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Recognition mechanism of D- and L-tryptophan enantiomers using 2-hydroxypropyl-α- or β-cyclodextrins as chiral selectors

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ABSTRACT

This work investigates the chiral recognition of D- and L-tryptophan enantiomers using 2-hydroxypropyl-α- or β-cyclodextrins (HPαCD and HPβCD) as selectors. Capillary electrophoresis experiments showed that only the α-form was effective for enantiomeric separation. NMR measurements (T_1 and ROESY) and circular dichroism experiments were carried out to provide information about the topologies of interaction between the tryptophan isomers and cyclodextrins. For HPβCD, the inclusion of both isomers was verified, while for HPαCD only D-tryptophan was inserted in the cavity. These results agree with the stability constant values obtained from NMR diffusion experiments. We were able to determine that, together with charged groups of tryptophan (NH_3^+ and COO^-), the indole moiety is the third guest interaction point with the cyclodextrin.

1. Introduction

The use of cyclodextrins (CDs) allows the enantiomeric separation of some substances of biochemical interest.[1] Due to the limited water solubility of native cyclodextrins, derivatives such as the hydroxypropylated form are used,[2] since they present the possibility of enhancing chiral resolution by increasing chiral selector concentration. The 2-hydroxypropyl-cyclodextrin form is obtained by the hydroxyalkylation of the primary OH groups in D-glucose,[3] and its high water solubility is the result of amorphization after hydroxypropylation.[2]

In several studies, the stability constant of the complex has been calculated to quantify the supramolecular interaction in solution between the organic guest and the cyclodextrin host. To obtain such information, several approaches have been described, such as isothermal titration calorimetry,[4] solubility diagrams[5] and multivariate analysis,[6] as well as Scott/Scatchard plots, which were applied to data obtained from UV absorption spectroscopy,[7] capillary electrophoresis (CE)[8] and high performance liquid chromatography (HPLC).[9] The stability constants could also be calculated from diffusion coefficient data obtained by diffusion ordered spectroscopy (DOSY) experiments.[10]

Cyclodextrins are known for their properties of molecular recognition, which are useful for enantiomeric separations of amino- and hydroxy-acids. Past reports have already studied the interactions between some amino acids, such as tryptophan and cyclodextrins.[11] α-Cyclodextrins are commonly used for the enantiomeric resolution of DL-tryptophan by means of capillary electrophoresis.[12] CE chiral separations of α-hydroxy acids were verified when mediated by the hydroxypropylated β-form while aromatic acids were separated by a biphasic method employing hydroxyalkylated and methylated cyclodextrins.[13]

UV circular dichroism has been used as a tool for the structural elucidation of inclusion compounds. For non-chiral organic compounds, induced circular dichroism by cyclodextrin hosts can give information about the orientation of the guest.[14] This technique also shows how tryptophan bound cyclodextrins can act as molecular sensors for non-optically active compounds.[15] As a complement, NMR spectroscopy is a powerful tool for obtaining structural information about host–guest interaction. Overhauser and relaxation experiments such as ROESY 2D and spin–lattice relaxation times (T_1) can be used to provide information on the complex stereochemistry[16] while DOSY experiments allow us to evaluate the binding strength.[10]

Herein we report the origin of chiral recognition of D- and L-tryptophan using 2-hydroxypropyl-α- or β-cyclodextrins. Evidence of enantiomeric resolution, when possible, was collected using capillary electrophoresis technique. Structural information about each cyclodextrin–tryptophan complex formation and the stability constant values for these complexes were acquired using UV circular dichroism and NMR spectroscopy.

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2. Results and discussion

Figure 1 shows the electropherograms acquired according to the procedure in Section 4. Runs A and B represent the use of HPβCD in the background electrolyte. As observed, no chiral separation was seen when using this cyclodextrin, even when 2.0×10^{-1} mol L^{-1} concentration was applied (Fig. 1B). On the other hand, runs C and D, with HPαCD, show enantiomeric resolution. However, when the temperature was increased from 25 °C to 50 °C (Fig. 1C and D, respectively), the resolution dropped from 1.7 to 0.8, but the enantiomer peaks remained resolved. Yuexian et al.[17] reported that the stability constants of α-cyclodextrin with amino acids are gradually reduced with the temperature increase. At higher temperatures, the molecular motion is more intense, causing the inclusion complexes to dissociate. As a result, at 50 °C a poorer resolution was obtained due to a minor binding ability of tryptophan to be complexed by HPαCD, but complexes were still formed.

The electrophoretic migration time observed for chiral separation in Figure 1C was L (29 min)→D (31 min), which was determined by injection of the enantiomerically pure tryptophan isomers. This result suggests that the D-enantiomer has a greater ability to bind HPαCD than L, since the higher migration time is generated by lower charge/mass relationship, which is the case when an inclusion complex is formed.

In order to understand the dependency of the chiral recognition mechanism on cyclodextrin cavity size, NMR spectroscopy experiments were carried out. Diffusion ordered spectroscopy (DOSY) was applied to D_2O solutions of DL-tryptophan and complexes to give their diffusion coefficients (D) and the stability constants of the inclusion compounds (Table 1). For the complexes, the observed diffusion coefficients, D_{obs}, are a weighted average of those for the individual species, bound and free tryptophan:

$$D_{obs} = f_{free}D_{free} + f_{bound}D_{bound} \quad (1)$$

where $f_{free} + f_{bound} = 1$; D_{free} and D_{bound} are determined from DOSY experiments of DL-tryptophan and complexes solutions, respectively. Here, it was considered that the diffusion coefficient of the bound species is the same as that of cyclodextrin. Considering a 1:1 stoichiometric ratio to calculate the stability constants of inclusion compounds, K, then

$$K = \frac{[IC]}{[HPCD][Trp]} \quad (2)$$

where [IC], [HPCD] and [Trp] are the equilibrium concentrations of inclusion compound, cyclodextrin and tryptophan, respectively. As no reagent was used in excess, [HPCD] = [Trp], which gives

$$K = \frac{[IC]}{[Trp]^2} \quad (3)$$

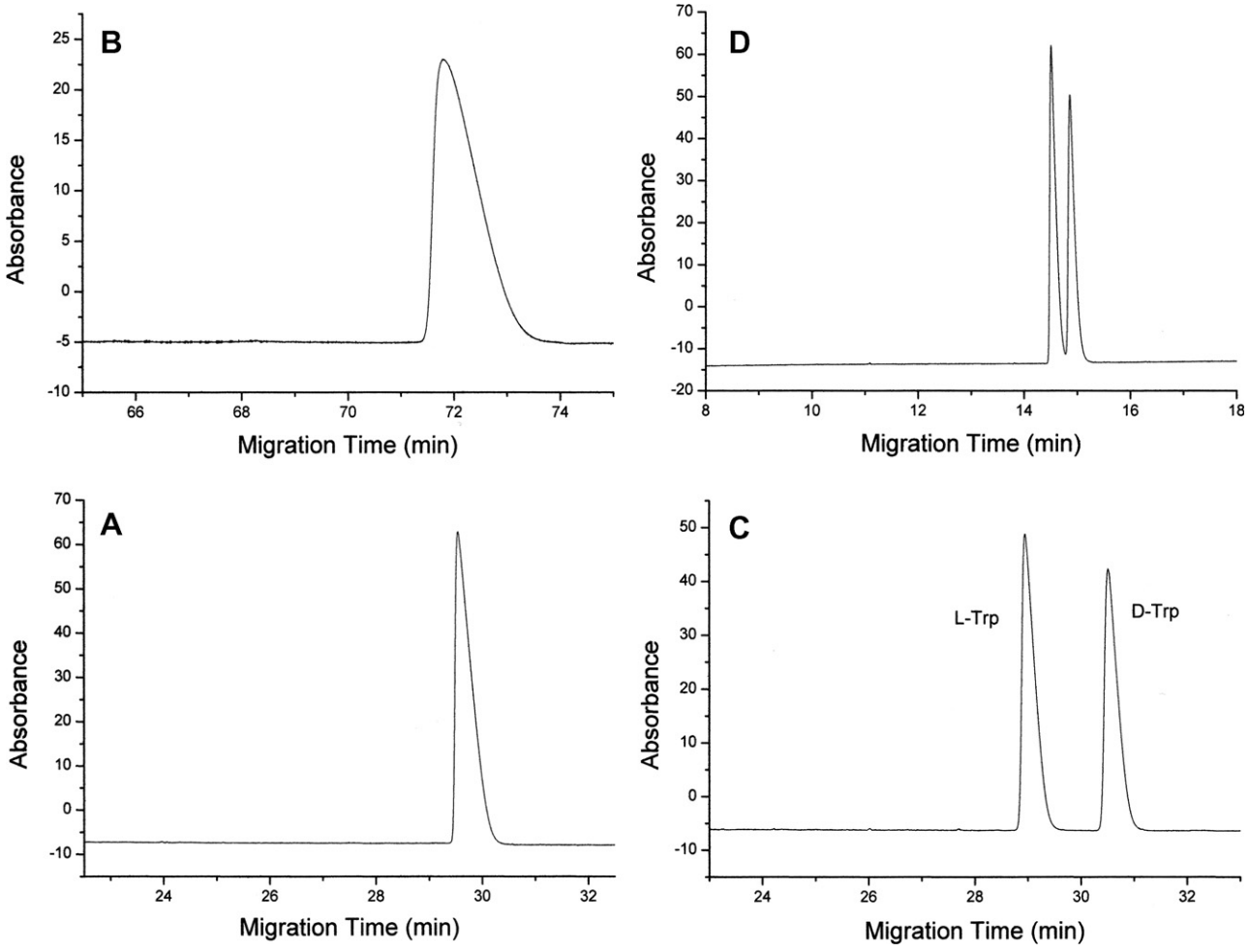

Figure 1. Electrophoretic runs of injected DL-Trp using HPβCD concentrations of 7.5×10^{-2} mol L^{-1} (A) and 2.0×10^{-1} mol L^{-1} (B) both at 25 °C; and HPαCD concentration of 7.5×10^{-2} mol L^{-1} at 25 °C (C) and 50 °C (D). Applied voltage: 30 kV; column: 50 mm (DI)X 56 cm fused silica capillary; background electrolyte: 5×10^{-2} M phosphate buffer (pH 2.5); detection wavelength: 200 nm.

Table 1
Diffusion coefficients (D) and stability constant values (K) for inclusion compounds and DL-Trp

	D ($\times 10^{-10}$ m^2 s^{-1})	K
DL-Trp	5.91 (D_{free})	—
HPαCD in HPαCD-DTrp	2.37 (D_{bound})	39
Trp in HPαCD-DTrp	4.27 (D_{obs})	
HPβCD in HPβCD-DTrp	2.03 (D_{bound})	29
Trp in HPβCD-DTrp	4.30 (D_{obs})	
HPαCD in HPαCD-LTrp	2.35 (D_{bound})	28
Trp in HPαCD-LTrp	4.48 (D_{obs})	
HPβCD in HPβCD-LTrp	1.91 (D_{bound})	31
Trp in HPβCD-LTrp	4.24 (D_{obs})	

Diffusion coefficients were measured by increasing the amplitude of the field gradient pulses in eight steps (0.68–13.62 G cm^{-1}). The duration of field gradient pulse (10 ms) and the diffusion time (20 ms) were constant. 40 mmol L^{-1} solutions of inclusion compounds and 4 mmol L^{-1} solution of tryptophan racemate were used.

but $[IC] = f_{bound}[Trp]_o$ and $[Trp] = f_{free}[Trp]_o$, in which $[Trp]_o$ is the initial concentration of tryptophan (4×10^{-2} mol L^{-1}), then

$$K = 25\left(\frac{f_{bound}}{f_{free}^2}\right) \quad (4)$$

which was used together with (1) to calculate the values of K in Table 1.

As shown in Table 1, the HPαCD-DTrp complex presents the highest value of stability constant and HPαCD-LTrp has the lowest K. This discrepancy explains the chiral recognition towards tryptophan racemate as demonstrated in capillary electrophoresis, while no enantiomeric resolution was observed when HPβCD was used, since its corresponding complexes gave very similar stability constants. Figure 2 shows the ^{1}H NMR spectra of the aromatic region of tryptophan. For all peaks in HPαCD-DTrp spectrum, broad lines were observed indicating that this complex has the most intense binding.

In order to obtain some structural information on the complex's stereochemistry and interaction topology, we measured the hydrogens T_1 relaxation times for tryptophan free and complexed, with cyclodextrins (Table 2). The ΔT_1 values were calculated for the Trp complexed in relation to free tryptophan. H3 and H4 show the largest ΔT_1, suggesting that these hydrogens are included in HPCD cavity.

To confirm the inclusion complex's topologies suggested by T_1 measurements, ROESY 2D spectra were acquired. Figure 3A presents the spectrum for HPαCD-DTrp, which shows a strong intra- or intermolecular interaction between hydrogens in terminal $-CH_3$ of the cyclodextrin hydroxypropyl moiety (1.085 ppm) and H3 of HPCD (3.950 ppm), which is an inside-cavity hydrogen. All other complexes presented this feature, which means that it is independent of guest and cavity size. This configuration was suggested by Haskins et al.[3] and consists of the hydroxypropyl moiety fitting its own cyclodextrin cavity. It is acceptable since the HP hydroxyl group can form a hydrogen bond with any of the cyclodextrins secondary OHs, stabilizing the structure.

Figure 3B and Table 3 display the cross peaks between tryptophan indole and cyclodextrin hydrogens. For HPαCD-DTrp, correlations between HPCD H3 and indolic H1 and H4 (Fig. 3B and Table 3) suggest that the indole moiety lies parallel to the cyclodextrin axis (Fig. 4). For the other complexes, due to the low signal-to-noise relationship, especially for cyclodextrin's H3 cross peaks, only a few cross peaks were picked from these spectra. This diffi-

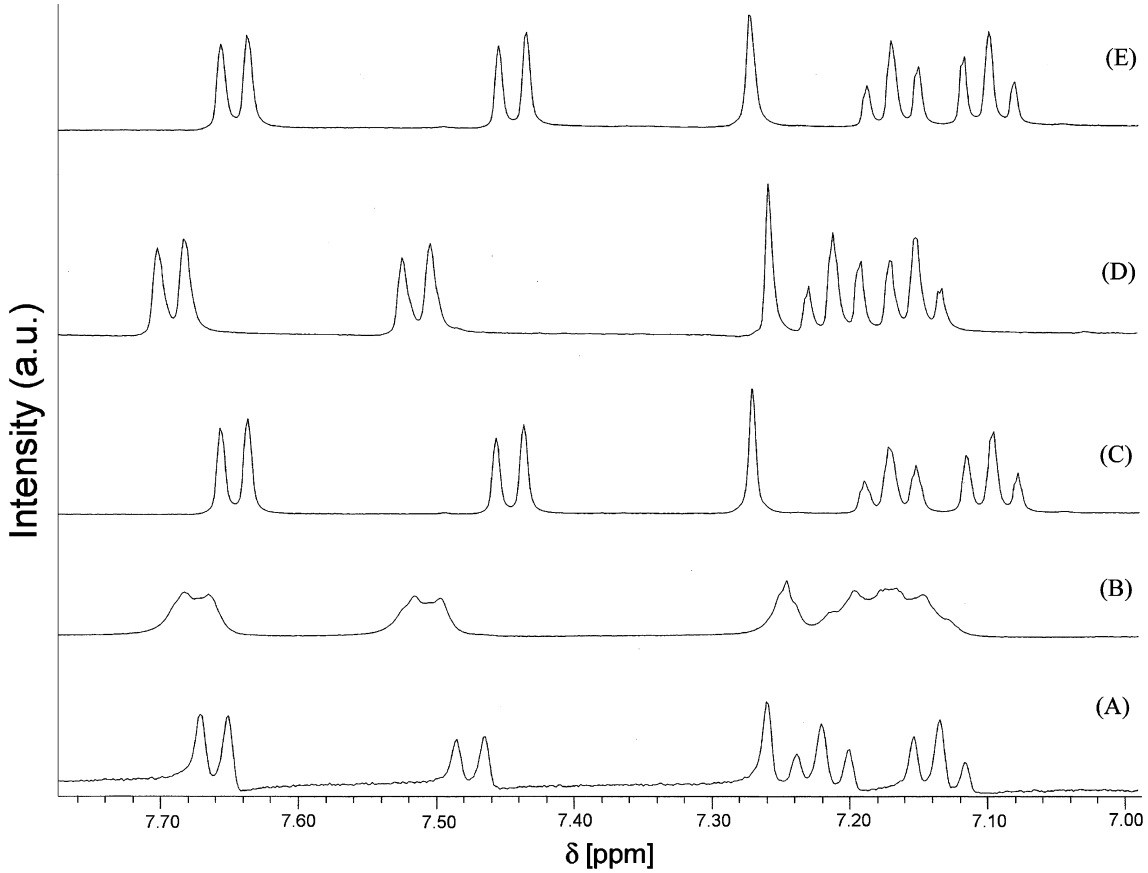

Figure 2. ^{1}H NMR spectra of indole region of tryptophan. (A) DL-Trp; (B) HPαCD—DTrp (C) HPβCD—DTrp; (D) HPαCD—LTrp and (E) HPβCD—LTrp.

Table 2
T_1 relaxation time values of tryptophan aryl hydrogens for inclusion compounds and DL-Trp

Hydrogen	DLTrp	HPαCD-D	ΔT_1	HPβCD-D	ΔT_1	HPαCD-L	ΔT_1	HPβCD-L	ΔT_1
H1	1.57	0.90	−0.67	0.90	−0.67	0.90	−0.67	0.90	−0.67
H2	1.57	0.98	−0.59	0.98	−0.59	0.90	−0.67	0.90	−0.67
H3	2.16	0.98	−1.18	0.98	−1.18	0.90	−1.26	0.90	−1.26
H4	2.50	1.02	−1.48	1.27	−1.23	1.23	−1.27	1.27	−1.23

T_1 values were measured using the standard inversion-recovery program. 40 mmol L^{-1} solutions of inclusion compounds and 4 mmol L^{-1} solution of tryptophan racemate were used.

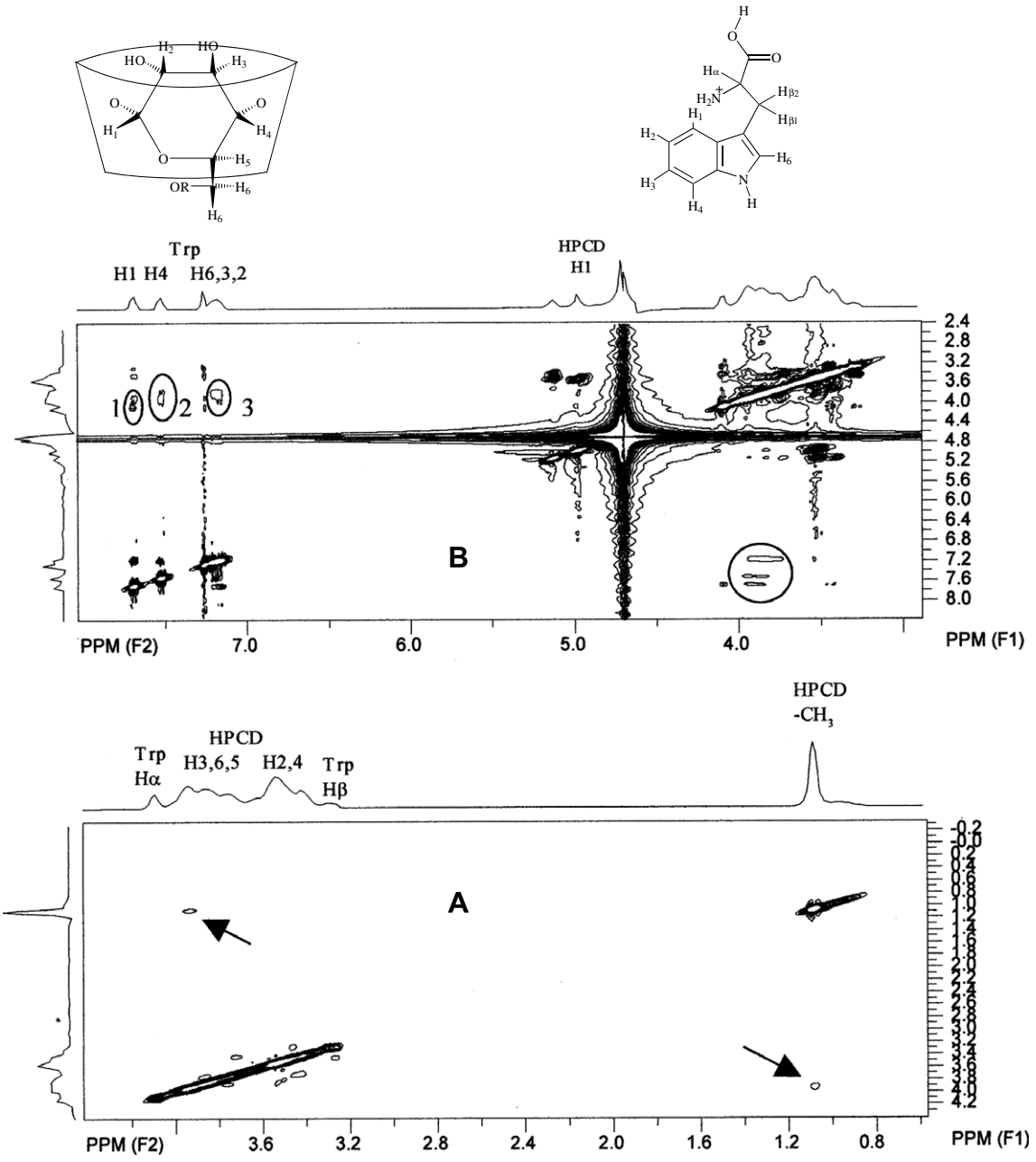

Figure 3. ROESY 2D spectrum for HPαCD-DTrp. (A) Arrows indicate the intramolecular correlation between the terminal $-CH_3$ of the cyclodextrin hydroxypropyl moiety (1.085 ppm) and H3 of HPCD (3.950 ppm); (B) circles involve cross peaks between tryptophan indole and HPαCD hydrogens: 1—HPCD (H3, H6) X Trp (H1); 2—HPCD (H3, H6) X Trp (H4); 3—HPCD (H3, H5, H6) X Trp (H2, H3).

culty is related to the low fraction of complexed tryptophan, since their stability constants are about 10 units lower than that for HPαCD-DTrp. In Table 3, correlations of indolic hydrogens with cyclodextrin H6 are probably related to the fact that this latter signal corresponds to two hydrogens, hence the corresponding ROE intensities tend to be intense.

UV circular dichroism spectra were acquired to complement the analysis of host–guest configurations (Fig. 5). Harata and Kodaka

Table 3
Intermolecular interactions between tryptophan indole and cyclodextrin hydrogens obtained from ROESY experiments

Complex	Intermolecular correlation
HPαCD-DTrp	HPCD (H6) Trp (H1, H2, H3, H4) HPCD (H5) Trp (H2, H3) HPCD (H3) Trp (H1, H2, H3, H4)
HPβCD-DTrp	HPCD (H3) Trp (H2)
HPαCD-LTrp	HPCD (H6) Trp (H1, H2)
HPβCD-LTrp	HPCD (H6) Trp (H1, H2, H4) HPCD (H3) Trp (H2)

Spectra were recorded with a mixing time of 500 ms and 4096 data points in F2 and 400 in F1. 40 mmol L^{-1} solutions of inclusion compounds were used.

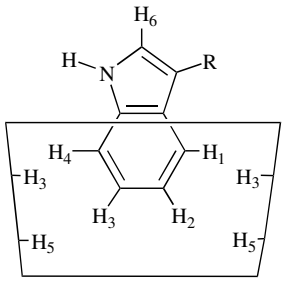

Figure 4. Suggested topology of HPαCD-DTrp, based on NMR and circular dichroism results.

rules[18,19] for induced circular dichroism say that inside and outside cavity interactions produce signals of opposite sign when the angle (ϕ) between cyclodextrin main axis and the dipole moment of indole $\pi \rightarrow \pi^*$ transition,[20] relative to the band centred at 220 nm (Fig. 6), is constant. As a consequence, half-included chromophores would produce no signal at all. If indole is outside the cavity, the ϕ angle above or below 54.7° produces a negative or positive ICD, respectively. The opposite is true when the interaction is detected inside of the cavity.

Spectra in Figure 5 show that only HPαCD-LTrp (Fig. 5C) and HPβCD-DTrp (Fig. 5B) produce ellipticity deviations as cyclodextrin is added.

For HPβCD-DTrp, a positive deviation was obtained. Hence, there are two possible situations: (i) inside-cavity interaction at $\phi < 54.7°$; or (ii) outside-cavity interaction at $\phi > 54.7°$. According to the ROESY data shown in Table 3, if the indole hydrogen H2 correlates with H3 of HPCD, the existence of a ϕ angle higher than 54.7° cannot be considered, since this would keep indole H2 outside the cavity (Fig. 7A). This suggests that the indole moiety is totally included in HPβCD and $\phi < 54.7°$ (Fig. 7B).

For HPαCD-LTrp, a negative deviation was observed. Two configurations are possible: (i) inside-cavity interaction at $\phi > 54.7°$; or (ii) outside-cavity interaction at $\phi < 54.7°$. Considering that the volume of α-cyclodextrin torus is not big enough to make the whole indole moiety at $\phi > 54.7°$, as considered by Kodaka in a similar system with naphthalene,[19] an outside cavity interaction at $\phi < 54.7°$ may be suggested in this case.

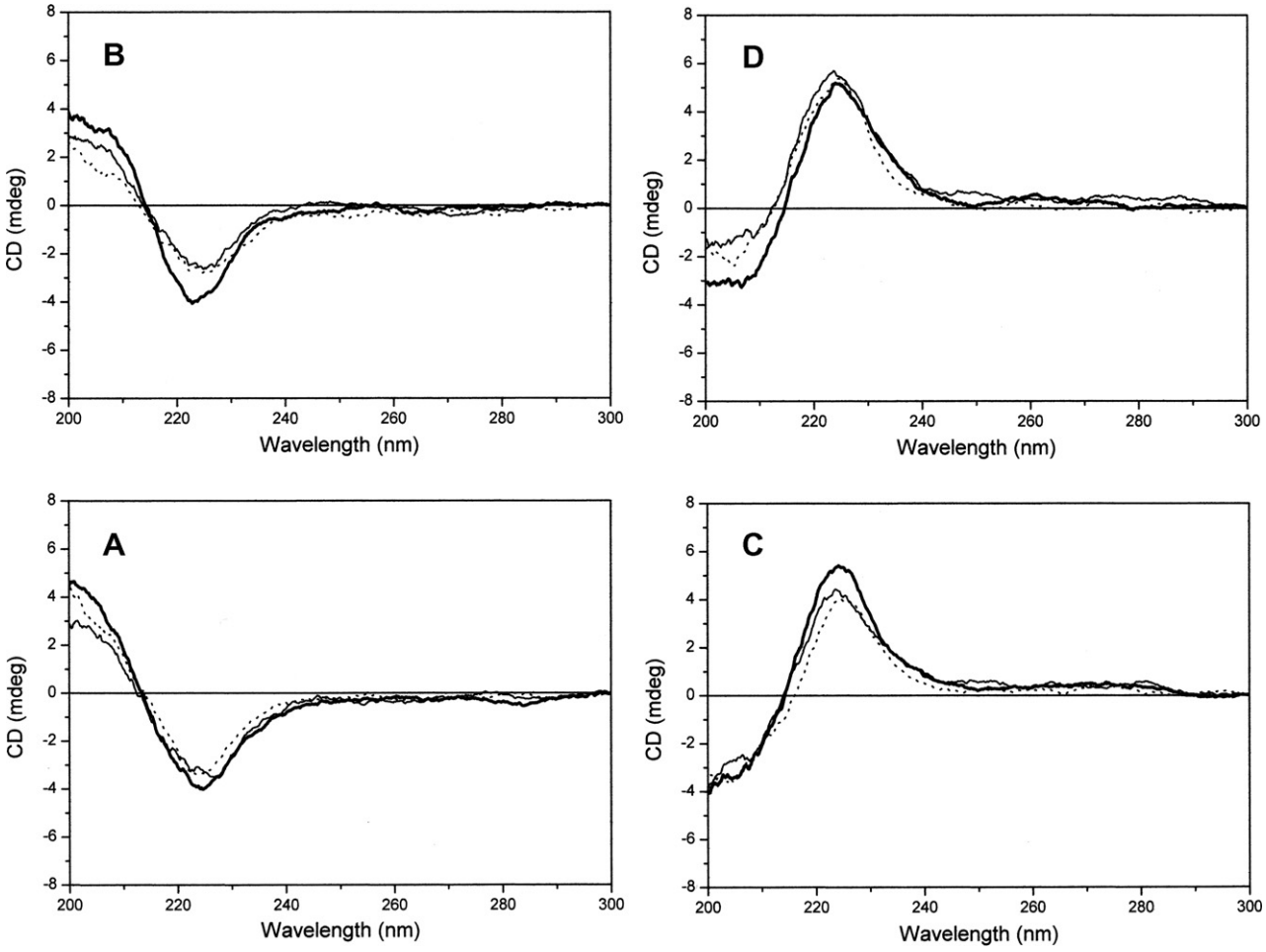

Figure 5. UV circular dichroism spectra for (A) HPαCD-DTrp; (B) HPβCD-DTrp (C) HPαCD-LTrp and (D) HPβCD-LTrp. Cyclodextrin concentrations: (—) zero; (–) 7.5; and (···) 10×10^{-2} mol L^{-1}. See Section 4 for more details.

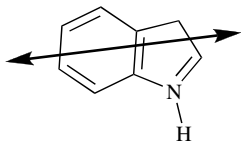

Figure 6. Transition dipole moment (μ) direction for the $\pi \rightarrow \pi^*$ indole band at 220 nm.

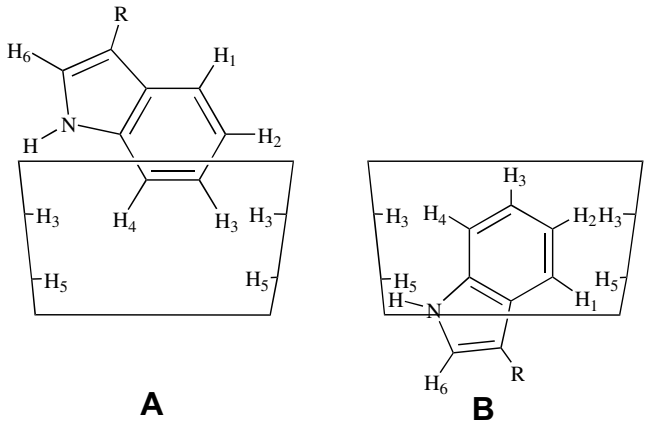

Figure 7. Suggested configurations of HPβCD-DTrp according to circular dichroism results. (A) $\phi = 60°$; (B) $\phi = 30°$.

For HPαCD-DTrp (Fig. 5A) and HPβCD-LTrp (Fig. 5D), no differences were observed in circular dichroism spectra when HPCD was added. This is supported by the correlations in Table 3, especially for HPαCD-DTrp, which suggests a half inclusion of the indole moiety for both complexes.

3. Conclusions

The mechanism to resolve tryptophan racemate by capillary electrophoresis using 2-hydroxypropyl-α-cyclodextrin as a chiral selector involves the half inclusion of the indole moiety of the D-isomer while the L-form is positioned outside the cavity. This means that the HPαCD-DTrp complex tends to be more stable, with a greater stability constant (K) than HPαCD-LTrp, as *K* values obtained from DOSY experiments point out. Using the β-form of cyclodextrin, both isomers are included in cyclodextrin cavity and no enantiomeric separation was observed, since the populations of the encapsulated D- and L-tryptophan are similar, as indicated by the very close stability constant values of HPβCD-DTrp and HPβCD-LTrp. The present work complements our previous studies,[21] where it was proposed that the complexation of DL-Trp by HPCD collapses the intermolecular NH_3^+–COO^- hydrogen bonds. As a result, the indole moiety represents the third interaction point needed for enantiomeric resolution of the DL-tryptophan racemate.

4. Experimental

4.1. Chemicals

2-Hydroxypropyl-β-cyclodextrin (HPβCD) and 2-hydroxypropyl-α-cyclodextrin (HPαCD), both with a substitution degree of 0.6, were purchased from Fluka (Buchs, Switzerland). Sigma Chemical Company (St.Louis, MO, USA) and Riedel-de Haën (Seelze, Germany) supplied D- and L-tryptophan (D- and L-Trp), respectively. Phosphate buffer (5×10^{-2} mol L^{-1}, pH 2.5) and 1 mol L^{-1} NaOH were acquired from Agilent Technologies (US).

4.2. Capillary electrophoresis

CE equipment, model Agilent 3D (Agilent technologies, USA) equipped with a diode array detector, and fused silica capillary, with 50 μm (ID) × 56 cm dimensions, were used for chiral separation. Analyses were performed at 30 kV with detection at 200 nm, using phosphate buffer cited before as the background electrolyte (BGE). Runs were performed at 25° and 50 °C. Cyclodextrins were dissolved in BGE in order to have 7.5×10^{-2} mol L^{-1} concentration for each form.

Before each run, the capillary was washed with NaOH 1 mol L^{-1} and ultra pure water (Agilent Technologies) for 10 min each, and phosphate buffer for another 30 min. DL-Tryptophan mixture (1.5 mg mL^{-1}), dissolved in the phosphate buffer, was hydrodynamically injected at 50 mbar s. Resolution (*R*) between the enantiomers was calculated using the following equation:

$$R = \frac{(t_1 - t_2)}{[0.5(W_1 + W_2)]} \quad (5)$$

where t_1 and t_2 are the migration times of enantiomers and W_1 and W_2 are the respective widths at the base of the peaks. Proper separations, that is, baseline resolved, are achieved for *R* values greater that or equal to 1.5.

4.3. NMR spectroscopy

All NMR spectra were carried out at 25 °C on a Bruker DRX-400 spectrometer using a 5 mm broadband inverse probe with a single gradient (*Z*). The samples (40 mmol L^{-1} solutions of HPαCD-DTrp, HPβCD-DTrp, HPαCD-LTrp and HPβCD-LTrp; and 4 mmol L^{-1} solution of DL-tryptophan) were prepared in D_2O. T_1 values were measured using the standard inversion-recovery programme. Pulse field gradient (PFG) NMR spectra[22–24] were acquired using a pulse gradient stimulated echo using bipolar gradient pulses for diffusion. Diffusion coefficients were measured by incrementing the amplitude of the field gradient pulses in eight steps (0.68–13.62 G cm^{-1}). The duration of the field gradient pulse (10 ms) and the diffusion time (20 ms) were constant. The spectra were recorded with eight scans in a 2D mode for each measurement, with a recycle time of 1.5 s between scans. Rotating frame Overhauser spectroscopy (ROESY)[25,26] experiments were recorded with a mixing time of 500 ms and 4096 data points in F2 and 400 in F1.

4.4. UV circular dichroism

HPβCD or HPαCD were added to 2.1×10^{-4} mol L^{-1} D- or L-Trp solutions in phosphate buffer. Cyclodextrin concentrations were 7.5 and 10×10^{-2} mol L^{-1}. Circular dichroism spectra were recorded using a Jasco J-715 spectropolarimeter (Jasco Corporation, Tokyo, Japan) with 1.00 mm path-length quartz cells at 25 °C. All spectra were collected in the 200–300 nm interval with three accumulations at a 50 nm min^{-1} scan rate and further subtracted from the respective blank spectrum.

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