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<td>Author(s)</td>
<td>SAWADA, Masami, YAMAUCHI, Yasuhiro, SHIZUMA, Motohiro, TAKAI, Yoshio, NAKANO, Kazurou, KURODA, Masao, ARAKAWA, Ryuichi</td>
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A Comparison with Capillary Electrophoresis

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Chiral recognition of the host–guest complexations between the (RRRR)-18-crown-6-tetraacarboxylic acid (18C6TCA) host (H) and α-amino acid or their ester derivative guests (G) has been systematically determined using both FAB mass spectrometry (MS) and capillary zone electrophoresis (CE). A comparison of these two techniques for the same series of guests was then done for the first time. In the case of 18C6TCA, we found that there is no correlation between the chiral recognition obtained from FABMS (i.e., the IRIS value) and from CE (i.e., the a value in an aqueous buffer solution) covering three sets of guests including amino acids, amino acid esters, and primary aromatic amines. On the other hand, the former showed a good agreement with that from NMR (i.e., the Ks/Ka value; the ratio of the corresponding equilibrium constants), supporting our earlier conclusion that FABMS is a good measurement to predicting differences (ΔJC values) in the respective chiral H–G interactions in solution. We also found that when a given guest changes from an amino acid to its corresponding amino acid ester, the a value by CE dramatically changes from α > 1.0 to α < 1.0, though the IRIS value by FABMS does not show any such changes. These findings were considered to be due to the characteristic contributions of the dissociable host’s COOH functions under the experimental pH conditions in CE.

1. Introduction

The chiral recognition of host (H)/guest (G) or substrate/receptor binding processes has recently been attracting much interest in the analytical/biological/organic chemistry fields.14–16 The enantioselective binding processes have nowadays been widely applied in chiral separations using capillary zone electrophoresis (CE)17,18 and high performance liquid chromatography (HPLC),19–21. Especially, CE is known as one of the highly sensitive detection methods for an aqueous buffer solution. Most recently, fast atom bombardment (FAB) mass spectrometry (MS)22–24 compared with electrospray ionization (ESI) MS25 or matrix assisted laser desorption ionization (MALDI) MS,26 has been deemed as a sensitive screening method and extensively used to detect the chiral recognition properties of various chiral host compounds (NMR matrix). thereon, a 1 : 1 mixture of an unlabeled (S)-enantiomer and a deuteriuated labeled (S)-enantiomer guest has been utilized as a characteristic guest pair (the enantiomer-labeled (EL) guest method).

A chiral crown ether compound, 18-crown-6-tetraacarboxylic acid (18C6TCA) 1, which was first synthesized by Lehn et al.,15,16 has been continuously applied as a representative crown host. Examples are (i) a chiral selector in CE,16,21 (ii) a catalyst in peptide synthesis,22 (iii) a chiral discriminating agent in NMR,23 (iv) a neutral carrier in an ion-selective membrane electrode,24–27 and (v) a chiral stationary phase in LC.28 Among them, we were quite interested in its application to CE where highly sensitive chiral separations of amino acids had been achieved by Kuhn et al.17,19,19 Further, in CE, a given host compound was simply utilized without any structural modifications such as fixations to chiral stationary phases in HPLC, and then, a direct comparison with FABMS was thought to be possible.

In this study, we selected the host-guest combinations including host 1 and various amino acid guests, their esters, and primary aromatic amines, compared the corresponding measures of the chiral recognition derived from FABMS and CE, and then investigated for the first time a correlation between them covering a wide range of amine guests. If a correlation was found, it would be very useful for practical applications.

2. Experimental

2.1 Materials

For chiral host 1, (RRRR)(+-)(18-crown-6)-2,3,11,12-
tetracarboxylic acid, a commercial sample (Aldrich) was used without further purification: mp 210–212°C.
All chiral guest compounds (both deuterium-labeled (S)-enantiomer and unlabeled (R)-enantiomer ones), including the hydrochloride salts of α-amino acids, α-amino acid alkyl esters, 1-phenylethylamine, and 1-(1-naphthyl)ethylamine, were commercial or synthetic samples which have already been described else-
where.10, 13, 29

2.2 FAB mass spectrometry: the enantiomer-
labeled guest method
Positive ion FAB mass spectra were obtained using a JEOI M600H mass spectrometer: acceleration volt, 3 kV; mass range, m/z 20–1,000; beam, Xe; emission, 20 mA; source pressure, ca. 10^-5 Torr.

2.2.1 Preparation of the sample solutions for the
amino ester salt guests As a standard method,10 the next three solutions were mixed using microsyringes and vibrated using an ultrasonic mixer. A 1 µL aliquot of the mixed solution was deposited on an FAB probe tip. The three solutions were as follows: (i) 5 µL of a 1.33 M MeOH solution of a 1:1 mixture of (R)-unlabeled and (S)-labeled amino acid ester hydrochloride guests, (ii) 5 µL of a 0.20 M CHCl3 (or MeOH) solution of a chiral host such as I, and (iii) 15 µL of the NBA matrix.

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<tr>
<th>Guest Class</th>
<th>Abbreviation</th>
<th>Detection method&lt;sup&gt;a&lt;/sup&gt; pH</th>
<th>Migration time (min)</th>
<th>α-Value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>IRIS-Value&lt;sup&gt;d&lt;/sup&gt;</th>
<th>K&lt;sub&gt;s&lt;/sub&gt;/K&lt;sub&gt;s&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt;</th>
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<td>D</td>
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<td>25.21</td>
<td>22.04</td>
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<td>10.98</td>
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<td>9.64</td>
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<td>17.18 (single peak)</td>
<td>1.00</td>
<td>1.39</td>
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</table>

<sup>a</sup> I: the indirect method, D: the direct method (see the experimental section).
<sup>b</sup> α = [migration time of R-isomer]/[migration time of S-isomer] in an aqueous Tris/citrate buffer solution. The value in parenthesis indicates the α value in a non-aqueous formamide solution.<sup>32</sup>  
<sup>c</sup> IRIS = I<sub>α</sub>/I<sub>α</sub>.  
<sup>d</sup> The ratio of equilibrium constants (K<sub>s</sub> and K<sub>s</sub>).  
<sup>e</sup> Pgly: phenylglycine.  
<sup>f</sup> 1-PEA = 1-phenylethylamine; CI salt was employed for CE.  
<sup>g</sup> 1-NEA = 1-(1-naphthyl)ethylamine; SCN salt was employed for CE.  
<sup>b</sup> Estimated assignment.  
<sup>h</sup> Ref. 29.  
<sup>i</sup> Solvent: CD<sub>3</sub>OD, Temp: 25°C.<sup>34</sup>  
<sup>k</sup> K<sub>s</sub> = 3.6×10<sup>4</sup> M<sup>-1</sup>, K<sub>s</sub> = 2.3×10<sup>4</sup> M<sup>-1</sup> were obtained from the competitive complexation system. Solvent: CD<sub>3</sub>OD, Temp: 25°C.
After evaporation of the solvents, the concentrations in the NBA were calculated to be \([H]=0.0677 \, M\) and \([G_9]= [G_3]=0.222 \, M\); these corresponded to the concentration condition B.\(^{13b}\) The accuracy of the 1/1 equivalent concentration of the \((R)\)- and \((S)\)-enantiomer guests was confirmed by checking whether the IRIS value with an achiral host \((18\text{-crown-6})\) was experimentally obtained as unity \((1.00 \pm 0.03)\) for every preparation of the mixed guest solutions. The relative peak intensity data were mostly averaged from the 10\(^{th}\) to 40\(^{th}\) scans \((n=30)\). The correction on the basis of the natural abundance of isotopes was performed for the methyl ester guests, as already described,\(^{10}\) but not for the ethyl and isopropyl ester guests.

### 2.2.2 Preparation of the sample solutions for the amino acid \(p\)-toluenesulfonic acid salt guests

The procedures for the sample preparation were the same as those already reported:\(^{18}\) \([H]=0.033 \, M\), \([G_9]= [G_3]=0.111 \, M\); these corresponded to the concentration condition C.\(^{13a}\) Commercially available deuterium labeled \((S)\)-amino acids\(^{9}\) were used without purification. Usually, somewhat noisy mass spectra were obtained in the case of the amino acid guests compared with amino acid ester guests.

In this paper, we regularly treated the \((S)\)-enantiomers as the deuterium labeled ones \((G_9_{de})\), and then used a 1/1 mixture of \(G_9\) and \(G_9_{de}\), as a given guest pair. The peak intensity ratio of the diastereomeric host–guest complex ions, which appeared simultaneously with an \(\kappa\) mass-unit difference in the FAB mass spectrum, was considered as the measure \((IRIS \text{ value})\) of the chiral recognition.\(^{10,12}\)

\[
I([H+G_9]+1)/I([H+G_9_{de}]+1) = I_{S_9}/I_{S_9_{de}} = IRIS
\]

The IRIS values obtained from the various host–guest combinations are summarized in Table 1 (for the host 1 series). A typical FAB mass spectrum is shown in Fig. 1.

### 2.3 Capillary electrophoresis

All capillary electrophoretic experiments were carried out using a Beckman P/ACE 5000 CE system. Electrophoresis was performed with an anode polarity at 25 \(V\) in a neutral coating capillary tube (Beckman eCAP Neutral Capillary) by applying a potential of 13.5 \(kV\); the dimensions of the tube were 45 cm (effective 38 cm) \(x\) 50 \(\mu\)m. The detection wavelength was mostly 214 \(nm\), but sometimes 234 \(nm\) for a series of tryptophan guests. A Beckman P/ACE station system was used for the data acquisition and processing.

All experiments for the direct \((D)\) detection mode in Table 1 were carried out in a tris/citrate buffer solution.\(^{17,19}\) Citric acid \((22.9 \, m\)M) was added to an aqueous solution of tris(hydroxymethyl)aminomethane \((Tris)\) \(10 \, m\)M containing host 1 \(10 \, m\)M to adjust the pH to 2.5; more citric acid \((52.5 \, m\)M) was similarly added to adjust the pH to 2.2. For the separation experiments in the indirect \((I)\) detection mode toward guests having non-aromatics, a visualized reagent of benzyltrimethylammonium chloride \((BTA)\) was used.\(^{17}\) Citric acid \((10.3 \, m\)M) was added to an aqueous solution of Tris \((5 \, m\)M) containing BTA \((20 \, m\)M) and host 1 \(5 \, m\)M to adjust the pH to 2.5. The pH measurements were performed using a Horiba D-11 pH meter and Horiba 6069-10C/4163-10T pH electrodes.

The chiral guests employed in the CE were \((i)\) amino acids, \((ii)\) the HCl salts of amino acid esters and \(1\)-phenylethylamine \((PEA)\), and \((iii)\) the HSCN salt of 1-(1-naphthyl)ethylamine \((1-NEA)\). Each aqueous guest’s solution was prepared by mixing the \((R)\)- and \((S)\)-enantiomers with different amounts (for example, \([IR]: [IS] = 1:2\) or \(2:1\), so that each enantiomer peak in the electropherogram was directly identified from the relative peak intensity. Usually, a 5 \(m\)M aqueous guest solution was prepared for the direct detection mode (a 30 \(m\)M aqueous solution for the indirect one) and hydrodynamically \((20 \, psi)\) injected (ca. 5 \(nL\)) for 1s. As an exception, a much more diluted \((0.5 \, m\)M) aqueous solution of Trp-OPr\(^{+}\) was prepared because of peak broadening.
Here, the suffixes R and S refer to the guests of the (R)- and (S)-enantiomers, respectively. Therefore, $\alpha > 1.0$ means that an (R)-enantiomer host is a relatively stronger binding one with host 1, and $\alpha < 1.0$ the opposite, e.g., an (R)-enantiomer is a relatively weaker binding one with host 1. The migration times and the $\alpha$ values in CE are summarized in Table 1. The reproducibility of the $\alpha$ value with different prepared buffer solutions was within ±0.01. A typical electropherogram is shown in Fig. 2.

2.4 NMR titration

The equilibrium constants ($K_R$ and $K_S$) between host 1 and each enantiomer ([R] or [S]) of the phenylglycine methyl ester hydrochloride (Pgly-OMe$^-$) were simultaneously determined using a racemic (RS)-Pgly-OMe$^-$Cl$^-$ guest with a standard (non-linear) NMR titration method in MeOH-$d_4$ at 25°C under the competitive complexation conditions$^{19,20}$: $[G] = 2.0$ mM, $[H] = 0.14$–2.5 mM. The resulting $K_R/K_S$ value is tabulated (Table 1) as the conventional measure of the chiral recognition in solution. A typical $^1$H-NMR spectral change is shown in Fig. 3. Enantiomer assignment of the two singlets (CH$_3$ protons of the guest enantiomers) was simply performed by further addition of the (R)-enantiomer guest, resulting in an increase in the intensities of the corresponding singlet: the CH$_3$ proton of the Pgly-OMe$^+$ ion accompanied by the (R)-enantiomer guest shifted more upfield.

3. Results and Discussion

The chiral recognition of host 1 toward the three sets of organic ammonium ion guests was studied using both capillary electrophoresis and FAB mass spectro-

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Fig. 2. An electropherogram for the enantiomer separation of Trp-OMe$^-$Cl$^-$ at a Tris/citrate buffer solution of pH=2.2 (25°C): detected at 254 nm.

Fig. 3. A $^1$H-NMR spectral change for the competitive complexation between host 1 and a racemic guest (RS)-Pgly-OMe$^-$ (Cl$^-$) in MeOH-$d_4$ at 25°C. The host 1 was successively added as solid (before adding host 1, HCl gas was introduced into the MeOH solution); ([RS]-Pgly-OMe$^-$)=2 mM and (a) [host 1]=0, (b) [host 1]=2.5 mM.
Fig. 4. A plot of the α values by CE against the IRIS values by FABMS for the combinations between host 1 and various guests: ▲ (amino acids), ■ (amino acid esters), and ● (aromatic amines).

Fig. 5. A plot of the α values by CE for the combinations between host 1 and amino acid ester guests against those between host 1 and amino acid guests.

Both measures of the chiral recognitions obtained from CE and FABMS are considered to be related to ΔΔG°, the difference in the complexation free energy by the two enantiomeric guests, as [i] ΔΔG° = RT ln α17 and [ii] ΔΔG° = RT ln (IRIS).120 Therefore, the α values obtained from CE (at pH 2.5) are plotted versus the IRIS values obtained from FABMS in Fig. 4. It is surprisingly evident that there exists no correlation between the two sets of chiral recognitions measured for an entire plot. Even if the given guests are restricted to the application of a class of amino acids or amino acid esters, a quite scattered plot is also obtained. Since chiral recognition should result from sensing molecular asymmetry in the respective chiral host–guest combination, the scattering reminds us of the prediction that the present host 1 possibly acts as a somewhat different molecular species under the two sets of measurements.

Another surprising finding is the correlation between the two sets of α values in CE. An (S)-enantiomer of an amino acid such as Leu, Phe, Trp, or Tyr was observed as a faster eluting one. On the other hand, the corresponding (R)-enantiomer of the amino acid ester inversely became a slower eluting one. Figure 5 shows such a behavior in the plot of the α values for amino acid esters versus those for the corresponding amino acids under the conditions of pH 2.5; a linear correlation with a negative and a unit slope (with an intercept of α = ca. 1.1). The larger the α value of an amino acid (α > 1.0), the smaller the α value of the corresponding amino acid ester (α < 1.0). The change in the direction of chiral recognition (the migration times) occurred for the amino acids having relatively larger alkyl substituents.

It has been understood that an increasing change in pH of mobile phase changes the negative charge of host 1 produced by the successive dissociation of the four COOH functions21–31 and then, significantly changes the migration times in CE. The incorporated COO function, the amount of which is controlled by the pH, may act as a stronger hydrogen acceptor, and may behave as a rather larger substituent due to hydration, and will cause a change in the degree and sometimes the direction of chiral recognition toward guests.

The pH effects are effective in CE, but need not be considered in FABMS. Recently, Ueno et al. examined the CE in a nonaqueous solution32 where the complication possible from such pH effects could be omitted. The α values of 1-NEA (1.24) and Phe (1.03) in formamide were in good agreement with the present IRIS values (1.23 and 1.01, respectively), indicating the specificity of host 1 in the aqueous CE experiments. The agreement in values strongly suggests the possible compatibility of the chiral recognition between the nonaqueous CE and the FABMS methods. When a new non-dissociative and water-soluble crown ether host will be developed in future, the chiral recognitions between the aqueous CE and the FABMS methods will be directly compared33; we are now intending to develop these types of new hosts for the aqueous CE experiments.

On the other hand, the relative equilibrium constants (the Kr/Ks value) in solution were determined using 1H-NMR spectrometry as a measure of the relative thermodynamic stability (chiral recognition). The Kr/Ks values were 1.3 for 1-NEA+ (MeOH, 25°C)34 and 1.6 for Pgly-OMe− (MeOH, 25°C), being in good agreement with the obtained IRIS values of 1.2 and 1.4, respectively. A plot of the IRIS values from FABMS versus the Kr/Ks values from the NMR (or UV) is illustrated in Fig. 6, which has been constructed using both the present and the previously reported host–guest combination sets.35 The present host 1–guest sets (i and j marks in Fig. 6) fell on a good correlation line with a slope of unity covering a wide range of host changes. This agreement further supports our earlier conclusions35, 36, 37 that the IRIS value under our FABMS conditions is a straightforward measure of chiral recognition (Ks/Kr) in solution, which is es-
Fig. 6. A plot of the IRIS values obtained from FABMS against the $K_R/K_S$ values obtained from NMR or UV. The figure notes correspond, in turn, to host, guest, and [solvent].

4. Conclusion

The chiral recognition of host 1 toward organic primary amine guests was extensively investigated using both CE in an aqueous buffer solution and FABMS in an NBA matrix. The IRIS values from FABMS did not show any correlation with the $a$ values from CE covering a wide range of amine guests. However, the former provided a good agreement with the $K_R/K_S$ values from the NMR in MeOH and also with the literature $a$ values from an alternative CE in a nonaqueous solution (formamide). The above scattering observation was attributed to a certain disturbance by the dissociable COOH functions of host 1, being particularly effective in an aqueous buffer solution. The present entire comparison with the chiral recognition by CE has further supported our earlier conclusion that FABMS is a facile and straightforward measurement tool for screening and quantitatively or semi-quantitatively evaluating various chiral host-guest interactions in solution.

References
10) M. Sawada, Y. Takai, H. Yamada, S. Hirayama, T.
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Keywords: FAB mass spectrometry, Capillary electrophoresis, Chiral recognition, Chiral crown ether, Amino acid ester, Deuterium labeling, Host-guest complex, Non-covalent chiral interactions.