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Mechanistic study of enantiomeric recognition of primary amino compounds using an achiral crown ether with cyclodextrin by capillary electrophoresis and nuclear magnetic resonance

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Abstract

A model and theoretical equations are presented to investigate the enantiomeric recognition mechanism of primary amino compounds using an achiral crown ether with cyclodextrin by capillary electrophoresis (CE) and nuclear magnetic resonance (NMR). Association constants were calculated from CE and ^{1}H NMR experiment results on the basis of the model and the equations. The key step of chiral recognition was identified from those values. Using CE analyses of three primary amino compounds [1-(1-naphthyl)ethylamine; 1-aminoindan; 1,2,3,4-tetrahydro-1-naphthylamine], the key step was identified with the equilibrium where the complex of a primary amino compound and 18-crown-6 becomes associated with 2,6-di-*O*-methyl-β-cyclodextrin for all the three compounds. From the ^{1}H NMR analyses of 1-(1-naphthyl)ethylamine, the key step was identified with the equilibrium where the complex of 1-(1-naphthyl)ethylamine and 18-crown-6 becomes associated with β-cyclodextrin.

Keywords: Enantiomeric separation; Nuclear magnetic resonance spectrometry; Crown ethers; Cyclodextrins; Naphthylethylamines; Aminoindan; Tetrahydronaphthylamines; Amines

1. Introduction

β-Cyclodextrins (β-CDs) are widely used for enantiomeric separations of various compounds as chiral selectors by capillary electrophoresis (CE), but some primary amino compounds can not be separated enantiomerically or show poor resolution with β-CDs only. Chiral crown ethers, such as (+)-18-crown-6-tetracarboxylic acid (18C6H$_4$), have a good ability of enantiomer resolution of primary amino compounds and have become the first choice in CE and high-performance liquid chromatography (HPLC) [1–3]. However, 18C6H$_4$ is very expensive. Recently, it has been reported that adding an achiral crown ether (18-crown-6) to β-CD-based separation media in CE could lead to or promote enantiomeric separations of primary amino compounds when the enantiomers could not be separated or showed poor resolution with β-CDs alone [4–7]. Enantiomeric separations of four primary amino compounds were achieved or enhanced by our capillary electrochromatography (CEC) with allyl carbamoylated β-CD-bonded negatively charged polyacrylamide gel-filled capillaries using a mobile phase containing 18-

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crown-6 [8]. These methods showed that the primary amino compound combines with the 18-crown-6 and β-CD moiety, and the formation of this complex is assumed to result in a more selective chiral interaction [4,6]. However, the chiral recognition mechanism of this system has not been studied or reported in detail to our knowledge.

Various studies have been reported on noncovalent interactions such as protein–sugar [9,10], antigen–antibody [11], protein–substrate [12], micelle–substrate [13], chiral selector–substrate [13–15] by CE and nuclear magnetic resonance (NMR). The measurement of association constants occupies a central position in those studies. More reliable correlations for estimating the association constants should be expected between CE and NMR because the interactions occur in a similar or even identical environment (free solution) in both techniques.

Our aim in this study is to gain more insight into the enantiomeric recognition related to the complex conformation mentioned above. In this paper, we tried to identify the key step of the enantiomeric recognition of primary amino compounds using both 18-crown-6 and CD on the basis of the association constants calculated by CE and ^{1}H NMR experiments, respectively.

2. Model and theory

The following model is proposed for CE and is used as a working hypothesis for enantiomeric recognition of primary amino compounds using 18-crown-6 with cyclodextrin:

$$\underset{\mu_f}{A} + CD \overset{K_1}{\rightleftarrows} \underset{\mu_{A\cdot CD}}{A\cdot CD}$$

$$\underset{\mu_f}{A} + \text{18-crown-6} \overset{K_2}{\rightleftarrows} \underset{\mu_{A\cdot 18\text{-crown-6}}}{A\cdot \text{18-crown-6}}$$

$$\underset{\mu_{A\cdot 18\text{-crown-6}}}{A\cdot \text{18-crown-6}} + CD \overset{K_3}{\rightleftarrows} \underset{\mu_{A\cdot 18\text{-crown-6}\cdot CD}}{A\cdot \text{18-crown-6}\cdot CD}$$

$$\underset{\mu_{A\cdot CD}}{A\cdot CD} + \text{18-crown-6} \overset{K_4}{\rightleftarrows} \underset{\mu_{A\cdot 18\text{-crown-6}\cdot CD}}{A\cdot \text{18-crown-6}\cdot CD}$$

$$K_1 = [A\cdot CD]/[A][CD] \quad (1)$$

$$K_2 = [A\cdot \text{18-crown-6}]/[A][\text{18-crown-6}] \quad (2)$$

$$K_3 = [A\cdot \text{18-crown-6}\cdot CD]/[A\cdot \text{18-crown-6}][CD] \quad (3)$$

$$K_4 = [A\cdot \text{18-crown-6}\cdot CD]/[A\cdot CD][\text{18-crown-6}] \quad (4)$$

where μ_f is the electrophoretic mobility of substrate A in the electrophoretic solution, $\mu_{A\cdot CD}$ is that of the A–CD complex, $\mu_{A\cdot 18\text{-crown-6}}$ is that of the A–18-crown-6 complex, $\mu_{A\cdot 18\text{-crown-6}\cdot CD}$ is that of the A–18-crown-6-CD complex, and K_1, K_2, K_3 and K_4 are association constants. Fig. 1 shows the model schematically. μ_A, which is the apparent electrophoretic mobility of the substrate A, is as shown below based on reference to previous papers [13,16,17]:

$$\mu_A = \frac{[A]\mu_f + [A\cdot CD]\mu_{A\cdot CD} + [A\cdot \text{18-crown-6}]\mu_{A\cdot 18\text{-crown-6}} + [A\cdot \text{18-crown-6}\cdot CD]\mu_{A\cdot 18\text{-crown-6}\cdot CD}}{[A] + [A\cdot CD] + [A\cdot \text{18-crown-6}] + [A\cdot \text{18-crown-6}\cdot CD]} \quad (5)$$

Substituting Eqs. (1), (2) and (3) into Eq. (5) yields the following equation:

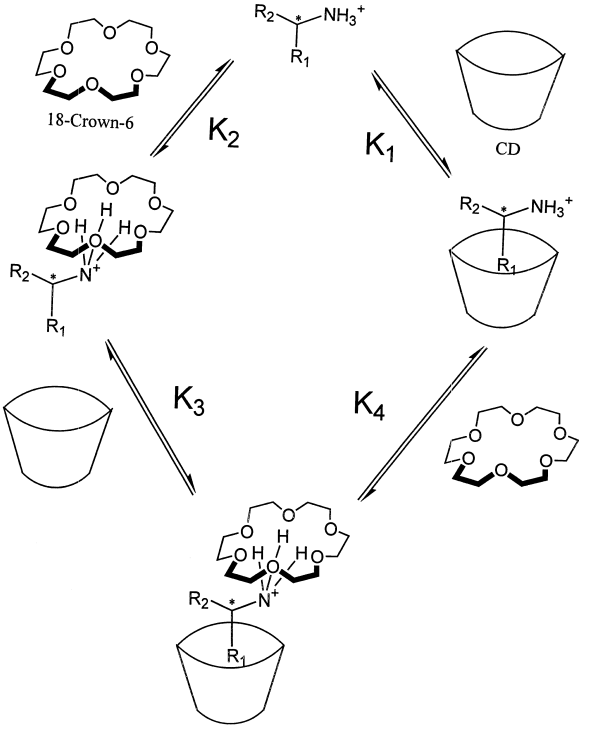

Fig. 1. Proposed schematic association model of the complex consisting of 18-crown-6, primary amino compound and CD.

$$\mu_A = \frac{\mu_f + K_1[CD]\mu_{A\cdot CD} + K_2[18\text{-crown-6}]\mu_{A\cdot 18\text{-crown-6}} + K_2K_3[18\text{-crown-6}][CD]\mu_{A\cdot 18\text{-crown-6}\cdot CD}}{1 + K_1[CD] + K_2[18\text{-crown-6}] + K_2K_3[18\text{-crown-6}][CD]} \quad (6)$$

and, similarly substituting Eqs. (1), (2) and (4) into Eq. (5) yields the following equation:

$$\mu_A = \frac{\mu_f + K_1[CD]\mu_{A\cdot CD} + K_2[18\text{-crown-6}]\mu_{A\cdot 18\text{-crown-6}} + K_1K_4[18\text{-crown-6}][CD]\mu_{A\cdot 18\text{-crown-6}\cdot CD}}{1 + K_1[CD] + K_2[18\text{-crown-6}] + K_1K_4[18\text{-crown-6}][CD]} \quad (7)$$

In this study we can assume that $K_1 \fallingdotseq K_{1R} \fallingdotseq K_{1S}$, $K_2 = K_{2R} = K_{2S}$, $K_{3R} \neq K_{3S}$ and $K_{4R} \neq K_{4S}$ where K_{1R}, K_{1S}, K_{2R}, K_{2S}, K_{3R}, K_{3S}, K_{4R} and K_{4S} are the association constants of *R*- and *S*-form enantiomers of the primary amino compounds tested, respectively. The difference in the apparent electrophoretic mobility of each enantiomer ($\Delta\mu$) is $\mu_{AR} - \mu_{AS}$, where μ_{AR} and μ_{AS} are used as the apparent electrophoretic mobilities of *R*- and *S*-form enantiomers, respectively. Therefore, with Eq. (6), the numerator of $\Delta\mu$ is shown as follows:

$$\begin{aligned}\text{numerator of } \Delta\mu = {} & [CD][18\text{-crown-6}]K_2(K_{3R} - K_{3S}) \\ & \times \{(\mu_{A\cdot 18\text{-crown-6}\cdot CD} - \mu_f) \\ & + [CD]K_1(\mu_{A\cdot 18\text{-crown-6}\cdot CD} - \mu_{A\cdot CD}) \\ & + [18\text{-crown-6}]K_2(\mu_{A\cdot 18\text{-crown-6}\cdot CD} - \mu_{A\cdot 18\text{-crown-6}})\}\end{aligned} \quad (8)$$

With Eq. (7), the numerator of $\Delta\mu$ is similarly shown as follows:

$$\begin{aligned}\text{numerator of } \Delta\mu = {} & [CD][18\text{-crown-6}]K_1(K_{4R} - K_{4S}) \\ & \times \{(\mu_{A\cdot 18\text{-crown-6}\cdot CD} - \mu_f) \\ & + [CD]K_1(\mu_{A\cdot 18\text{-crown-6}\cdot CD} - \mu_{A\cdot CD}) \\ & + [18\text{-crown-6}]K_2(\mu_{A\cdot 18\text{-crown-6}\cdot CD} - \mu_{A\cdot 18\text{-crown-6}})\}\end{aligned} \quad (9)$$

If the electrophoretic mobilities of the free and complexed enantiomers are different, and if the two enantiomers have different association constants, that is K_{3R} and K_{3S} are different and K_{4R} and K_{4S} are different, it is clear from Eqs. (8) and (9) that enantiomeric separation is possible.

Also, Eqs. (8) and (9) show that the enantiomeric separation of primary amino compounds is related to the difference of association constants in a pair of enantiomers when the complex of a primary amino compound and 18-crown-6 becomes associated with CD, $K_{3R} - K_{3S}$, and to the difference of association constants when the complex of a primary amino compound and CD becomes associated with 18-crown-6, $K_{4R} - K_{4S}$. Thus, we can identify the key step of recognition with the equilibrium where the complex of a primary amino compound and 18-crown-6 becomes associated with CD (enantiomeric association) or the equilibrium where the complex of a primary amino compound and CD becomes associated with the 18-crown-6 (diastereomeric association), by comparing the absolute value of ($K_{3R} - K_{3S}$) with the absolute value of ($K_{4R} - K_{4S}$).

In ^{1}H NMR, the model and theory mentioned above can be used with the chemical shift (δ) in place of electrophoretic mobility [13].

$$\delta_{obs} = \frac{\delta_0 + K_1[CD]\delta_{A\cdot CD} + K_2[18\text{-crown-6}]\delta_{A\cdot 18\text{-crown-6}} + K_2K_3[18\text{-crown-6}][CD]\delta_{A\cdot 18\text{-crown-6}\cdot CD}}{1 + K_1[CD] + K_2[18\text{-crown-6}] + K_2K_3[18\text{-crown-6}][CD]} \quad (10)$$

$$\delta_{obs} = \frac{\delta_0 + K_1[CD]\delta_{A\cdot CD} + K_2[18\text{-crown-6}]\delta_{A\cdot 18\text{-crown-6}} + K_1K_4[18\text{-crown-6}][CD]\delta_{A\cdot 18\text{-crown-6}\cdot CD}}{1 + K_1[CD] + K_2[18\text{-crown-6}] + K_1K_4[18\text{-crown-6}][CD]} \quad (11)$$

$$\begin{aligned}\text{numerator of } \Delta\delta = {} & [CD][18\text{-crown-6}]K_2(K_{3R} - K_{3S}) \\ & \times \{(\delta_{A\cdot 18\text{-crown-6}\cdot CD} - \delta_0) \\ & + [CD]K_1(\delta_{A\cdot 18\text{-crown-6}\cdot CD} - \delta_{A\cdot CD}) \\ & + [18\text{-crown-6}]K_2(\delta_{A\cdot 18\text{-crown-6}\cdot CD} - \delta_{A\cdot 18\text{-crown-6}})\}\end{aligned} \quad (12)$$

$$\begin{aligned}\text{numerator of } \Delta\delta = {} & [CD][18\text{-crown-6}]K_1(K_{4R} - K_{4S}) \\ & \times \{(\delta_{A\cdot 18\text{-crown-6}\cdot CD} - \delta_0) \\ & + [CD]K_1(\delta_{A\cdot 18\text{-crown-6}\cdot CD} - \delta_{A\cdot CD}) \\ & + [18\text{-crown-6}]K_2(\delta_{A\cdot 18\text{-crown-6}\cdot CD} - \delta_{A\cdot 18\text{-crown-6}})\}\end{aligned} \quad (13)$$

where δ_0 is the chemical shift of the substrate A, $\delta_{A\cdot CD}$ is that of the A–CD complex, $\delta_{A\cdot 18\text{-crown-6}}$ is

that of A–18-crown-6 complex, $\delta_{A\cdot 18\text{-crown-}6\cdot CD}$ is that of the A–18-crown-6–CD complex and δ_{obs} is the apparent chemical shift of the substrate A.

3. Experimental

3.1. Chemicals

Dimethyl sulfoxide (DMSO), 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CD) and distilled water were purchased from Nacalai Tesque (Kyoto, Japan). (*R*)-(+)-1-(1-Naphthyl)ethylamine, (*S*)-(–)-1-(1-naphthyl)ethylamine, DL-1-(1-naphthyl)ethylamine, 1-aminoindan and 18-crown 6-ether (18-crown-6) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Boric acid and β-cyclodextrin (β-CD) were from Wako (Osaka, Japan). Tris(hydroxymethyl)-aminomethane (Tris) was from Sigma (St. Louis, MO, USA). Deuterium chloride 37% (w/w) solution in deuterium oxide (^{2}HCl) and 1,2,3,4-tetrahydro-1-naphthylamine hydrochloride were purchased from Aldrich (Milwaukee, WI, USA). Deuterium oxide (^2H_2O) was obtained from Cambridge Isotope Labs. (Andover, MA, USA). 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) was from Stohler Isotope Chemicals.

3.2. CE

3.2.1. Instrument

CE experiments were carried out on a Beckman P/ACE system 5510 (Beckman Instruments, Fullerton, CA, USA). A coated capillary (CElect-N, Supelco, Bellefonte, PA, USA) was used as the separation tube [47 cm (effective length 40 cm)×50 μm I.D.×360 μm O.D.]. The temperature of the capillary tubing was maintained at 25°C with a liquid coolant. The applied voltage was held at constant 200, 300 or 400 V/cm. The detection wavelength was 214 nm.

3.2.2. Solution

In consideration of the solubility in buffer, DM-β-CD was used as a chiral selector. The electrophoretic solutions used for calculating the association constants were: for K_1, 200 m*M* Tris–300 m*M* boric acid buffer (pH 7.0) containing 0, 2, 5, 8, 10, 20, 30 or 50 m*M* DM-β-CD, respectively; for K_2, 200 m*M* Tris–300 m*M* boric acid buffer (pH 7.0) containing 0, 50, 100, 200, 300 or 500 m*M* 18-crown-6, respectively; for K_3, 200 m*M* Tris–300 m*M* boric acid buffer (pH 7.0) containing 10 m*M* 18-crown-6 and 0, 5, 10, 20, 30 or 50 m*M* DM-β-CD, respectively; for K_4, 200 m*M* Tris–300 m*M* boric acid buffer (pH 7.0) containing 10 m*M* DM-β-CD and 0, 10, 20, 50, 100, 200 or 500 m*M* 18-crown-6, respectively. Each analyte shown in Fig. 2 was dissolved in 50 m*M* Tris–phosphate buffer (pH 2.0) containing a small amount of DMSO and was introduced into the anodic end of the capillary by the pressure mode (0.5 p.s.i., 3 s; 1 p.s.i.=6894.76 Pa). DMSO was used as a neutral marker.

3.3. ^{1}H-NMR

3.3.1. Instrument

^{1}H NMR spectra were obtained on a Varian Unity Inova NMR spectrometer (Varian, USA) operating at 500 MHz for ^{1}H. A total of 512 transients was collected with a frequency range of 7500 Hz to give a digital resolution of 0.61 Hz/point. Chemical shifts were reported in parts per million (ppm) relative to internally added DSS. All measurements were done at 20°C, and sample tubes of 5 mm I.D. (Wilmad, Buena, NJ, USA) containing 500 μl of sample solution were employed.

3.3.2. Solution

β-CD was used as a chiral selector. DSS was used as an internal standard at the concentration of 20

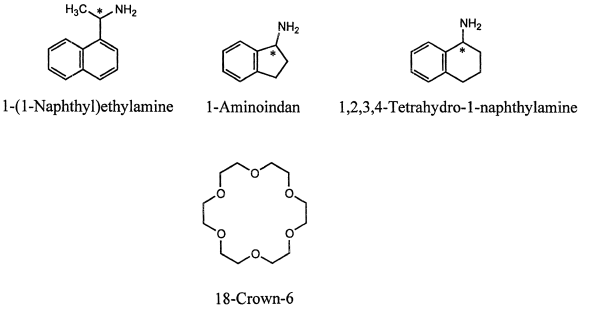

Fig. 2. Chemical structures of the primary amino compounds tested and 18-crown-6.

μ*M*. Standard solutions of 1-(1-naphthyl)ethylamine were prepared by dissolving it in a small amount of ^{2}HCl followed by dilution with ^2H_2O. The solutions used for calculating the association constants were: for K_1, ^{2}HCl–^2H_2O solution containing 10 μ*M* DL-1-(1-naphthyl)ethylamine and 0, 100, 200, 300, 500, 800 or 1000 μ*M* β-CD, respectively; for K_2, ^{2}HCl–^2H_2O solution containing 10 μ*M* DL-1-(1-naphthyl)ethylamine and 0, 20 000, 30 000, 50 000, 80 000, 100 000, 200 000 or 300 000 μ*M* 18-crown-6, respectively; for K_3, ^{2}HCl–^2H_2O solution containing 10 μ*M* (*R*) or (*S*)-1-(1-naphthyl)ethylamine, 10 000 μ*M* 18-crown-6 and 0, 2, 5, 10, 50, 100, 500 or 1000 μ*M* β-CD, respectively; for K_4, ^{2}HCl–^2H_2O solution containing 10 μ*M* (*R*) or (*S*)-1-(1-naphthyl)ethylamine, 10 000 μ*M* β-CD and 0, 1000, 2000, 3000, 5000, 8000 or 10 000 μ*M* 18-crown-6, respectively. The pH of the sample solutions was around 6.5.

3.4. Calculation

In CE experiments, the electrophoretic mobility (μ) was calculated in the usual manner with:

$$\mu = (lL/V) \cdot [(t_0 - t)/(t \cdot t_0)]$$

where l is the effective length of the capillary (cm), L is the total length of the capillary (cm), V is the applied voltage (V/cm), t is the migration time of a substrate (s) and t_0 is the migration time of the neutral marker of DMSO (s).

K_1, K_2, $\mu_{A\cdot DM\text{-}\beta\text{-}CD}$ and $\mu_{A\cdot 18\text{-crown-}6}$ in CE were calculated with well-known equations and plotting forms [10,13,14,17] as shown below:

$$\frac{1}{\mu_A - \mu_f} = \frac{1}{(\mu_{A\cdot DM\text{-}\beta\text{-}CD} - \mu_f)K_1} \cdot \frac{1}{[DM\text{-}\beta\text{-}CD]} + \frac{1}{\mu_{A\cdot DM\text{-}\beta\text{-}CD} - \mu_f} \quad (14)$$

$$\frac{1}{\mu_A - \mu_f} = \frac{1}{(\mu_{A\cdot 18\text{-crown-}6} - \mu_f)K_2} \cdot \frac{1}{[18\text{-crown-}6]} + \frac{1}{\mu_{A\cdot 18\text{-crown-}6} - \mu_f} \quad (15)$$

where the association constant could be obtained from (intercept/slope), and $(\mu_{A\cdot DM\text{-}\beta\text{-}CD} - \mu_f)$ or $(\mu_{A\cdot 18\text{-crown-}6} - \mu_f)$ from the reciprocal of the intercept.

In ^{1}H NMR, K_1, K_2, $\delta_{A\cdot\beta\text{-}CD}$ and $\delta_{A\cdot 18\text{-crown-}6}$ were calculated with similar equations and plotting forms as shown below:

$$\frac{1}{\delta_{obs} - \delta_0} = \frac{1}{(\delta_{A\cdot\beta\text{-}CD} - \delta_0)K_1} \cdot \frac{1}{[\beta\text{-CD}]} + \frac{1}{\delta_{A\cdot\beta\text{-}CD} - \delta_0} \quad (16)$$

$$\frac{1}{\delta_{obs} - \delta_0} = \frac{1}{(\delta_{A\cdot 18\text{-crown-}6} - \delta_0)K_2} \cdot \frac{1}{[18\text{-crown-}6]} + \frac{1}{\delta_{A\cdot 18\text{-crown-}6} - \delta_0} \quad (17)$$

where the association constant could be obtained from (intercept/slope), and $(\delta_{A\cdot\beta\text{-}CD} - \delta_0)$ or $(\delta_{A\cdot 18\text{-crown-}6} - \delta_0)$ from the reciprocal of the intercept.

To calculate K_3 and K_4, a non-linear least-squares method [18,19] using one of the functions of the Microsoft Excel for Windows was applied to Eqs. (6), (7), (10) and (11) in CE and ^{1}H NMR experiments.

4. Results and discussion

4.1. Enantiomeric separation of primary amino compounds by CEC with β-CD-bonded charged polyacrylamide gels and a mobile phase containing 18-crown-6

Recently, we prepared allyl carbamoylated β-CD-bonded charged polyacrylamide gel-filled capillaries and achieved enantiomeric separations of various compounds by CEC with them [8,20]. However, the CEC study yielded poor or no resolution of some primary amino compounds. To obtain the enantiomer resolution of the primary amino compounds, we investigated the use of a mobile phase containing 18-crown-6, having the structure shown in Fig. 2, on the basis of information from Refs. [4–6]. Finally, enhancements of the enantiomeric separations of four primary amino compounds were obtained [8]. A typical electrochromatogram of 1-(1-naphthyl)-ethylamine is shown in Ref. [8].

In this method, it is assumed that the formation of

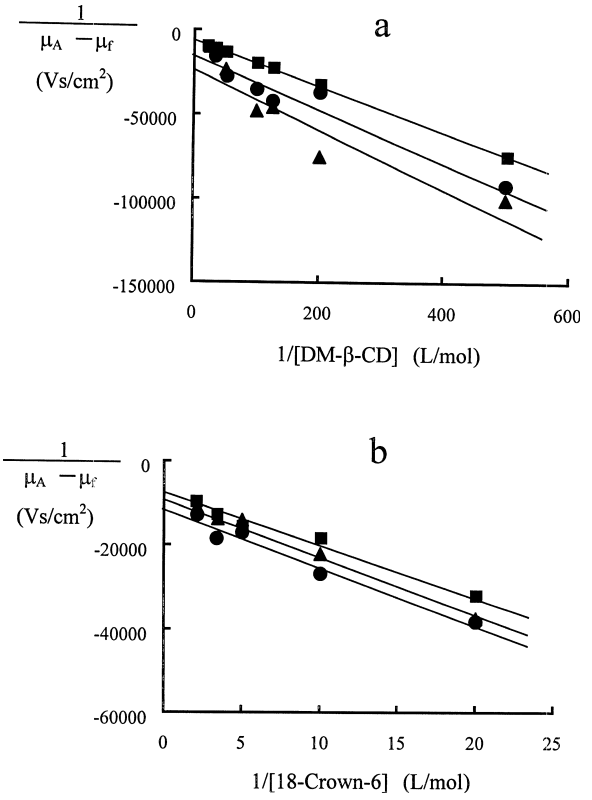

Fig. 3. Plot of $(\mu_A - \mu_f)^{-1}$ versus [DM-β-CD] or [18-crown-6] for the calculation of the association constants according to Eqs. (14) or (15). Symbols: ■, 1-(1-naphthyl)ethylamine; ▲, 1-aminoindan; ●, 1,2,3,4-tetrahydro-1-naphthylamine. See Section 3 for further details.

the three-composition complex results in a more selective chiral interaction, although the chiral recognition mechanism of this system has not been studied and reported in detail. We decided to try to identify the key step of the enantiomeric recognition of primary amino compounds in this system with CE and ^{1}H NMR.

4.2. Identification of the key step of the enantiomeric recognition by CE

4.2.1. Measurements of K_1, K_2, μ_f, $\mu_{A\cdot DM\text{-}\beta\text{-}CD}$ and $\mu_{A\cdot 18\text{-}crown\text{-}6}$

Three primary amino compounds, 1-(1-naphthyl)-ethylamine, 1-aminoindan and 1,2,3,4-tetrahydro-1-naphthylamine, were chosen as test compounds to identify the key step by CE experiments. Low enantiomeric resolutions of 1-(1-naphthyl)-ethylamine and 1,2,3,4-tetrahydro-1-naphthylamine were obtained with 30 or 50 m*M* DM-β-CD, and the migration times of the first eluted enantiomer of the two compounds were used to calculate the mobilities. The plots according to Eqs. (14) and (15) are shown in Fig. 3. The straight lines were drawn according to the least-squares method. The association constants and electrophoretic mobilities calculated from the values of the slope and intercept of the line are shown in Table 1.

4.2.2. Measurements of K_3 and K_4

It is presumed that K_3 is the largest among the four kinds of association constants because of the hydrophobicity of the complex between a primary aromatic amino compound and 18-crown-6 must be large. However, stabilizing complexes would not be always related to an increase in enantiomeric selectivity as shown in Eqs. (8) and (9): then measurements of K_3 and K_4 were carried out to identify experimentally the key step of the enantiomeric recognition of the three primary amino compounds. Changes in apparent electrophoretic mobility of the three primary amino compounds were examined by varying the concentrations of DM-β-CD or 18-crown-6 in the electrophoretic solution containing 10 m*M* 18-crown-6 or DM-β-CD, respectively, and were plotted against them. Fig. 4 shows the results.

Table 1
Experimental values of association constants and electrophoretic mobilities[a]

Compound	Association constant [(mol/l)$^{-1}$]		Electrophoretic mobility (V s/cm^2)		
	K_1	K_2	μ_f	$\mu_{A\cdot DM\text{-}\beta\text{-}CD}$	$\mu_{A\cdot 18\text{-}crown\text{-}6}$
1-(1-Naphthyl)ethylamine	43	7	$2.00\cdot10^{-4}$	$2.47\cdot10^{-5}$	$7.56\cdot10^{-5}$
1-Aminoindan	105	5	$2.07\cdot10^{-4}$	$1.54\cdot10^{-4}$	$7.66\cdot10^{-5}$
1,2,3,4-Tetrahydro-1-naphthylamine	78	9	$2.03\cdot10^{-4}$	$1.19\cdot10^{-4}$	$1.15\cdot10^{-4}$

[a] See Section 3 for further details.

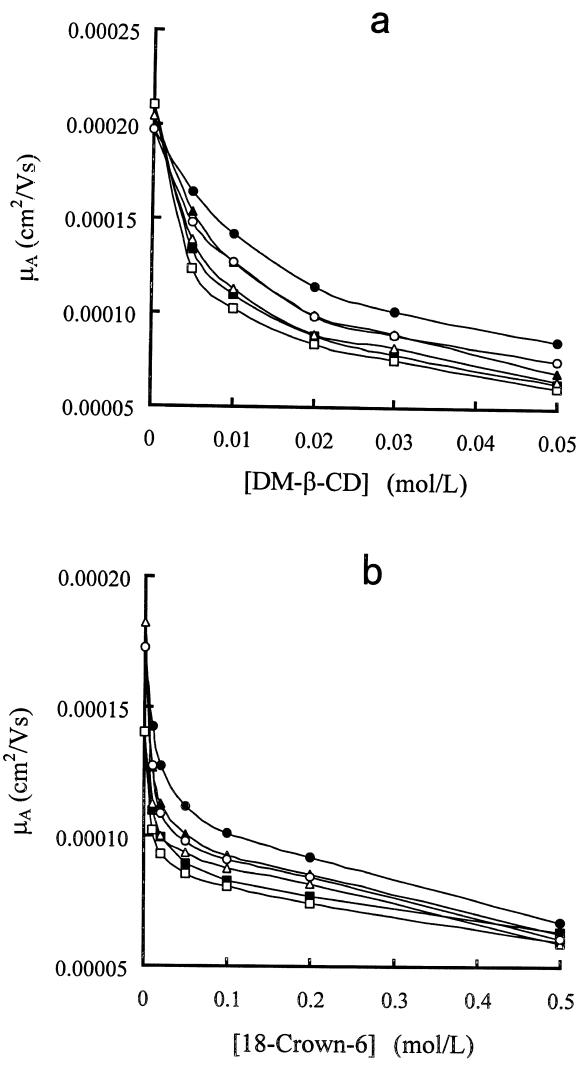

Fig. 4. Plot of μ_A versus [DM-β-CD] or [18-crown-6] for the calculation of the association constants. Symbols: ■, (*R*)-1-(1-naphthyl)ethylamine; □, (*S*)-1-(1-naphthyl)ethylamine; ▲, 1-aminoindan (the first eluted enantiomer); △, 1-aminoindan (the second eluted enantiomer); ●, 1,2,3,4-tetrahydro-1-naphthylamine (the first eluted enantiomer); ○, 1,2,3,4-tetrahydro-1-naphthylamine (the second eluted enantiomer). See Section 3 for further details.

The mobility decreased with increasing concentrations of DM-β-CD or 18-crown-6. Such decreases in the mobility indicate increased formation of the three-composition complex. The values of K_1, K_2, μ_f, $\mu_{A\cdot DM\text{-}\beta\text{-}CD}$ and $\mu_{A\cdot 18\text{-crown-}6}$ measured in Section 4.2.1 and six couples of the measured values of μ_A and the concentrations of DM-β-CD were substituted into Eq. (6), and the optimization of K_3 was done using a non-linear least-squares method. The values measured in Section 4.2.1 and seven couples of the measured values of μ_A and the concentrations of 18-crown-6 were substituted into Eq. (7) to obtain K_4 similarly. The values of K_3 and K_4 obtained are summarized in Table 2. A typical enhanced enantiomeric separation of 1-aminoindan is shown in Fig. 5.

As can be seen from Table 2, K_{31} and K_{32} are different ($K_{31}-K_{32}\neq 0$) for all the three compounds, where K_{31} and K_{32} are the association constants of the first and second eluted enantiomers of the primary amino compounds tested, respectively, when the complex of a primary amino compound and 18-crown-6 becomes associated with DM-β-CD. K_{41} and K_{42} are also different ($K_{41}-K_{42}\neq 0$) for all the three compounds, where K_{41} and K_{42} are those of the first and second eluted enantiomers, respectively, when the complex of a primary amino compound and DM-β-CD becomes associated with 18-crown-6. The values of $\mu_{A\cdot 18\text{-crown-}6\cdot DM\text{-}\beta\text{-}CD}$ of them were $6\text{–}7\cdot 10^{-5}$ ($cm^2/V\,s$) (data are not shown in Table 2). These results support the enantiomeric separations in this system on the basis of Eqs. (8) and (9). In addition to them, K_{31} and K_{32} are the largest among the four kinds of association constants from Tables 1 and 2, which is in accord with the expectations mentioned above.

The absolute values of ($K_{31}-K_{32}$) were larger than those of ($K_{41}-K_{42}$) for all the three compounds from the results of Table 2. Therefore, the key step of the enantiomeric recognition of the three compounds was identified with the equilibrium of the enantiomeric association where the complex of a primary amino compound and 18-crown-6 is associated with DM-β-CD rather than the diastereomeric association.

4.3. Identification of the key step of the enantiomeric recognition by ^{1}H NMR

1-(1-Naphthyl)ethylamine was chosen as a test compound to identify the key step by ^{1}H NMR experiments. Since the CH proton bonded to the asymmetric carbon atom of 1-(1-naphthyl)-ethylamine overlapped the H_2O proton from the solvent and β-CD, the CH_3 proton next to the

Table 2
Experimental values of association constants[a]

Compound	Association constant $[(mol/l)^{-1}]$[b]			
	K_{31}	K_{32}	K_{41}	K_{42}
1-(1-Naphthyl)ethylamine	1400 (*R*-form)	2100 (*S*-form)	240 (*R*-form)	340 (*S*-form)
1-Aminoindan	2100	2700	160	280
1,2,3,4-Tetrahydro-1-naphthylamine	510	800	50	130

[a] See Section 3 for further details.
[b] K_{31} and K_{41} are the association constants of the first eluted enantiomers and K_{32} and K_{42} are those of the antipodes.

asymmetric center was observed. A ^{1}H NMR spectrum of ^{2}HCl–^2H_2O solution containing 100 μ*M* 1-(1-naphthyl)ethylamine, 10 m*M* β-CD and 18-crown-6, respectively, expanded for the CH_3 proton is shown in Fig. 6. Splitting of the resonance line attributed to the enantiomeric recognition was observed.

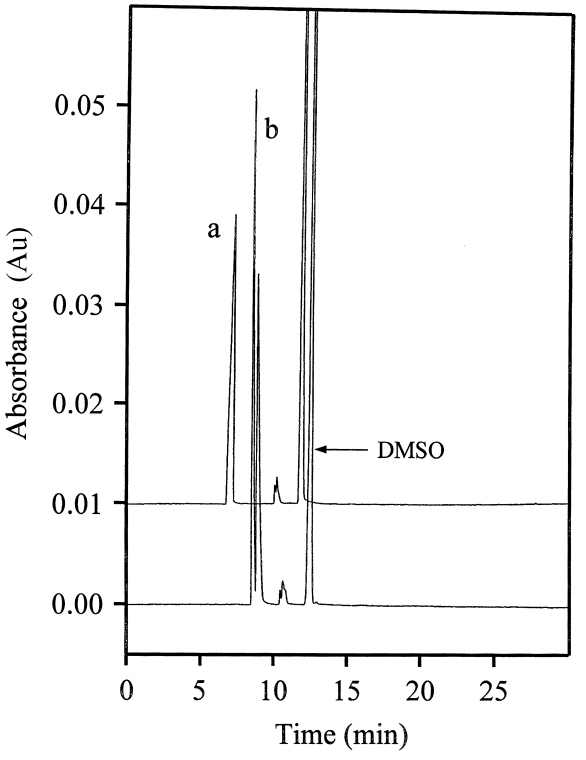

Fig. 5. Enantiomeric separation of 1-aminoindan by capillary zone electrophoresis. (a) A electrophoretic solution consisting of 200 m*M* Tris–300 m*M* boric acid buffer (pH 7.0) containing 10 m*M* DM-β-CD. (b) A electrophoretic solution consisting of 200 m*M* Tris–300 m*M* boric acid buffer (pH 7.0) containing 10 m*M* DM-β-CD and 18-crown-6, respectively.

4.3.1. Measurements of K_1, K_2, δ_0, $\delta_{A\cdot\beta\text{-}CD}$ and $\delta_{A\cdot 18\text{-}crown\text{-}6}$

The plots according to Eqs. (16) and (17) are shown in Fig. 7. The straight lines were drawn according to the least-squares method. The association constants and chemical shifts calculated from the value of the slope and intercept of the line are shown in Table 3.

4.3.2. Measurements of K_3 and K_4

Changes in the apparent chemical shift of 1-(1-naphthyl)ethylamine were examined by varying the concentrations of β-CD or 18-crown-6 in the solu-

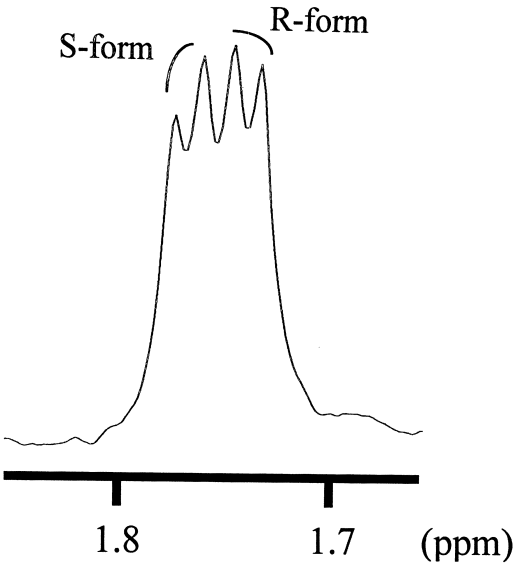

Fig. 6. ^{1}H NMR spectrum of enantiomeric recognition of 1-(1-naphthyl)ethylamine expanded for the CH_3 proton. Sample solution: ^{2}HCl–^2H_2O solution containing 100 μ*M* DL-1-(1-naphthyl)ethylamine, 10 m*M* β-CD and 18-crown-6, respectively.

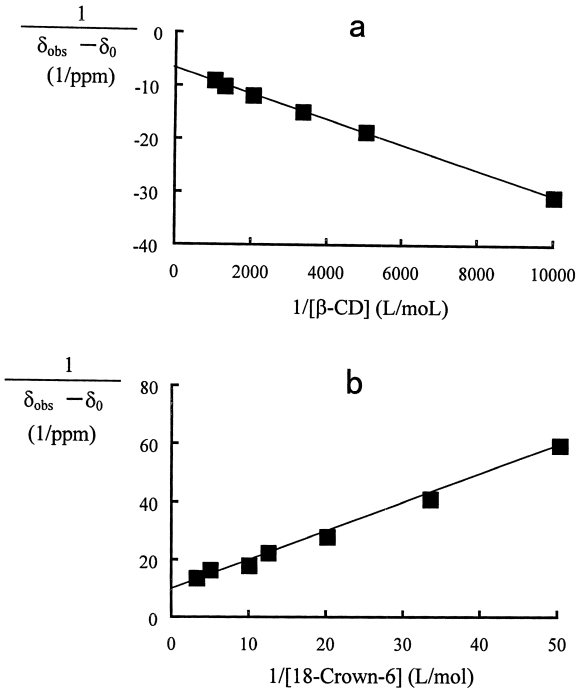

Fig. 7. Plot of $(\delta_{obs}-\delta_0)^{-1}$ versus [β-CD] or [18-crown-6] for the calculation of the association constants of 1-(1-naphthyl)ethylamine according to Eqs. (16) or (17). See Section 3 for further details.

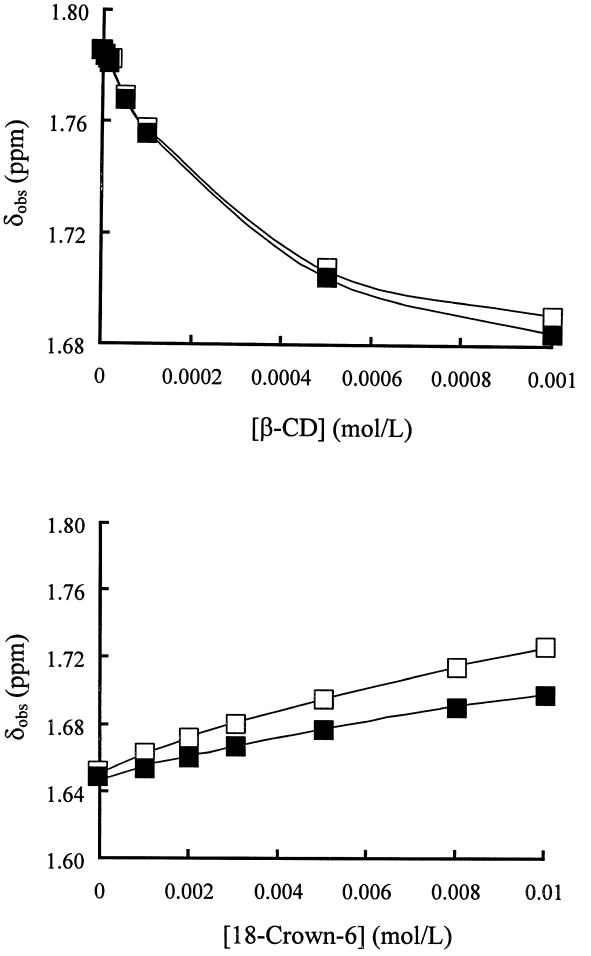

Fig. 8. Plot of δ_{obs} versus [β-CD] or [18-crown-6] for the calculation of the association constants. Symbols: ■, (*R*)-1-(1-naphthyl)ethylamine; □, (*S*)-1-(1-naphthyl)ethylamine. See Section 3 for further details.

tion containing 10 000 μ*M* 18-crown-6 or β-CD, respectively, and were plotted against them. Fig. 8 shows the results. The values of K_1, K_2, δ_0, $\delta_{A\cdot\beta\text{-CD}}$ and $\delta_{A\cdot 18\text{-crown-6}}$ obtained in Section 4.3.1 and eight couples of the measured values of δ_A and the concentrations of β-CD were substituted into Eq. (10), and the optimization of K_{3R} and K_{3S} was done using a non-linear least-squares method. The values obtained in Section 4.3.1 and seven couples of the measured values of δ_A and the concentrations of 18-crown-6 were substituted into Eq. (11) to similarly obtain K_{4R} and K_{4S}. The values obtained are summarized in Table 4.

As can be seen from Table 4, K_{3R} and K_{3S} are

Table 3
Experimental values of association constants and chemical shifts[a]

Compound	Association constant $[(mol/l)^{-1}]$		Chemical shift (ppm)		
	K_1	K_2	δ_0	$\delta_{A\cdot\beta\text{-CD}}$	$\delta_{A\cdot 18\text{-crown-6}}$
1-(1-Naphthyl)ethylamine	2780	9	1.79	1.63	1.89

[a] See Section 3 for further details.

Table 4
Experimental values of association constants[a]

Compound	Association constant [(mol/l)$^{-1}$]			
	K_{3R}	K_{3S}	K_{4R}	K_{4S}
1-(1-Naphthyl)ethylamine	10 000	12 800	200	180

[a] See Experimental section for further details.

different ($K_{3R} - K_{3S} \neq 0$) and K_{4R} and K_{4S} are different ($K_{4R} - K_{4S} \neq 0$). The values of $\mu_{A\cdot 18\text{-crown-}6\cdot\beta\text{-CD}}$ were 1.73–1.77 (ppm) (data are not shown in Table 4). These results support the enantiomeric recognition in this system on the basis of Eqs. (12) and (13). K_{3R} and K_{3S} are the largest among the four kinds of association constants from Tables 3 and 4, which is in accord with the expectations.

The absolute values of ($K_{3R} - K_{3S}$) were larger than those of ($K_{4R} - K_{4S}$) from the results of Table 4, and therefore the key step of the enantiomeric recognition was identified with the equilibrium of the enantiomeric association where the complex of 1-(1-naphthyl)ethylamine and 18-crown-6 is associated with β-CD rather than the diastereomeric association.

From the results mentioned above, the values of the association constants of 1-(1-naphthyl)ethylamine found by ^{1}H NMR were larger than those found by CE, about 64 times with regard to K_1 and 6–7 times with regard to K_3. These results could be due to the difference in the kind of CD used, because of the solubility and the difference in the analytical conditions such as the composition of solution. The sign of ($K_{4R} - K_{4S}$) of 1-(1-naphthyl)ethylamine obtained by ^{1}H NMR experiments was opposite to that obtained by CE experiments. This seems to be due to experimental error caused by the small increase of chemical shift with increasing concentrations of 18-crown-6 when K_4 values were measured by ^{1}H NMR experiments. However, both the CE and ^{1}H NMR methods supported the same key step for 1-(1-naphthyl)ethylamine, therefore, the two analytical methods are useful for identifying the key step of enantiomeric recognition.

5. Conclusion

A model and theoretical equations are presented to investigate the enantiomeric recognition mechanism of primary amino compounds using 18-crown-6 with CD by CE and ^{1}H NMR. Association constants were calculated by CE and ^{1}H NMR experiments on the basis of the model. The key step of the chiral recognition identified from those values was the equilibrium of the enantiomeric association where the complex of a primary amino compound and 18-crown-6 is associated with CD rather than the equilibrium of the diastereomeric association where the complex of a primary amino compound and CD is associated with 18-crown-6. The model to calculate association constants shown in this paper was shown be advantageous for the analysis of not only the enantiomeric recognition mechanism but also various associations. Association constants calculated by this method can offer interesting information about various three-composition complexes.

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