delta\Delta G$ values are extremely small. The enantiomeric separation of mandelic acid and its related compounds[10] and amino acids[11] by CZE using cationic CDs is such a case. The highly sensitive enantiomeric separation in CZE is due to the extremely large number of theoretical plates of this method.

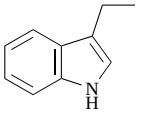
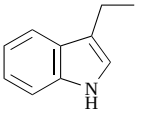
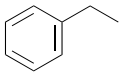
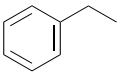
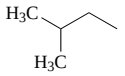
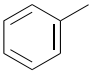
The present study is focused on the chiral recognition of α-amino acid derivatives by charged CDs through Coulomb interaction between host and guest. Although several attempts at chiral recognition of the amino acids by CDs have been

Table 1 Structures and abbreviations of cyclodextrin derivatives



X	Y	*n*	Abbreviation
OH	OH	6	α-CD
OH	OH	7	β-CD
NH_3^+	NH_3^+	6	Per-NH_3^+-α-CD
OH	NH_3^+	7	Mono-NH_3^+-β-CD
NH_3^+	NH_3^+	7	Per-NH_3^+-β-CD
$SCH_2CO_2^-$	$SCH_2CO_2^-$	6	Per-CO_2^--α-CD
OH	$SCH_2CO_2^-$	7	Mono-CO_2^--β-CD
$SCH_2CO_2^-$	$SCH_2CO_2^-$	7	Per-CO_2^--β-CD

Table 2 Structures and abbreviations of amino acid derivatives

R	R′	
		AcTrp / TrpME
		AcPhe / PheME
		AcLeu / PGlyME
		AcVal
		AcAla

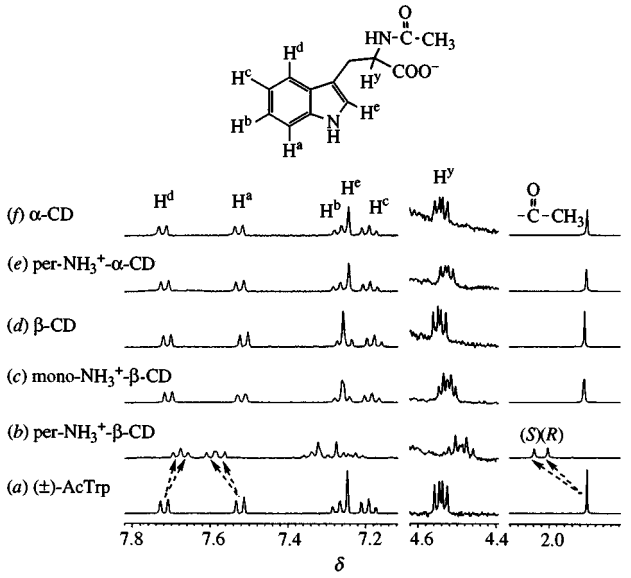


Fig. 1 ^{1}H NMR spectra of (±)-AcTrp (2×10^{-3} mol dm^{-3}) in D_2O at pD 6.0 in the absence and the presence of various CDs (8×10^{-3} mol dm^{-3})

carried out,[12] detailed spectroscopic studies have not been reported. NMR spectroscopy was applied in this study and the results demonstrate the advantage of the use of Coulomb interaction for chiral recognition in host–guest chemistry.

Results and discussion

Chiral recognition by cationic cyclodextrins – ^{1}H NMR spectroscopy

The chiral recognition of *N*-acetylated α-amino acids was studied by means of ^{1}H NMR spectroscopy. Per-NH_3^+-α-, per-NH_3^+-β- and mono-NH_3^+-β-CDs were used as the cationic hosts. The pK_a values of mono- and per-aminated β-CDs were reported to be 8.49[8] and 6.9–8.5,[13] respectively. Since the NMR spectra were measured at pD 6.0, the protonated host cation and the dissociated guest anion exist under the present conditions. Fig. 1 shows the ^{1}H NMR spectra of (±)-AcTrp in D_2O in the presence of four-equivalents of the CDs at 25 °C. Amongst

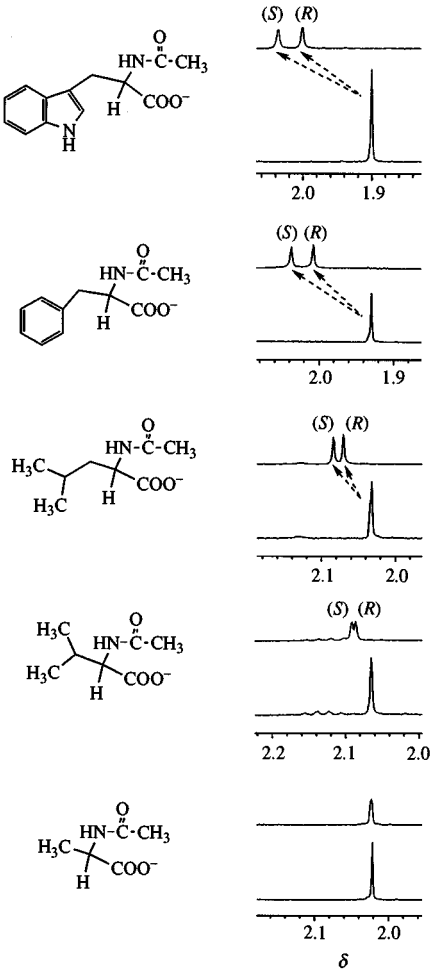


Fig. 2 ^{1}H NMR signals of the CH_3 protons of the acetyl groups of various *N*-acetylated amino acids (2×10^{-3} mol dm^{-3}) in the absence (lower) and the presence of per-NH_3^+-β-CD (8×10^{-3} mol dm^{-3}) (upper) in D_2O at pD 6.0 and 25 °C

α-, β-, mono-NH_3^+-β-, per-NH_3^+-α- and per-NH_3^+-β-CDs, only per-NH_3^+-β-CD causes significant shifts of the signals of anionic AcTrp. The signals of the CH_3 protons of the AcTrp enantiomers are significantly shifted to lower magnetic fields and the complexation-induced shift in the chemical shift (CIS) of the (*S*)-enantiomer is more remarkable than that of the (*R*)-enantiomer. The difference in CIS between the enantiomers was also observed with other protons of AcTrp. These results indicate the unique ability of per-NH_3^+-β-CD to recognize the chirality of AcTrp. Although all signals of (±)-AcTrp were broadened upon addition of per-NH_3^+-α-CD, no evidence for chiral discrimination by such a cationic CD having a smaller cavity size was obtained. In the case of mono-NH_3^+-β-CD, there is a small splitting of the signal at H^e to give two peaks due to the formation of the (*R*)- and (*S*)-AcTrp complexes upon addition of a large excess (16 equivalents) of mono-NH_3^+-β-CD, $\Delta\delta_{enant}$ being 0.007 where $\Delta\delta_{enant} = |\delta_R - \delta_S|$. The *K* value for complexation of AcTrp with mono-NH_3^+-β-CD is much smaller than that for the per-NH_3^+-β-CD complex (*vide infra*).

Similar effects of per-NH_3^+-β-CD on the NMR spectra were observed for other amino acids such as AcPhe, AcLeu and AcVal, but not for AcAla. Fig. 2 shows the ^{1}H NMR signals due to the CH_3 protons of the *N*-acetylated amino acids in the absence and the presence of per-NH_3^+-β-CD. The $\Delta\delta_{enant}$ value is reduced by reducing the size of the hydrophobic part of the amino acid. The signal of (±)-AcAla is broadened in the presence of per-NH_3^+-β-CD, which suggests that the smaller amino acid is bound to this host through Coulomb interactions, though the complexation is not enantioselective.

Table 3 Binding constants K and enantioselectivities $\Delta\Delta G$ for complexation of anionic *N*-acetylated amino acids with cationic amino-cyclodextrins in D_2O at 25 °C[a]

Host	Guest	K/dm^3 mol^{-1}	$\Delta\Delta G$/ kJ mol^{-1} [b]
Per-NH_3^+-β-CD	(*S*)-AcTrp	2310 ± 90	−1.21
	(*R*)-AcTrp	1420 ± 50	
	(*S*)-AcPhe	2180 ± 130	−0.21
	(*R*)-AcPhe	2000 ± 130	
	(*S*)-AcLeu	2480 ± 130	−0.10
	(*R*)-AcLeu	2380 ± 110	
	(*S*)-AcVal	2090 ± 160	−1.16
	(*R*)-AcVal	1310 ± 80	
	AcAla	*c*	
Mono-NH_3^+-β-CD	(*S*)-AcTrp	99 ± 4	−1.08
	(*R*)-AcTrp	64 ± 6	
	(*S*)-AcPhe	67 ± 8	−0.49
	(*R*)-AcPhe	55 ± 7	
	(*S*)-AcLeu	58 ± 4	−0.37
	(*R*)-AcLeu	50 ± 3	
	AcVal	*c*	
	AcAla	*c*	

[a] Binding constants were determined from ^{1}H NMR titrations ([*N*-acetylamino acids] = 1×10^{-3} mol dm^{-3}) in D_2O at pD 6.0. Errors indicated are standard deviations. [b] $\Delta\Delta G = \Delta G_S - \Delta G_R$. [c] Binding constant cannot be determined because of very small $\Delta\delta$.

The NMR spectrum of native Trp, having a zwitterionic structure was scarcely affected by β-CD, mono-NH_3^+-β-CD or per-NH_3^+-β-CD. A very small splitting of the signal due to the H^a proton of Trp was observed upon addition of α-CD. Similar NMR behaviour of the α-CD-(±)–Trp system has already been reported by Lipkowitz *et al.*[12g] The NMR data indicate very weak or no interaction of zwitterionic Trp with β-CD, mono-NH_3^+-β-CD or per-NH_3^+-β-CD.

Binding constants and thermodynamic parameters for complexation of cationic cyclodextrins

The K values for complexation of the acetylated amino acids with per-NH_3^+-β-CD and mono-NH_3^+-β-CD were determined from the ^{1}H NMR titration curves which were analyzed by a non-linear least-squares method. The results are summarized in Table 3. In all cases, the K values for the complexes of the (*S*)-enantiomers of the *N*-acetylated amino acids are larger than those of the corresponding (*R*)-enantiomers. Very small K values have been reported for the complexation of Phe and the derivatives of phenylglycine with native α-CD (K = 10–20 dm^3 mol^{-1}).[12a,b] Zwitterionic CDs also show very small K values for native Trp in a zwitterionic form.[5] Meanwhile, native Ala, Phe and Trp form extremely stable complexes through coordination to the copper complexes of functionalized CDs (log K = 7–16).[14] The complexes of the *N*-acetylated amino acids and per-NH_3^+-β-CD show intermediate K values. The complexes of mono-NH_3^+-β-CD are less stable than those of per-NH_3^+-β-CD. Our previous study revealed that per-NH_3^+-β-CD forms a very stable inclusion complex with the *p*-methylbenzoate anion through Coulomb interactions (K = 15000 dm^3 mol^{-1}) while the mono-NH_3^+-β-CD complex of the *p*-methylbenzoate anion is relatively unstable (K = 660 dm^3 mol^{-1}).[15] The difference in the K values between these two cationic CDs was ascribed to the difference in the ΔS values for complexation, ΔS for the per-NH_3^+-β-CD–*p*-methylbenzoate system being positive and much larger than that for the mono-NH_3^+-β-CD system.[15] We determined the ΔH and ΔS values to be −0.56 ± 1.88 kJ mol^{-1} and 63.1 ± 5.9 J mol^{-1} K^{-1}, respectively, for the per-NH_3^+-β-CD–(*S*)-AcTrp system and 0.07 ± 0.65 kJ mol^{-1} and 60.5 ± 2.2 J mol^{-1} K^{-1}, respectively, for the per-NH_3^+-β-CD–(*R*)-AcTrp system.† As for the *p*-methylbenzoate anion, the complexation of anionic AcTrp with cationic per-NH_3^+-β-CD is entropically dominated. Such an entropy-dominating complexation involving Coulombic interaction and inclusion can be interpreted in terms of dehydration of both host and guest upon association.[15,16]

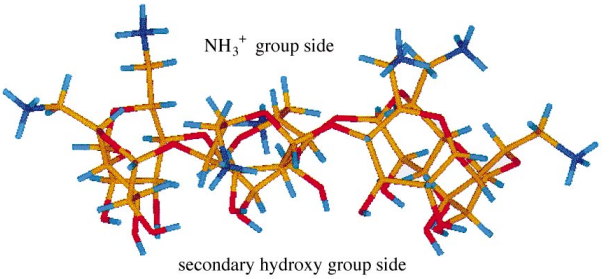


Fig. 3 Optimized structure of per-NH_3^+-β-CD in water derived from the MM–MD calculations

Structures of cationic cyclodextrin complexes

The structure of per-NH_3^+-β-CD itself can be predicted from molecular mechanics–molecular dynamics (MM–MD) calculations. Fig. 3 shows the calculated structure of per-NH_3^+-β-CD where the effects of water have been considered. The NH_3^+-group side of this host is expanded because of the electrostatic repulsion. Fluorescence spectroscopy indicates that such a polycationic CD cannot include neutral guests such as pyrene and naphthalene while its partially cationic form at higher pH can complex with these guests.

However, per-NH_3^+-β-CD forms relatively stable inclusion complexes with oppositely charged guests (see Table 3). The changes in the chemical shifts of the protons of per-NH_3^+-β-CD upon addition of the (*R*)- and (*S*)-acetylated amino acids are shown in Fig. 4 where $\Delta\delta = \delta_{free} - \delta_{complex}$. In all cases, the signals of the H^3 and H^5 protons of per-NH_3^+-β-CD significantly shift to higher magnetic fields upon complexation with the acetylated amino acids, indicating that the hydrophobic parts of the amino acids are located inside the CD cavity. As shown in Fig. 4, no marked difference in the pattern of CIS was observed between the enantiomers of each amino acid. The ROESY spectrum of the per-NH_3^+-β-CD-(*S*)-AcLeu system is shown in Fig. 5 which clearly indicates the correlation between the CH_3 protons of the 2-methylpropyl group of AcLeu and the H^3 proton of the host and the CH_3 protons of the acetyl group of AcLeu and the H^6 proton of the host. A similar ROESY spectrum was recorded for the per-NH_3^+-β-CD-(*R*)-AcLeu system, suggesting that the structure of the (*S*)-enantiomer complex is similar to that of the (*R*)-enantiomer.‡ The structures of the per-NH_3^+-β-CD–AcLeu complexes deduced from the ROESY spectrum and the MM–MD calculations are shown in Figs. 6 and 7, respectively. The NMR data clearly indicate the complexation through the cooperative effects of Coulomb interaction and inclusion. Similar cooperative binding was previously found in the triamino-per-*O*-methyl-α-cyclodextrin-benzyl phosphate[17] and peraminated β-CD-nucleotide systems.[18] MM–MD calculations suggest that the guest is embedded in a distorted cavity of per-NH_3^+-β-CD (see Fig. 7). In both enantiomer complexes, the carboxylate anion group of the guest is located at the center of the ammonium group side of the host toroid because the bulky alkyl group of the guest is situated inside of the CD cavity. Hence the −$NHCOCH_3$ group of the amino acid sits on the rim of the cavity. The Coulomb interaction, the inclusion and the steric hindrance might

† The K values for determination of the thermodynamic parameters were measured to be 2390, 2310, 2250, 2080, 2210 and 2380 dm^3 mol^{-1} at 20, 25, 30, 35, 40 and 50 °C, respectively, for the (*S*)-enantiomer complex and 1420, 1420, 1400, 1420, 1470 and 1400 dm^3 mol^{-1} at 20, 25, 30, 35, 40 and 50 °C, respectively, for the (*R*)-enantiomer complex.
‡ Although we tried to measure the ROESY spectra of the AcTrp and AcPhe systems, no reliable results were obtained because of the low proton concentrations of these guests.

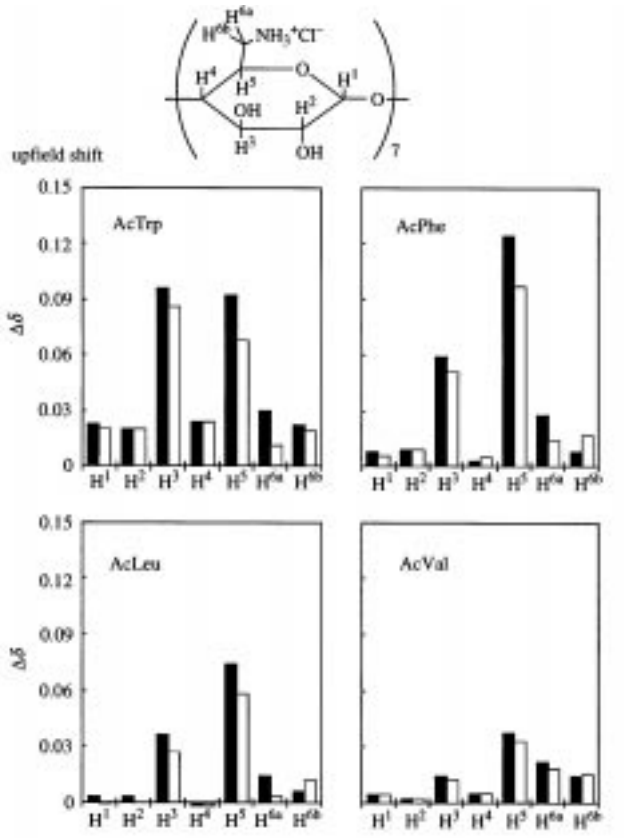


Fig. 4 Changes in the ^{1}H NMR chemical shifts of per-NH_3^+-β-CD (1×10^{-3} mol dm^{-3}) in D_2O at pD 6.0 and 25 °C upon addition of (*R*)- and (*S*)-amino acids (1×10^{-3} mol dm^{-3}), (■) (*S*)-enantiomer, (□) (*R*)-enantiomer

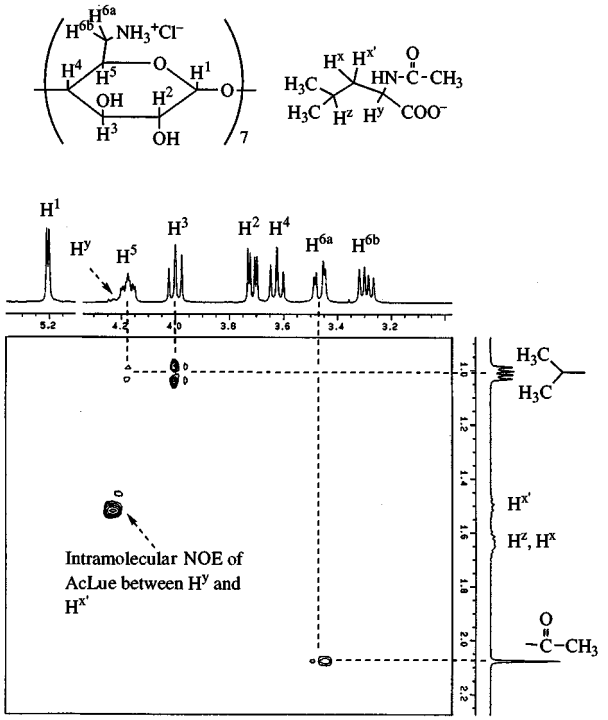


Fig. 5 ROESY spectrum of the per-NH_3^+-β-CD-(*S*)-AcLeu system in D_2O at pD 6.0 and 25 °C. The spectrum was measured for the solution of a mixture of per-NH_3^+-β-CD (8×10^{-3} mol dm^{-3}) and (*S*)-AcLeu (4×10^{-3} mol dm^{-3}) in N_2-saturated D_2O. The mixing time for the ROESY measurement was 250 ms.

determine the structure and hence the stability of each enantiomer complex. The calculations do not suggest the formation of a hydrogen bond between the −NHCO− group of the guest

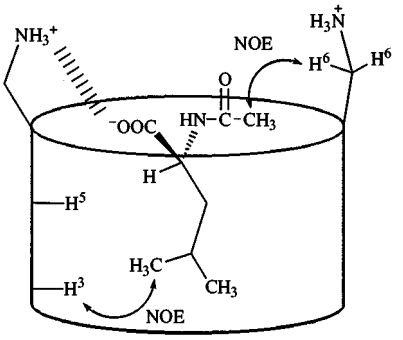


Fig. 6 A plausible structure of the per-NH_3^+-β-CD–(*S*)-AcLeu complex deduced from ^{1}H NMR spectroscopy

and the NH_3^+ group of the host. In the structures shown in Fig. 7, all NH_3^+ groups of the host are located some distance from the CO_2^- group of the guest. The interaction between point charges (Coulomb force of attraction) is proportional to R^{-1} where R is the distance between the point charges. Such a long distance Coulomb interaction might occur in the present system.

Chiral recognition by anionic cyclodextrins

Similar chiral recognition using Coulomb interaction can also be expected for anionic CD. We studied the enantioselective complexation of cationic amino acids with anionic CDs. Per-CO_2^--α-, per-CO_2^--β- and mono-CO_2^--β-CDs were used as the anionic hosts. The pK_a values of per-CO_2^--β-CD have been reported to be 3.6–5.6.[13] The amino acid methyl esters were used as the guest molecules. Since the NMR spectra were recorded at pD 7.0, the host and the guest have opposite charges under the present conditions. Fig. 8 shows the ^{1}H NMR spectra of (±)-TrpME in D_2O at pD 7.0 in the presence of four equivalents of CDs. Except for α-CD, the CDs used in this work affect the signals of the CH_3 and H^y protons of the TrpME. The singlet peak of the CH_3 protons of (±)-TrpME is split into two singlet peaks, which are assigned to the complexes of the TrpME enantiomers, upon complexation. Therefore, it can be concluded that β-, per-CO_2^--β-, mono-CO_2^--β- and per-CO_2^--α-CDs recognize the chirality of TrpME spectroscopically. However, the enantiomers of TrpME as well as PheME and PGlyME were separated from each other by CZE only when per-CO_2^--β-CD was used as a chiral selector (*vide infra*). Enantiomer separation by CZE is achieved when there is a difference in K values between the enantiomers. Meanwhile, a spectroscopic difference is caused by a difference in structures even if the K values of enantiomers are the same. The results of CZE predict that the difference in the K values between the enantiomers of the α-amino acid methyl esters exists only in the case of per-CO_2^--β-CD. The K values obtained by the NMR titrations are listed in Table 4. As for cationic CDs, very weak enantioselectivity is found in the complexation with per-CO_2^--β-CD.

Anionic CDs can be used as chiral selectors in CZE,[19] while peraminated CDs in their cationic forms do not work because of their strong adsorption onto the fused silica surface of a capillary. The separation factor α in CZE is defined in eqn. (1),

$$\alpha = (t_2 - t_{free})/(t_1 - t_{free}) \quad (1)$$

where t_1 and t_2 are the retention times of the enantiomers and t_{free} is the retention time of a standard (methanol) which does not interact with CD. The α values obtained in CZE of the amino acid methyl esters using per-CO_2^--β-CD as a chiral selector are shown in Table 4. The system having the largest $\Delta\Delta G$ (TrpME) shows the largest α value. No enantiomer separation was observed when CDs other than per-CO_2^--β-CD were used and/or the native amino acids were used as the guests for per-CO_2^--β-CD as well as other CDs. These results clearly indi-

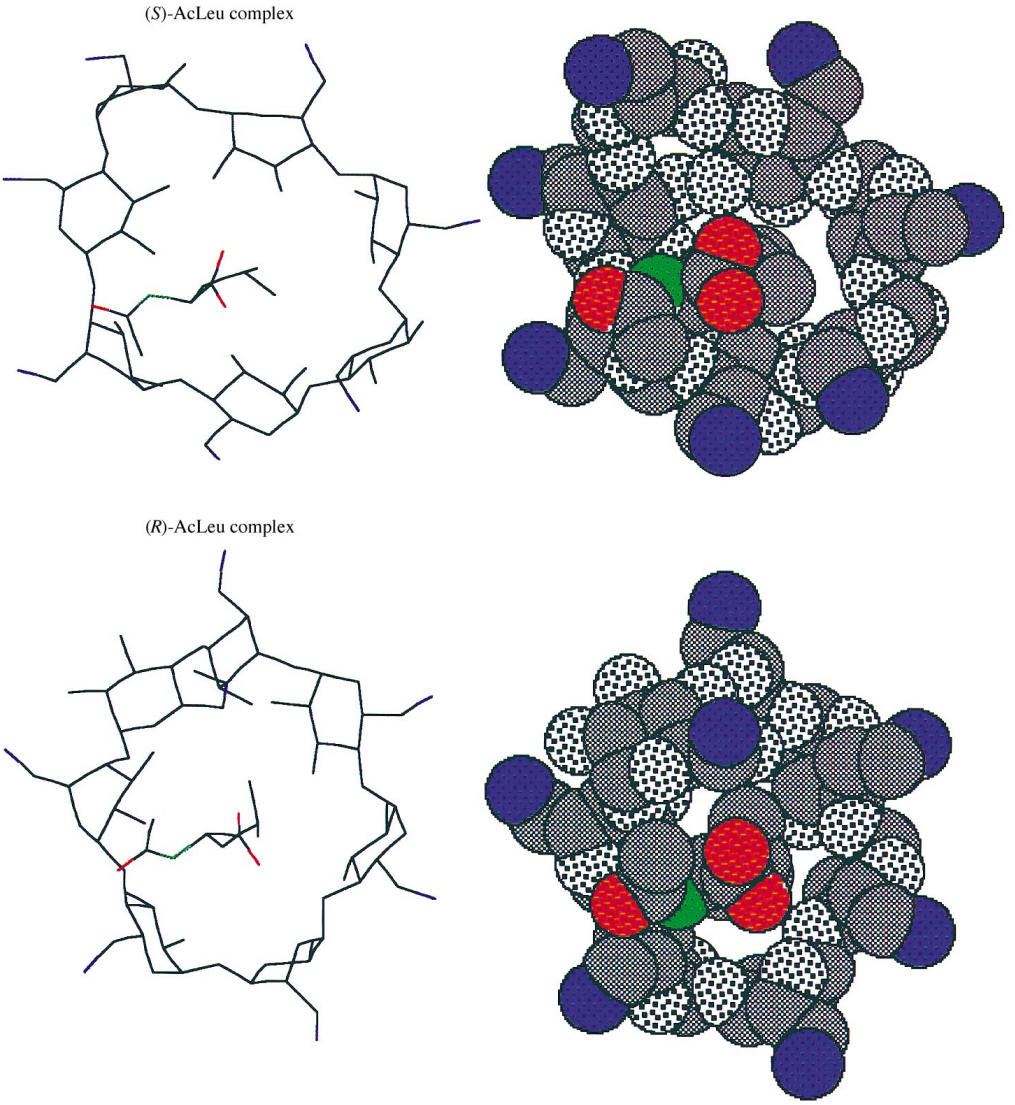


Fig. 7 Structures of the per-NH_3^+-β-CD-(*S*)- and (*R*)-AcLeu complexes derived from the MM–MD calculations: red, oxygen in the guest; green, nitrogen in the guest; blue, nitrogen in the host

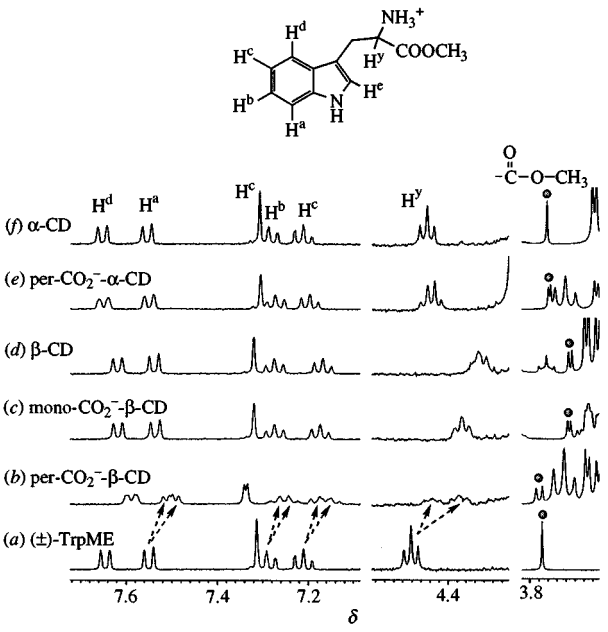


Fig. 8 ^{1}H NMR spectra of (±)-TrpME (2×10^{-3} mol dm^{-3}) in D_2O at pD 7.0 and 25 °C in the absence and the presence of various CDs (8×10^{-3} mol dm^{-3})

Table 4 Binding constants (*K*) and enantioselectivities (ΔΔ*G*) for complexation of cationic amino acid methyl esters with anionic per-CO_2^--β-CD in D_2O at 25 °C[a] and retention times (*t*) and separation factors (*α*) in CZE[b]

Guest	K/dm^3 mol^{-1}	ΔΔG/kJ mol^{-1}[c]	t/s	α
(*S*)-TrpME	380 ± 20		34.2	
(*R*)-TrpME	550 ± 30	0.92	38.3	1.12
(*S*)-PheME	1520 ± 150		58.2	
(*R*)-PheME	1570 ± 160	0.08	59.6	1.02
(*S*)-PGlyME	260 ± 10		31.3	
(*R*)-PGlyME	280 ± 10	0.18	32.0	1.02

[a] Binding constants were determined from ^{1}H NMR titrations ([per-CO_2^--β-CD] = 2×10^{-3} mol dm^{-3}) in D_2O at pD 7.0. Errors indicated are standard deviations. [b] The method of the CZE measurements is described in Experimental section. [c] $\Delta\Delta G = \Delta G_S - \Delta G_R$.

cate the essential role of the Coulomb interaction between the host and the guest in chiral recognition.

Conclusions

Good artificial receptors of amino acids, which function in organic solvents, have been developed. For example, Mizutani *et al.*[20] prepared a chiral porphyrin zinc complex having C_2 symmetry which recognizes the chirality of amino acid esters in chloroform through the three-point attachment mechan-

ism. Mendoza and co-workers[21] reported an amino acid receptor composed of a chiral guanidinium cation, a crown ether and a naphthalene ring. Such a lipophilic receptor enantioselectively extracts zwitterionic amino acids in water. A similar receptor was also synthesized by Schmidtchen and co-workers.[22] Artificial receptors which function in organic solvents can be designed more easily than those which are used only in water. The aqueous system, however, has an advantage in host–guest complexation because hydrophobic interaction as well as strong van der Waals forces[23] are present in water. Although we tried to use Coulomb interactions as a point interaction, which is available in water, for chiral recognition by CDs, the results are disappointing. However, the present study suggests the advantage of the use of Coulomb interactions as an attractive force which participates in chiral recognition. Since CDs belong to a C_n point group, these oligosaccharides might be expected to show a low ability to recognize the central chirality. Indeed, native CDs such as α- and β-CDs exhibit a very low ability to discriminate between the enantiomers of the amino acids. However, the function of CDs was significantly improved by using Coulomb interactions. We plan to continue further studies before we conclude that CDs do not exhibit high ability to recognize central chirality.

Experimental

α- and β-CDs (Nacalai) were purchased and an antioxidant contained in these materials was extracted with THF using a Soxhlet extractor. Per-NH_3^+-β-CD was the same as that reported previously.[24] Per-NH_2-α-CD,[25] mono-NH_2-β-CD[26] and the *N*-acetylated amino acids[27] were prepared according to the procedures described in the literature. The amino acid methyl esters (Aldrich) were purchased. D_2O (99.8%) and [$^2H_6$]DMSO (99.8%) were obtained from CEA. Water was distilled by a Yamato Auto Still Glass Model WAG 220 and further purified by a Yamato MILLIPORE WQ500 Auto Pure.

The ^{1}H NMR data were collected using a JEOL JNM-A400 (400 MHz) using sodium 3-(trimethylsilyl) [2,2,3,3-$^2H_4$]propionate (TSP, Aldrich) as an external standard. The CZE measurements were carried out using a JASCO capillary electrophoresis system CE-800 with a 300 mm (effective length) × 50 μm fused-silica capillary cartridge (non-coated). The capillary was filled with the 0.033 mol dm^{-3} phosphate buffer at pH 7.0 with per-CO_2^--β-CD (5×10^{-3} mol dm^{-3}) and the sample (4×10^{-4} mol dm^{-3}) in the same buffer solution was introduced into the capillary by applying the potential for 10 s. The applied voltage was 4.1 kV where the current was *ca*. 15 μA in the case of per-CO_2^--β-CD. The MM–MD calculations including the effects of water were carried out as previously reported.[28]

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