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ENANTIOSELECTIVE HYDROPHOBIC ENTANGLEMENT OF ENANTIOMERIC SOLUTES WITH CHIRAL FUNCTIONALIZED MICELLES BY ELECTROKINETIC CHROMATOGRAPHY

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SUMMARY

With chiral micellar systems consisting of surfactants functionalized with L-amino acid residues, sodium N-dodecanoyl-L-amino acidates, it was possible to resolve enantiomeric N-acylated amino acid esters by electrokinetic chromatography. This method provides a sophisticated means for assessing enantioselectivity based on hydrophobic entanglement of a solute with chiral functionalized micelles, owing to the absence of any solid support to hold the liquid stationary phase in place. The chiral micelles of sodium N-dodecanoyl-L-valinate most effectively made possible the resolution of N-3,5-dinitrobenzoylated racemic amino acid isopropyl esters. The steric effects of the amino acid residue of chiral surfactants and derivatization of the amino acids to be resolved on enantiomer resolution are discussed.

INTRODUCTION

Many separation processes mediated by the presence of surfactant organized assemblies have been developed over the past decade[1,2], especially in reversed-phase liquid chromatography with a micellar mobile phase, and this mode of separation has thus been termed micellar chromatography. This method is a versatile means of separation, which applies a secondary distribution equilibrium between the micellar mobile phase and the solute[1–8]. The micelle can cause a neutral solute to become entangled with the hydrophobic core consisting of hydrocarbon chains as a third liquid phase[5–8]. There is, however, primary equilibrium between mobile and hydrophobic stationary bonded phases. With micellar electrokinetic chromatography, whose development was started initially by Terabe and co-workers[9–14], there is no solid support to hold the stationary phase in place and the method is thus based on the distribution of a solute between the micellar and aqueous phases. This type of chromatography should therefore reveal the essential hydrophobic binding affinity of micelles toward a solute, subordinated as the secondary equilibrium in micellar chromatography, and provide a sophisticated means for assessing the micellar selectivity of various solutes[15–17].

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We recently found that chiral amide-terminated monolayers anchored to a silica gel surface afforded a hydrophobic interfacial phase so as to diminish the liquid–solid interfacial area under aqueous media, where there hydrogen-bond association occurs which leads to enantiomer resolution[18]. This prompted us to incorporate chiral hydrogen bonding amide functionality into the micellar hydrophobic core so as to provide some clarification of the enantioselective entanglement of the micelle with a solute molecule. For this purpose, micellization of chiral surfactants, N-dodecanoyl-L-amino acid sodium salts, was carried out. These chiral micelles are instantaneous media ordered by the surrounding aqueous solution, as well as the interfacial phase on the bonded phase, and are thus distinct from a structured chiral cavity of cyclodextrin with which a solute binds hydrophobically to form an inclusion complex[19–23]. Hydrophobic interactions between the chiral micelle and solute should be incapable of a tight fit, as seems to be the case in the inclusion complex. In this study, chiral functionalized micellar systems were each found to possess a distinct binding affinity toward enantiomeric amino acid derivatives for the inducement of their optical resolution in electrokinetic chromatography. It could therefore be postulated that enantioselectivity may be observed when a solute binds via hydrogen bonds to the amide functionality into the micellar interior core. The influence of the steric bulkiness of amino acid side-chains in the chiral surfactants and the effect of the derivatization of the amino acids to be resolved on the extent of the enantiomer resolution are discussed.

EXPERIMENTAL

Synthesis of sodium N-dodecanoyl-L-amino acidate

N-Dodecanoyl-L-valine was prepared from L-valine by treatment with the dodecanoic acid N-hydroxysuccinimide ester (m.p. 87°C; lit.[24] m.p. 75°C), according to the literature procedure[6]; m.p. 103–105°C (recrystallized from ethyl acetate), $[\alpha]_D^{26.5} = -2.6°$ ($c = 1.00$, methanol). This carboxylic acid was then converted to the corresponding sodium salt by treatment with methanolic sodium hydroxide and the small amount of residual carboxylic acid was extracted with acetone in a Soxhlet apparatus. Another chiral surfactant containing L-alanine was also prepared from the corresponding carboxylic acid by a procedure similar to the above; N-dodecanoyl-L-alanine, m.p. 82—84°C, $[\alpha]_D^{26.5} = -19.8°$ ($c = 1.00$, methanol). These two carboxylic acids were identified on the basis of ^{1}H NMR and IR spectra.

Chromatographic procedures

Electrokinetic capillary chromatography was performed according to Terabe *et al.*[9]. The capillary column consisted of a fused-silica tube (70 cm × 50 μm I.D.; 50 cm to the detection cell); Scientific Glass Engineering, North Melbourne, Australia). A regulated d.c. power supply delivering a maximum of 25 kV (Model HEL-25R0.1-TYU; Matusada Precision Devices, Otsu, Japan) was used as the source of a high voltage between the ends of the column filled with chiral micellar solution. Chromatography was carried out at a constant current of either 26 or 40 μA. The elution of a solute injected at the positive end of the column was monitored by on-column UV detection at the negative end. The variable-wavelength UV detector used was a Gasukuro Kogyo (Tokyo, Japan) Model 502U, provided with a specially modified cell holder which held the partially burned fused-silica tube so as to establish

an on-column UV cell with a slit width of 50 μm. The wavelength was set at either 230 or 260 nm.

Sodium dodecyl sulphate (SDS) (Tokyo Kasei, Tokyo, Japan) was recrystallized from ethanol prior to use. Chiral micellar solutions were prepared by dissolving the chiral surfactant alone or together with various amounts of SDS in borate–phosphate buffer (pH 7.0) solution containing 0.025 *M* sodium tetraborate and 0.05 *M* sodium dihydrogenphosphate solution. In all instances, the micellar solution was filtered through a 0.45-μm pore-size membrane filter followed by degassing in an ultrasonic bath for 3 min. The capillary column was rinsed with methanol and then water for several hours under application of the current. The samples to be resolved were prepared in our laboratory.

The capacity factor ($\tilde{k}'$) for a solute was calculated as follows[9]:

$$\tilde{k}' = (t_R - t_0)/\{t_0[1 - (t_R/t_{MC})]\}$$

where t_R is the retention time of a particular solute, t_0 that determined with methanol as the solute unsolubilized in a micelle and t_{MC} that with Sudan III as the solute completely solubilized in a micelle.

Fluorescence measurements of the microenvironment polarity

Steady-state fluorescence spectra were obtained with a Hitachi 650-60 spectrometer using excitation and emission slit widths each of 5 nm. The emission intensity was measured during excitation at 337 nm and at both 373 and 383 nm using pyrene (Tokyo Kasei; recrystallized from ethyl acetate) as a probe in the chiral surfactants dissolved in the borate–phosphate buffer solution. The ratio of the intensity at 383 nm (I_{383}) to that at 373 nm (I_{373}) reflected the microenvironment polarity around the probe[25].

RESULTS AND DISCUSSION

Table I shows the results for the resolution of enantiomeric N-acylated amino acid isopropyl ester with a chiral micellar solution consisting of sodium N-dodecanoyl-L-valinate (SDVal) at 0.025 *M*. The critical micellar concentration (CMC) of this micellar system in borate–phosphate buffer (pH 7.0) was determined to be $3 \cdot 10^{-3}$ *M* using the ratio of the intensity of the pyrene fluorescence peaks at 383 nm (I_{383}) to that at 373 nm (I_{373}). This negatively charged micellar phase migrates more slowly than the aqueous phase toward the negative end of the column, because the electroosmotic velocity of the aqueous phase is much stronger than the electrophoretic velocity of the micelle in the opposite direction. The dye Sudan III, tightly entangled by the micelle, migrates at the same velocity as the micellar phase itself. Hence the velocity of the dye is the difference between those of electroosmosis and electrophoresis and its capacity factor is infinite. The separation is thus based on the distribution of a solute between these two phases.

The 3,5-dinitrobenzoyl derivatives were the most effectively resolved in all the solutes examined, containing the corresponding 4-nitrobenzoyl and benzoyl derivatives, and also facilitated detection of the elution of the solute as a strong UV chromophore. A typical resolution of racemic N-3,5-dinitrobenzoylamino acid

TABLE I

RESOLUTION OF RACEMIC AMINO ACID DERIVATIVES WITH A CHIRAL MICELLAR SYSTEM CONSISTING OF SDVal WITH ELECTROKINETIC CHROMATOGRAPHY

Chromatographic conditions: column, fused-silica capillary tubing (Scientific Glass Engineering) (50 cm × 50 μm I.D. for affecting the separation); micellar solution, 0.025 *M* sodium N-dodecanoyl-L-valinate (SDVal) in 0.025 *M* sodium tetraborate–0.05 *M* sodium dihydrogenphosphate buffer (pH 7.0); current, 40 μA; total applied voltage, *ca.* 15 kV; temperature, ambient (*ca.* 20°C); detection, UV at either 230 or 260 nm.

Solute[a]	*Retention time (min)*		*Capacity factor*[b]		
	t_{RD}	t_{RL}	k'_D	k'_L	α
DNB-Ala-OPr^i	10.60	11.34	0.60	0.80	1.32
DNB-Val-OPr^i	14.09	15.18	1.69	2.15	1.27
DNB-Leu-OPr^i	18.08	19.30	3.93	5.04	1.28
DNB-Phe-OPr^i	19.76	20.42	5.55	6.41	1.15
NB-Ala-OPr^i	10.36	10.65	0.51	0.58	1.13
NB-Val-OPr^i	13.46	13.89	1.40	1.55	1.11
NB-Leu-OPr^i	17.08	17.64	3.10	3.48	1.12
NB-Phe-OPr^i	18.84	19.09	4.45	4.70	1.05
Bz-Ala-OPr^i	9.74	9.96	0.35	0.39	1.14
Bz-Val-OPr^i	12.36	12.68	1.05	1.15	1.09
Bz-Leu-OPr^i	15.94	16.40	2.50	2.75	1.10
Bz-Phe-OPr^i	17.54	17.76	3.46	3.62	1.05

[a] Abbreviations: DNB, 3,5-dinitrobenzoyl; NB, 4-nitrobenzoyl; Bz, benzoyl; Pr^i = isopropyl.
[b] t_0 = 7.82 min; t_{MC} = 27.43 min.

isopropyl esters is presented in Fig. 1. In the elution of the resolved amino acid derivatives, the D-enantiomer eluted faster than the corresponding L-enantiomer in all instances, indicating that the chiral micelle binds to the L-enantiomer having the same configuration as its chiral component to a greater extent than the D-enantiomer. Among the different amino acid derivatives, the alanine derivatives were the least and the phenylalanine derivatives the most strongly retained. Hence the elution order of

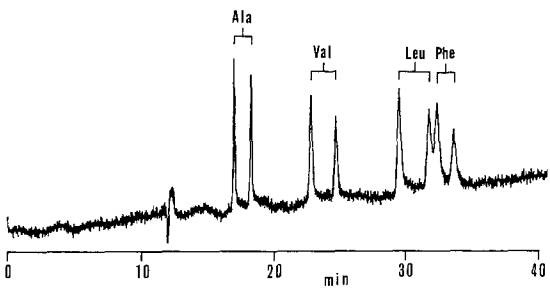

Fig. 1. Optical resolution of a mixture containing four enantiomeric pairs of amino acids as N-(3,5-dinitrobenzoyl) O-isopropyl ester derivatives by electrokinetic capillary chromatography. Chromatographic conditions: column, fused-silica tubing (Scientific Glass Engineering) (50 cm × 50 μm I.D. for affecting the separation); micellar solution, 0.025 *M* sodium N-dodecanoyl-L-valinate (SDVal) in 0.025 *M* borate–0.05 *M* phosphate buffer (pH 7.0); total applied voltage, *ca.* 10 kV; current, 26 μA; detection, UV at 230 nm; temperature, ambient (*ca.* 20°C).

each series of amino acid derivatives is dictated by the extent of the increase in the hydrophobicity of the amino acid side-chain in the solute following entanglement with the micellar interior core. The elution range from the alanine to phenylalanine derivatives varied considerably, in the order benzoyl, 4-nitrobenzoyl and 3,5-dinitrobenzoyl derivatives, as shown in Table I.

Esterification of N-acylated amino acids was found to be essential for enantiomer resolution. Racemic 3,5-dinitrobenzoylamino acids were insensitive to the chiral micelle and eluted faster than the corresponding isopropyl ester derivatives. With these carboxylic acids, the capacity factors with 0.025 *M* SDVal micellar solution obtained were 0.80 for the alanine, 0.73 for the valine, 0.70 for the leucine and 0.72 for the phenylalanine derivative.

As expected, dilution of the SDVal micellar solution with achiral SDS at a total concentration of 0.025 *M* gave a lower enantioselectivity with decrease in the fraction of the chiral surfactant SDVal. Fig. 2 illustrates this deterioration in the separation factors of the 3,5-dinitrobenzoyl and 4-nitrobenzoylalanine derivatives. The corresponding benzoyl derivative ceased to be resolvable when the SDVal molar fraction decreased to 32%, although the separation factors for this derivative were essentially the same as those for the 4-nitrobenzoyl derivative in the SDVal concentration range 50–100%.

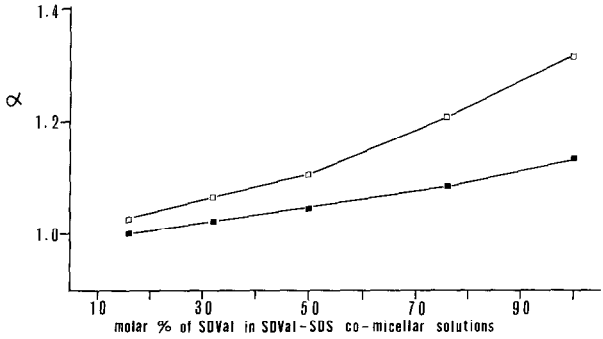

Fig. 2. Separation factor (α) between enantiomers as a function of molar percentage of SDVal in the co-micellar solution (total concentration 0.025 *M*). □, 3,5-Dinitrobenzoylalanine isopropyl ester; ■, 4-nitrobenzoylalanine isopropyl ester.

Next, a micellar solution consisting of sodium N-dodecanoyl-L-alaninate (SDAla) was used in place of the SDVal micellar solution in order to assess the influence of the steric bulkiness of amino acid side-chains in the chiral surfactants on the extent of enantiomer resolution. A 0.025 *M* SDAla micellar solution provided smaller separation factors for all the solutes examined in an SDVal micelle, as is partially evident from Table II for the 3,5-dinitrobenzoyl derivatives. This micelle had a CMC of $5 \cdot 10^{-3}$ *M*, which is higher than that of the SDVal micelle, possibly owing to the decrease in the hydrophobicity of SDAla having a methyl side-chain. The pyrene intensity ratio (I_{383}/I_{373}), however, increased from 0.67–0.70 to 1.07–1.11 at the CMC, and remained virtually constant above the CMC, as was observed for the SDVal micelle (1.10–1.11).

TABLE II

RESOLUTION OF RACEMIC 3,5-DINITROBENZOYLAMINO ACID DERIVATIVES WITH A CHIRAL MICELLAR SYSTEM CONSISTING OF SODIUM N-DODECANOYL-L-ALANINATE (SDAla)

Chromatographic conditions as in Table I except that a micellar solution containing 0.025 *M* SDAla in borate–phosphate buffer (pH 7.0) was used.

Solute[a]	*Retention time (min)*		*Capacity factor*[b]		
	t_{RD}	t_{RL}	$\tilde{k}'_D$	$\tilde{k}'_L$	α
DNB-Ala-OPr^i	11.91	12.46	0.99	1.16	1.17
DNB-Val-OPr^i	16.09	16.76	2.77	3.20	1.16
DNB-Leu-OPr^i	19.90	20.56	6.38	7.46	1.17
DNB-Phe-OPr^i	21.52	21.82	9.60	10.42	1.09

[a] Abbreviations as in Table I.
[b] t_0 = 7.72 min; t_{MC} = 26.46 min.

The intensity ratio reflects the microenvironment polarity around pyrene solubilized in the micellar interior core, and has been found to remain in the range 0.70–1.00 for various micellar systems, whereas pyrene in a pure hydrocarbon solvent has the ratio of the order of 1.7. The same order of the intensity ratios for two different micellar systems can be interpreted as essentially the same extent of penetration of water into both chiral micelles. Hence, the lower enantioselectivity in the SDAla micellar system may possibly be due to the smaller perturbation of the micellar structure resulting from the smaller steric bulkiness of SDAla when the amino acid derivative having the opposite configuration to the surfactant is intercalated between the densely packed pure enantiomeric surfactant. Thus, dilution of an SDVal micellar solution with SDS would decrease the density of the chiral barrier formed by the valine residue, resulting in a decrease in enantioselectivity. The amide functionality in the chiral micelles may possibly serve as a hydrogen bonding site for entrapping enantiomers into such an ordered shallow hydrophobic region. The intensity ratio of the chiral micelles was higher than that observed for the SDS micelle in borate–phosphate buffer solution (1.00–1.03). This may possibly arise from the smaller extent of penetration of water into the chiral micelles rather than into the SDS micelle. Consequently, chiral micellar systems appear to provide favourably ordered media for hydrogen bonding with solute enantiomers.

In electrokinetic chromatography, the resolution (R_s) was dependent on the $\tilde{k}'$ value of the solute[10], and a large $\tilde{k}'$ value is unfavourable for good resolution. In preliminary experiments using a co-micellar solution with equimolar amounts of SDVal and SDS on fused-silica tubing (Gasukuro Kogyo), a decrease in the total micellar concentration from 0.1 to 0.025 *M* led to a smaller $\tilde{k}'$ and virtually constant separation factors (α) for the amino acid derivatives. The $\tilde{k}'_D$ and α values observed for the 3,5-dinitrobenzoylalanine derivative were 1.19 and 1.11, respectively, in 0.025 *M* solution. In contrast, the corresponding phenylalanine derivative was the most hydrophobic and thus had the largest $\tilde{k}'_D$ value of 13.07, but its resolution even in the 0.025 *M* solution was poor.

The resolution of enantiomeric mixtures was improved by adding methanol to the co-micellar solution at 5–10% (v/v), resulting in a greater total elution range, as is evident from the values of t_0/t_{MC}[10]. With the 0.025 *M* co-micellar solution, an increase in the organic modifier concentration decreased t_0/t_{MC} from 0.20 (9.63/48.39) in the absence of methanol to 0.10 (11.03/109.63) in 10% (v/v) methanol. This corresponded to a decrease in $\tilde{k}'$ of the enantiomers and an increase in resolution (R_s), as was noted in particular for the phenylalanine derivative with the largest retention[26].

On using a chiral micelle consisting of SDVal alone on a fused-silica tube (Scientific Glass Engineering), the phenylalanine derivative was resolved almost completely, with a $\tilde{k}'_D$ value of 5.55 without the addition of methanol. When methanol was added to the micellar solution, the retention became smaller on increasing the methanol concentration to 5–10% (v/v), similarly to the results observed in the previous examination, as shown in Table III. The change in the total elution range (t_0/t_{MC}) was less than in the co-micellar system, being 0.29 in the absence of methanol and 0.25 in 10% (v/v) methanol. The total elution range was not particularly broad when using the SDVal–SDS co-micellar system (0.28–0.24 with equimolar amounts of the mixtures under the same conditions), and thus differences in the elution range arise from the different natures on the inner wall between the fused-silica tubes used rather than the particular features of the micellar systems.

The addition of an organic solvent to the micellar solution reduced the solute retention as measured by the capacity factor, as seemingly observed in reversed-phase liquid chromatography. This is due in part to the phase polarity determined by intercalation of the solvent in the hydrophobic interfacial phase in reversed-phase

TABLE III

$\tilde{k}'_D$ AND α VALUES OF AMINO ACID DERIVATIVES AS A FUNCTION OF METHANOL CONCENTRATION IN 0.025 *M* SDVal SOLUTION

Chromatographic conditions as in Table I except that a micellar solution was used.

Solute[a]	*Methanol concentration (%, v/v)*					
	0		*5*[b]		*10*[c]	
	$\tilde{k}'_D$	α	$\tilde{k}'_D$	α	$\tilde{k}'_D$	α
DNB-Ala-OPr^i	0.60	1.32	0.52	1.28	0.38	1.28
DNB-Val-OPr^i	1.69	1.27	1.41	1.27	0.99	1.23
DNB-Leu-OPr^i	3.93	1.28	3.44	1.30	2.27	1.27
DNB-Phe-OPr^i	5.55	1.15	4.78	1.19	3.01	1.15
NB-Ala-OPr^i	0.51	1.13	0.39	1.13	0.29	1.13
NB-Val-OPr^i	1.40	1.11	1.07	1.10	0.78	1.10
NB-Leu-OPr^i	3.10	1.12	2.51	1.11	1.75	1.11
NB-Phe-OPr^i	4.54	1.05	3.61	1.06	2.38	1.07
Bz-Ala-OPr^i	0.35	1.14	0.27	1.16	0.19	1.12
Bz-Val-OPr^i	1.05	1.09	0.87	1.09	0.63	1.08
Bz-Leu-OPr^i	2.50	1.10	1.71	1.09	1.20	1.09
Bz-Phe-OPr^i	3.46	1.05	2.65	1.05	1.80	1.04

[a] Abbreviations as in Table I.
[b] t_0 = 8.26 min; t_{MC} = 28.66 min.
[c] t_0 = 9.20 min; t_{MC} = 36.12 min.

chromatography[27]. The addition of methanol, however, to equimolar amounts of SDVal–SDS co-micellar solutions brought about no changes in the micropolarity of the interior core, as was expected. The intensity ratio of pyrene fluorescence peaks appeared essentially constant (I_{383}/I_{373} = 1.10–1.12) with 0–20% (v/v) methanol. This appears to support the notion that water-soluble alcohols predominately dissolve in the aqueous phase, causing the aggregation number of a surfactant to change according to the alcohol concentration[28]. Thus, the main effect of an organic modifier may be to change the micellar size, in addition to the electroosmotic flow, as suggested by Gorse *et al.*[16].

We are now examining better chiral surfactants containing SDVal congeners. The results will be presented in detail in the near future.

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