Optical Resolution of Racemic α-Hydroxycarboxylic Acids on a Dynamic Chiral Stationary Phase Derived from (S)-Leucinol by Ligand Exchange Chromatography†

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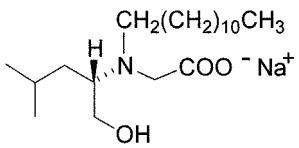
Liquid chromatographic direct separation of the enantiomers on chiral stationary phases (CSPs) have been considered as a most effective analytical tool for the rapid and accurate determination of enantiomeric composition of chiral compounds.[1] For example, CSPs based on cellulose derivatives,[2] cyclodextrins,[3] macro cyclic antibiotics,[4] chiral crown ethers[5] and low molecular mass π-acidic or π-basic chiral molecules[6] have been successfully utilized in the determination of enantiomeric composition of chiral compounds.

Ligand exchange CSPs have also been successfully employed for the direct separation of α-amino acid enantiomers. For example, Cu(II) complexes of optically pure α-amino acids and their derivatives have been applied as CSPs usually after binding covalently or hydrophobically to solid column support.[7] In this area, we have utilized copper (II) complexes of optically active amino alcohol derivatives hydrophobically adsorbed on octadecyl silica gel[8] or covalently bonded to silica gel[9] as CSPs for the direct separation of α-amino acid enantiomers. Especially, a dynamic CSP prepared by hydrophobically loading (S)-*N,N*-carboxymethyl dodecyl leucinol monosodium salt **1** onto a commercial octadecyl-silica gel column (Waters μ-Bondapak™ C_{18}, 3.9 × 300 mm) was very excellent in separating the two enantiomers of various racemic α-amino acids.[8d] However, the dynamic CSP based on **1** has not been applied in the direct separation of other racemic compounds.



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Optically active α-hydroxycarboxylic acids are important as biologically active substances, chiral building blocks or intermediates for the asymmetric synthesis of natural products.[10] Consequently, the determination of the enantiomeric composition of chiral α-hydroxycarboxylic acids is of increasing interest. Previously, liquid chromatographic Pirkle-type CSPs have been applied in separating the two enantiomers of α-hydroxycarboxylic acids as their achiral derivatives.[11] Ligand exchange CSPs have also been applied in the direct separation of α-hydroxycarboxylic acids without derivatization.[12] However, the dynamic CSP based on **1** was not utilized in the resolution of α-hydroxycarboxylic acids. In this study, we want to extend the use of the dynamic CSP based on **1** to the direct separation of the two enantiomers of racemic α-hydroxycarboxylic acids.

We examined, in this study, the resolution of nine different racemic α-hydroxycarboxylic acids shown in Figure 1 on the dynamic CSP based on **1**. Table 1 summarizes the results for the resolution of racemic α-hydroxycarboxylic acids on the dynamic CSP based on **1** with the variation of organic modifier content in aqueous mobile phase at the constant Cu(II) concentration. The representative chromatograms are illustrated in Figure 2. As shown in Table 1 and Figure 2, the resolution of α-hydroxycarboxylic acids on the dynamic CSP based on **1** is generally reasonable. In every case, the base line resolution can be expected under an appropriate mobile phase condition except for the resolution of mandelic acid **3**. Mandelic acid **3** showed only non-baseline resolution with marginal separation factor when 20% methanol in water was used as a mobile phase.

In addition, the resolution of α-hydroxycarboxylic acids on the dynamic CSP based on **1** is quite dependent of the type and the content of organic modifier in aqueous mobile

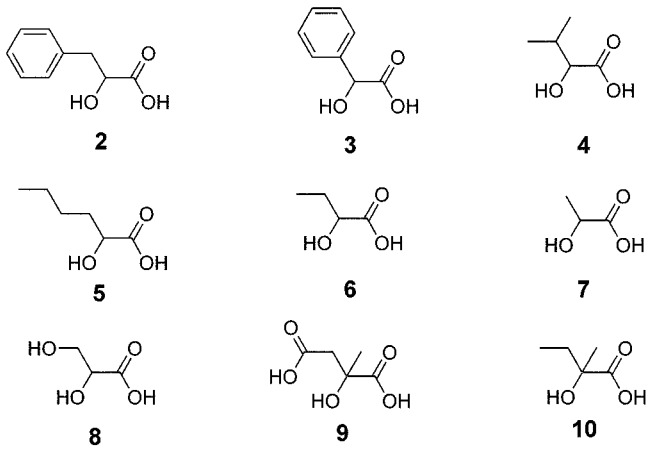

Figure 1. The structures of α-hydroxycarboxylic acids **2-10** used in this study.

†This paper is dedicated to Professor Yong Hae Kim for his distinguished achievements in organic chemistry.
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Table 1. Resolution of racemic α-hydroxycarboxylic acids **2-10** on the dynamic CSP based on **1** with the variation of organic modifier content in aqueous mobile phase at the constant Cu(II) concentration (2.5×10^{-4} M)[a]

	20% CH_3CN			10% CH_3CN			100% Water			10% CH_3OH			20% CH_3OH		
	k_1[b]	α[c]	R_S[d]	k_1[b]	α[c]	R_S[d]	k_1[b]	α[c]	R_S[d]	k_1[b]	α[c]	R_S[d]	k_1[b]	α[c]	R_S[d]
2	27.04(R)	1.24	1.88	52.29(R)	1.19	1.63	93.68(R)	1.24	2.06	54.27(R)	1.32	2.14	45.96(R)	1.42	3.01
3	21.37	1.00		33.18	1.00		54.41	1.00		35.71	1.00		34.69(R)	1.06	0.28
4	11.53(S)	1.08	0.79	15.65(S)	1.13	1.05	24.83	1.00		16.18	1.00		14.93(R)	1.12	0.77
5	28.79	1.36	2.50	52.25	1.38	3.14	88.61	1.21	1.76	50.31	1.20	1.28	40.68	1.09	0.33
6	4.04	1.17	1.03	5.30	1.20	1.63	7.39	1.10	0.79	5.01	1.04		5.14	1.00	
7	1.33(S)	1.35	1.17	1.67(S)	1.34	1.44	2.38(S)	1.24	1.26	1.60(S)	1.32	1.36	1.67(S)	1.31	1.11
8	0.89(S)	1.37	1.52	1.14(S)	1.29	1.53	1.31(S)	1.26	1.26	1.15(S)	1.36	1.68	1.29(S)	1.37	1.85
9	24.36	1.00		41.54	1.06		74.15(S)	1.16	0.57	38.49(S)	1.39	1.15	36.96(S)	1.31	1.13
10	7.47	1.34	2.20	10.91	1.34	2.62	14.64	1.44	2.88	9.91	1.42	2.73	10.32	1.51	3.04

[a]Flow rate: 0.8 mL/min. Temperature: 20 °C. Detection: 254 nm UV. [b]Capacity factor of the first eluted enantiomer. The absolute configuration of the first eluted enantiomer is presented in the parenthesis. The elution order was determined by injecting configurationally known samples. For the results, which do not contain elution orders, configurationally known samples were not available. [c]Separation factor. [d]Resolution factor.

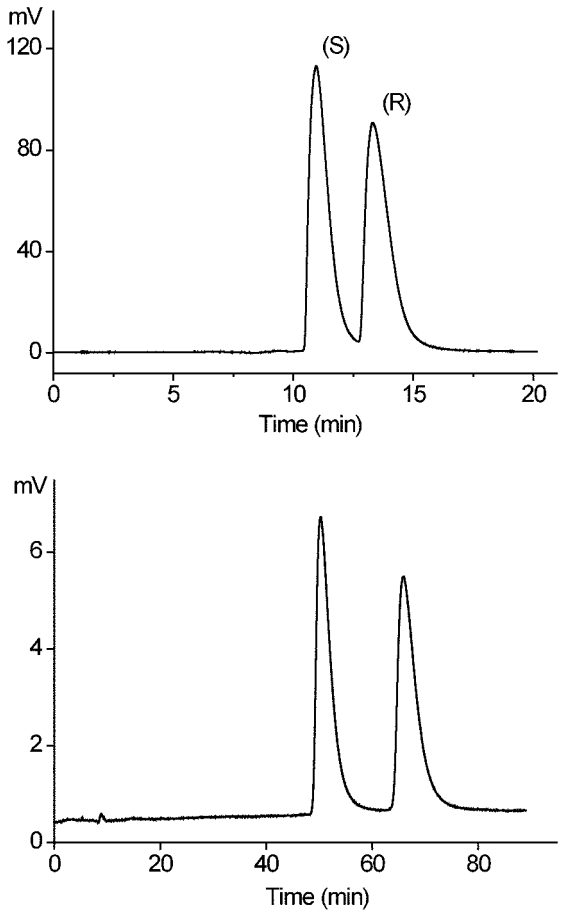

Figure 2. Representative chromatograms for the resolution of lactic acid **7** (top) and 2-hydroxy-2-methylbutyric acid **10** (bottom) on the dynamic CSP based on chiral selector **1**. Chromatographic conditions are identical to those given in the foot note of Table 1.

phase as shown in Table 1. In general, the retention factors (k_1) decrease as the organic modifier concentration in aqueous mobile phase increases. The decreasing trends of the retention factors (k_1) with the increase of the organic modifier concentration in aqueous mobile phase are more significant with acetonitrile than with methanol. The polarity decrease in the mobile phase has been known to usually diminish the retention of analytes on the column in the reverse phase chromatography.[8b] This generalization is exactly consistent with the retention data summarized in Table 1. As the organic modifier concentration in aqueous mobile phase increases, the polarity of the mobile phase decreases and consequently the retention of analytes decreases. Acetonitrile is less polar than methanol. Consequently, the retention of analytes decreases more significantly with the use of acetonitrile than with the use of methanol as an organic modifier in aqueous mobile phase.

In contrast, the separation (α) and the resolution factors (R_S) did not show consistent trends with the variation of the type and the content of organic modifier in aqueous mobile phase. For example, both of the separation (α) and the resolution factors (R_S) for the resolution of 3-phenyllactic acid **2** increases as the methanol concentration in aqueous mobile phase increases. However, both of the separation (α) and the resolution factors (R_S) for the resolution of 2-hydroxycaproic acid **5** decreases as the methanol concentration in aqueous mobile phase increases.

The elution orders for the resolution of α-hydroxycarboxylic acids on the dynamic CSP based on **1** are also not consistent. In the resolution of 3-phenyllactic acid **2** and mandelic acid **3**, the (R)-enantiomer is eluted faster than the (S)-enantiomer. However, in the resolution of lactic acid **7** and citramalic acid **9**, the (S)-enantiomer is eluted faster than the (R)-enantiomer. Interestingly, the elution order for the resolution of 2-hydroxy-3-methylbutyric acid **4** is reversed when the organic modifier in aqueous mobile phase is changed from acetonitrile to methanol. From these results, the energy difference between the two diastereomeric ternary complexes formed from the fixed ligand, (R)- or (S)-α-hydroxycarboxylic acids and Cu(II) is concluded to be quite subtle.

The structures of the two diastereomeric ternary complexes expected to be formed from the fixed ligand, (R)- or (S)-α-hydroxycarboxylic acids and Cu(II) are proposed from the

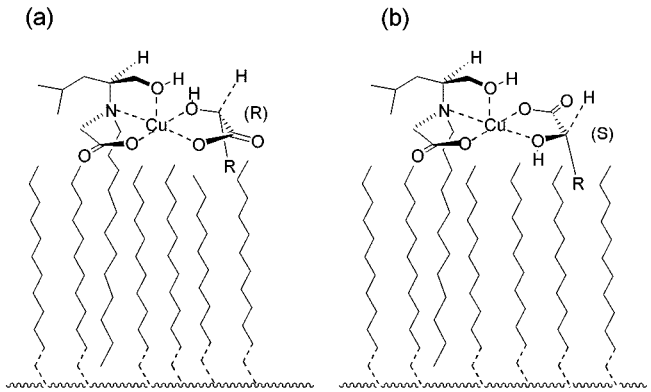

Figure 3. The proposed structures of the ternary complex formed from the fixed ligand [(S)-chiral selector **1**], Cu(II) and (a) (R)- and (b) (S)-hydroxycarboxylic acid.

study of chemical models as shown in Figure 3. In the model, the *N*-dodecyl chain of (S)-*NN*-carboxymethyl dodecyl leucinol monosodium salt **1** (chiral selector) is bounded between octadecyl chains of silica gel through the lipophilic interaction. The bounded chiral selector and the analyte coordinate around Cu(II) ion to form square planar ternary complex with the *cis*-conformation (Figure 3a) or with the *trans*-conformation (Figure 3b) of the carboxylic acid groups of the chiral selector and the analyte. Finally, the hydroxy functionality of the chiral selector coordinates to Cu(II) at the axial position of the square planar complex.

When the carboxylic acid groups of the chiral selector and the analyte coordinate around Cu(II) ion with the *cis*-conformation (Figure 3a), the alkyl group at the chiral center of (R)-α-hydroxycarboxylic acids should be intercalated between the octadecyl chains of silica gel under the reverse mobile phase condition. Similarly, when the two carboxylic acid groups of the chiral selector and the analyte coordinate around Cu(II) ion with *trans*-conformation, the alkyl group at the chiral center of (S)-α-hydroxycarboxylic acids should be intercalated between the octadecyl chains of silica gel under the reverse mobile phase condition as shown in Figure 3b. The elution order should be determined by the stability difference between the two diastereomeric ternary complexes shown in Figure 3.

In previous studies for the resolution of α-amino and α-hydroxycarboxylic acids on ligand exchange CSPs based on proline, the diastereomeric ternary complex with *trans*-configuration was proposed to be more stable than that with *cis*-configuration.[8a,12a,13] Similarly, the diastereomeric ternary complex with *trans*-configuration shown in Figure 3b is expected to be more stable than that with *cis*-configuration shown in Figure 3a under the reverse mobile phase condition. In this instance, the (R)-enantiomer should be eluted faster than the (S)-enantiomer. In the resolution of α-hydroxycarboxylic acids, which contain relatively more lipophilic side chain at the chiral center such as 3-phenyllactic acid **2** and mandelic acid **3**, our expectation turns out to be true, the (R)-enantiomers being eluted faster than the (S)-enantiomer. In the resolution of citramalic acid **9**, the methyl group at the chiral center is expected to intercalate between the octadecyl groups of the CSP while the hydrophilic carboxymethyl group at the chiral center is directed toward the bulk mobile phase in the chiral recognition model shown in Figure 3b. In this instance, the (S)-enantiomer is eluted faster than the (R)-enantiomer. However, in the resolution of 2-hydroxy-3-methylbutyric acid **4**, the stability difference between the two diastereomeric ternary complexes shown in Figure 3 seems to be quite small and dependent on the type of organic modifier. In the resolution of lactic acid **7**, the ternary complex with *cis*-configuration shown in Figure 3a seems to be preferable to that shown in Figure 3b even though the reason is not clear yet.

In conclusion, the dynamic CSP prepared by tentatively loading the copper (II) complex of (S)-*NN*-carboxymethyl dodecyl leucinol monosodium salt **1** onto a commercial octadecyl silica gel column can be successfully used for the resolution of racemic α-hydroxycarboxylic acids. Based on the chromatographic resolution results, a chiral recognition model utilizing the enantioselective formation of the ternary complex from fixed ligand **1**, hydroxycarboxylic acids and Cu(II) was proposed. However, it should be noted that the structures of the ternary complexes shown in the proposed chiral recognition models might be confirmed or improved further by spectroscopic and/or X-ray crystallographic analysis in the future.

Experimental Section

Chromatography was performed with an HPLC system consisting of a Waters model 510 HPLC pump, a Rheodyne model 7725i injector with a 20 μL sample loop, a Waters 486 Absorbance detector and a YoungLin Autochro Data Module (Software: YoungLin Autochro 2000). The temperature of the chiral column was maintained at 20 °C by using a JEIO TECH VTR-620 Circulator (Seoul, Korea).

Dynamic chiral column was obtained from the previous study.[8d] Racemic and optically active α-hydroxycarboxylic acids used in this study were purchased from Aldrich. To resolve racemic α-hydroxycarboxylic acids on the dynamic chiral column, a mobile phase, which was prepared by dissolving specified amount of $CuSO_4$ in deionized water containing specified amount of acetonitrile or methanol as an organic modifier, was passed through the column until the baseline (UV monitor, 254 nm) became stable to equilibrate the column and then, a methanolic solution (usually 3 μL) containing a racemic or optically enriched α-hydroxycarboxylic acid (usual concentration: 1.0 mg/mL) was injected. Column void volume (the elution time of an unretained solute) was measured from the solvent front of methanol.

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