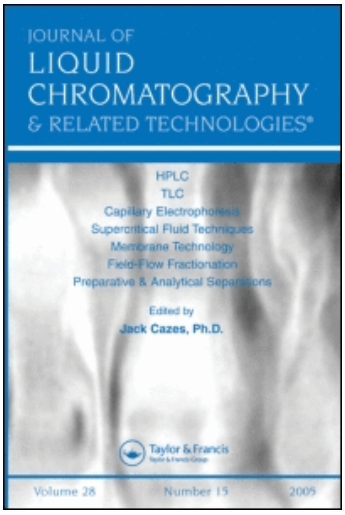

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Enantiomeric Separation by Micellar Electrokinetic Chromatography Using Saponins

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ENANTIOMERIC SEPARATION BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY USING SAPONINS

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ABSTRACT

Glycyrrhizic acid (GRA) and β-escin were employed in micellar electrokinetic chromatography (MEKC) for enantiomeric separation. Both GRA and β-escin are saponins and natural chiral surfactants. A mixed micelle consisting of GRA, octyl-β-D-glucoside, and sodium dodecyl sulfate (SDS) was successfully used for enantiomeric separation of dansylated DL-amino acids. β-Escin was also employed to prepare a mixed micelle with SDS and the mixed micelle was capable of separating phenylthiohydantoin-DL-amino acids under acidic conditions.

INTRODUCTION

To perform enantiomeric separation by capillary electrophoresis (CE), two techniques are mainly available: one is capillary zone electro-

phoresis (CZE) using chiral additives (1–3); the other is electrokinetic chromatography (EKC) using a chiral micelle (4–8), a cyclodextrin derivative (9), or a combination of achiral micelle and a cyclodextrin (10,11). Although CZE is applicable only to ionic compounds, EKC can separate both ionic and nonionic compounds (9).

Among the EKC techniques, micellar EKC (MEKC) is particularly useful and widely accepted (12). Enantiomeric separation by MEKC with a chiral micelle is a simple technique; that is, a chiral surfactant solution or a mixed micelle solution of a chiral and achiral surfactant can be employed instead of a simple salt buffer solution used in CZE.

Two classes of chiral surfactants have been successfully employed for enantiomeric separation by MEKC: one group of surfactants are optically active amino acid derivatives (4,5,13) and the other group natural surfactants such as bile salts (7,14) and digitonin (8). Nonionic surfactants such as digitonin must be used together with an ionic surfactant such as sodium dodecyl sulfate (SDS) to form a mixed micelle having an electrophoretic mobility (8). Even an ionic surfactant, e.g., sodium dodecanoyl L-valinate showed better resolution when it was used as a mixed micelle with SDS than when it was employed alone (8). Addition of methanol and/or urea often improved peak shapes and resolution (8).

Bile salts form a helical micelle having a reversed micelle conformation (15). Among various bile salts commercially available, deoxycholate and its taurine conjugate, taurodeoxycholate, have particularly been effective for enantiomeric separation (6,7). The mechanism of chiral discrimination by bile salts is not known. However, the fact that most of the racemic compounds optically resolved with the bile salt micelle have fused ring structures may be related to the relatively planar structure of bile acids.

Amino acid derivatives (5,13,16), benzoin (13), and warfarin (3) are examples of successfully resolved enantiomers by MEKC using amino acid–derived surfactants.

So far, the use of chiral micelles is not very successful for enantiomeric separation by MEKC. The main objective of the present work is to

find some other useful chiral surfactants for enantiomeric separation. In this paper, the use of glycyrrhizic acid (GRA) and β-escin is described for the separation of dansylated (DNS) or phenylthiohydantoin (PTH) DL-amino acids.

EXPERIMENTAL

MEKC was performed with a laboratory built CE instrument, which consisted of a Matsusada Precision Devices HPCZE-30PN0.25-LDSW (Kusatsu, Shiga, Japan) or a Bertan 230-30R (Hicksville, NY) high voltage dc power supply and a Jasco UVIDEC-100-V spectrophotometric detector (Tokyo, Japan) or a Jasco 821-FPS spectrofluorometric detector. The cell part of the fluorometric detector was modified to an immersed type to reduce the reflection of excitation light at the surface of the capillary, as described by Kurosu et al. (16). A cell block of the photometric detector was constructed with plastic instead of aluminum and an aperture made of stainless steel was grounded to diminish electrical noise. The fluorometric detector was operated at 330 nm for excitation and 550 nm for emission and the photometric detector at 220 nm. A fused silica capillary of 50 μm i.d. was obtained from Polymicro Technologies (Phoenix, AZ). Electropherograms or chromatograms were recorded with a Shimadzu Chromatopac C-R6A data processor (Kyoto, Japan). The capillary was cooled with a small electric fan during the CE run.

GRA monoammonium salt (hydrate), DNS-, and PTH-amino acids were purchased from Wako Chemical (Osaka, Japan). β-Escin was from Sigma (St. Louise, MO). SDS of protein research grade was obtained from Nacalai Tesque (Kyoto, Japan). The other reagents for preparing buffer solutions were of analytical-reagent grade. All the chemicals and reagents were used as they were received. Deionized water was purified with a Milli-Q system (Nippon Millipore, Tokyo, Japan) or a Toraypure LV-408 (Toray, Tokyo, Japan). All the separation solutions were filtered through a disposable membrane filter of 0.45 μm pore size, prior to use.

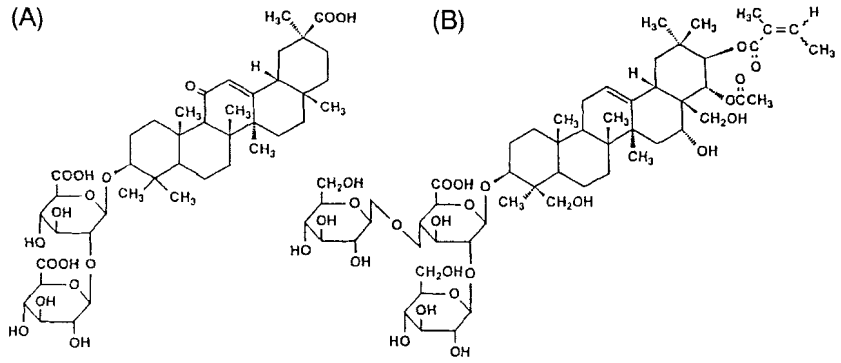

FIGURE 1. Molecular structure of glycyrrhizic acid (A) and β-escin (B).

RESULTS AND DISCUSSION

Molecular structures of GRA and β-escin are shown in **Figure 1**. Both are triterpene glucosides and commercially available from various suppliers. The D/E ring junction has *cis* configuration in both triterpenes and therefore, only the E-ring is located out of plane consisting of the other four fused rings. The triterpene moiety constitutes the hydrophobic part of the surfactant and the sugar moiety the hydrophilic part. Therefore, these triterpene glucoside surfactants have a relatively planar rigid hydrophobic moiety and a considerably bulky hydrophilic moiety in one molecule. GRA contains an α, β-unsaturated carbonyl group, which absorbs uv lights maximum at 248 nm. Therefore, GRA is not suitable for the uv detector.

GRA has three carboxyl groups in one molecule and soluble in an alkaline buffer. However, GRA solutions in buffer solutions easily gelled and they were not used for MEKC. Mixed solutions of GRA and SDS also tended to gel. The use of GRA together with a nonionic surfactant such as Tween 20 or octyl-β-D-glucoside was not suitable for MEKC because of gelation or a slow migration velocity of the micelle.

A successful solution for enantiomeric separation was a mixed micellar solution consisting of 30 mM GRA, 50 mM octyl-β-D-glucoside,

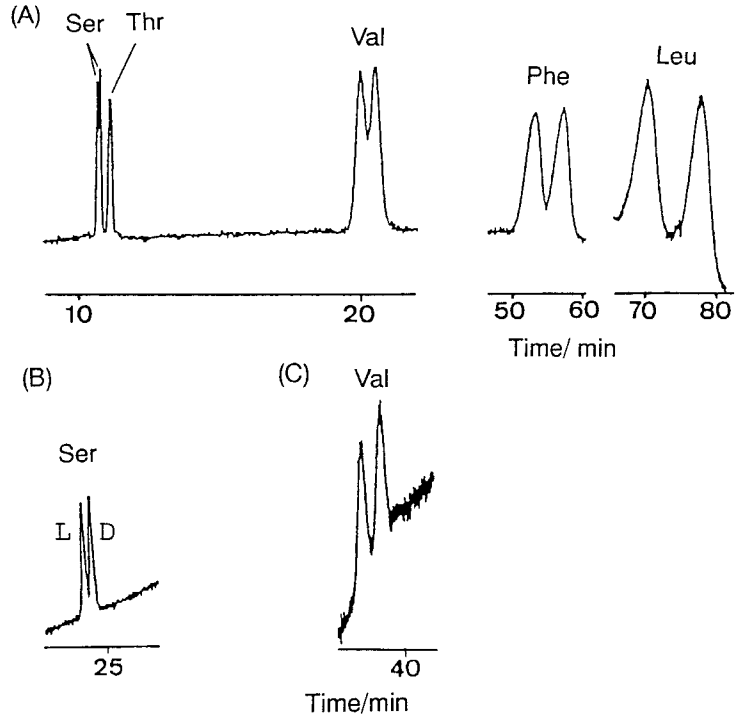

FIGURE 2. Enantiomeric separation of DNS-DL-amino acids by MEKC with glycyrrhizic acid: Each chromatograms were recorded in separate runs and the electroosmotic velocities were different among the runs; (A) t_0 = 4.7 min; (B) t_0 = 6.5 min; (C) t_0 = 5.0 min. Conditions: capillary, 52 μm i.d. x 80 cm (30 cm to the detector); separation solution, 30 mM glycyrrhizic acid, 50 mM octyl-β-D-glucoside, and 10 mM SDS in a 20 mM borate-10 mM phosphate buffer (pH 7.0); applied voltage, 25 kV; detection, fluorescence (ex. 330 nm, em. 550 nm).

and 10 mM SDS in a 20 mM borate-10 mM phosphate buffer (pH 7.0). Examples of enantiomeric separation of some DNS-DL-amino acids are shown in **Figure 2**. The unstable baseline was probably ascribed to the temperature change during the run, because the separation was performed in a room without air conditioning. The resolution of DNS-DL-Ser and DNS-DL-Val was improved when the electroosmotic velocity was

TABLE 1

Enantiomeric Separation of DNS-DL-Amino Acids by MEKC with Glycyrrhizic Acid [a].

DNS-Amino acid	Migration time/min			μ_{ep}*/mm^2 V^{-1} min^{-1}		R_s [c]
	t_0	t_L	t_D	$\mu_{ep,L}$	$\mu_{ep,D}$	
Ser	4.70	10.73	10.83	1.109	1.118	0.75
	6.50	23.60	24.19			1.30
Thr	4.70	11.18		1.225		0
Met	4.90	23.93	24.43	1.558	1.566	0.65
		40.45	41.16			0.90
Val	4.70	19.88	20.40	1.572	1.582	0.85
	5.00	37.11	38.48			1.40
Aba[b]	4.55	20.00		1.630		0
Phe	4.70	52.13	56.38	1.859	1.872	1.20
Leu	4.18	13.63		1.916	1.929	0
	4.68	69.95	76.95			1.50

[a] Conditions are the same as given in **Figure 1.** [b] α-Aminobutyric acid. [c] Calculated according to $R_s = 2(t_D - t_L)/(w_L + w_D)$, where w_L and w_D are peak width at the baseline.

reduced. DNS-D-Ser was eluted later than the corresponding L-isomer, that is, the D-isomer was incorporated into the micelle more than the L-isomer was. The elution orders of the other amino acid enantiomers were not confirmed, but they were assumed to be the same as those of DNS-DL-Ser. Although the capillary was rinsed with a 0.1 M sodium hydroxide solution prior to each run, the reproducibility of the migration time was poor, probably due to the variation of the electroosmotic velocity.

The results of enantiomeric separation with GRA are summarized in **Table 1.**

In MEKC the capacity factor (k') is defined as

$$k' = \frac{n_{mc}}{n_{aq}} \quad (1)$$

where n_{mc} and n_{aq} are the total moles of solute incorporated into the micelle and that in the surrounding aqueous phase, respectively. Since the migration time of the solute (t_R) is limited between that of the bulk solution (t_0) and that of the micelle (t_{mc}) in MEKC (18), the capacity factor can be related to the migration times as

$$k' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} \quad (2)$$

The migration times t_0 and t_{mc} are usually measured with relevant tracers for each migration, e.g., methanol for t_0 and Sudan III or IV for t_{mc}. In case of fluorometric detector, fluorescent tracers were required, especially for the micelle, but we did not use such a tracer of the micelle. The electroosmotic velocity was successfully measured with methanol or formamide as a fluctuation of the baseline.

When the migration time of the micelle is unavailable, we can use the effective mobility (19) (μ_{ep}^*) as a substitute,

$$\mu_{ep}^* = \frac{k'}{1 + k'} \mu_{ep}(mc) \quad (3)$$

where $\mu_{ep}(mc)$ is the electrophoretic mobility of the micelle. The migration velocity of the solute (v_s) is written with μ_{ep}^* as

$$v_s = \mu_s \frac{V}{L} = (\mu_{eo} + \mu_{ep}^*) \frac{V}{L} \quad (4)$$

where V is the applied voltage, L the total length of the capillary, and μ_{eo} the electroosmotic mobility. Although μ_{ep}* means an apparent electrophoretic mobility of the neutral molecules, which is proportional to the fraction of the solute incorporated into the micelle, μ_{ep}* does not give the capacity factor, unless μ_{ep}(mc) is known. The effective mobility of each enantiomer is given in **Table 1**. The reproducibility of the effective mobility was considerably poor and the values given in **Table 1** are averages of a few runs. The poor reproducibility can probably be attributed to a gradual change of the electroosmotic velocity during the run.

Resolution equation in MEKC is expressed as (20)

$$R_s = \frac{N^{1/2}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{1 + k_2'}\right)\left(\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})\,k_1'}\right) \qquad (4)$$

where R_s is resolution, N is the plate number, and α is the separation factor equal to k_2'/k_1'. The last term in the right-hand side of eq. 4 is ascribed to the limited migration time window. This term, therefore, is characteristic of MEKC and indicates the effect of the electroosmotic velocity on resolution. The smaller the t_0/t_{mc} value, the higher the resolution and hence, a slower electroosmotic velocity causes a higher resolution, because the mobility of the micelle is relatively independent of experimental conditions, except for viscosity. Viscosity does not affect the t_0/t_{mc} value.

A mixed micelle of β-escin and SDS was successfully used for enantiomeric separation of PTH-amino acids, as shown in **Figure 3**, where 25 mM β-escin and 50 mM SDS in a 50 mM phosphate buffer solution (pH 3.0) was employed. Under acidic conditions, electroosmosis was weak and the micelle migrated toward the positive electrode (21). Enantiomeric separation was unsuccessful under neutral conditions. Although PTH-DL-Trp was not resolved in **Figure 3**, a decrease in the SDS fraction of the mixed micelle led to a partial resolution as shown in **Figure 4**. The partially resolved PTH-DL-Nle was completely separated under the conditions given in **Figure 4**. However, the separation solution used in **Figure 4** was not stable and gelled easily, therefore the reproducibility of the migration time was poor.

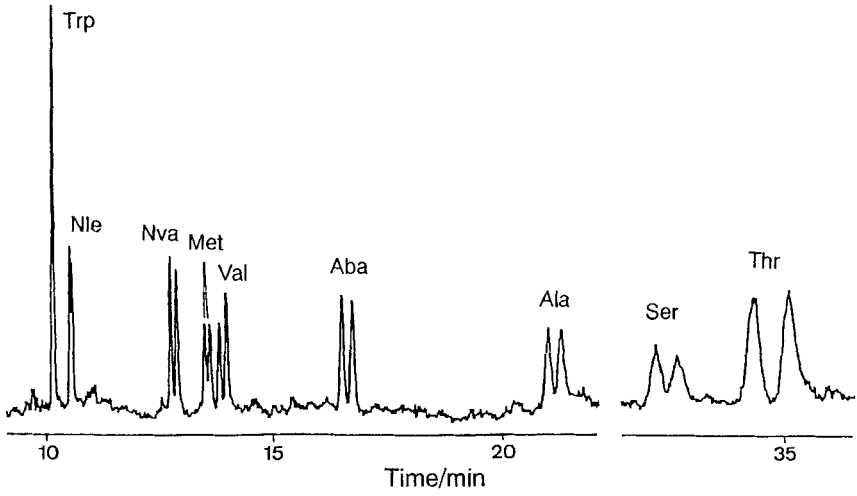

FIGURE 3. Enantiomeric separation of PTH-DL-amino acids by MEKC with β-escin. Conditions: capillary, 50 μm i.d. x 65 cm (50 cm to the detector); separation solution, 25 mM β-escin and 50 mM SDS in a 50 mM phosphate buffer (pH 3.0); applied voltage, 20 kV; detection, uv absorbance at 220 nm.

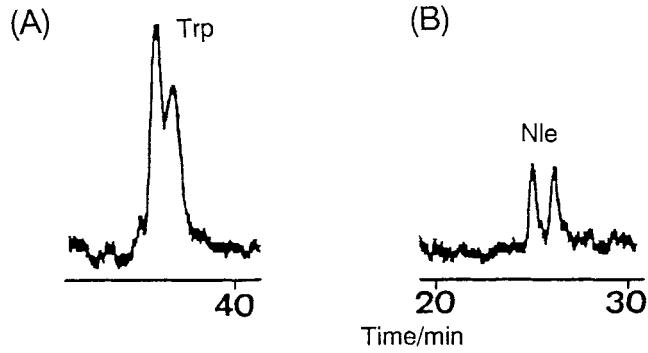

FIGURE 4. Enantiomeric separation of PTH-DL-Trp (A) and PTH-DL-Nle (B) by MEKC with β-escin. Chromatograms (A) and (B) were recorded in different runs. Conditions: capillary, 50 μm i.d. x 50 cm (30 cm to the detector); separation solution, 30 mM β-escin and 30 mM SDS in a 50 mM phosphate buffer (pH 3.0); applied voltage, 18 kV. Other conditions were the same as in **Figure 3.**

TABLE 2

Enantiomeric Separation of PTH-DL-Amino Acids by MEKC with β-Escin[a].

PTH-DL-Amino acid	$-t_1$	$-t_2$	k_1'	k_2'	α	R_s
Trp	10.20		12.41			0
Nle[b]	10.57	10.63	10.58	10.35	1.02	0.76
Nva[c]	12.75	12.89	6.07	5.93	1.02	1.29
Met	13.48	13.60	5.41	5.33	1.02	1.02
Val	13.81	13.95	5.18	5.08	1.02	1.20
Aba[d]	16.49	16.71	3.95	3.88	1.02	1.34
Ala	21.03	21.28	3.06	3.02	1.01	1.33
Ser	32.28	32.74	2.46	2.44	1.01	1.39
Thr	34.41	35.41	2.39	2.36	1.01	1.60

[a] Conditions are the same as in **Figure 3**; the elution order of each enantiomer was not identified; t_{mc} = −8.34 min and t_0 = 5.76 min except for Ser and Thr; t_{mc} = −8.64 min and t_0 = 5.63 min for Ser and Thr. [b] Norleucine; [c] Norvaline; [d] See **Table 1**.

Since the electroosmotic flow and the migration of the micelle were in the opposite directions, the migration time was assumed to be negative for the migration toward the positive electrode, when eq. 2 was employed to calculate the capacity factor. The migration times, capacity factors, and resolutions of PTH-DL-amino acids are summarized in **Table 2**. Under the conditions given in **Figure 3**, the solute having a larger capacity factor migrated faster toward the positive electrode, giving a shorter migration time. The migration time of the bulk solution (t_0) had to be measured in a separate run under acidic conditions, because the t_0 peak was not observed together with the peaks of PTH-amino acids. DNS-DL-amino acids were not resolved under the same conditions given in **Figure 3**.

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REFERENCES

1. E. Gassmann, J. E. Kuo, R. N. Zare, *Science*, **230**, 813–814 (1985)
2. S. Fanali, *J. Chromatogr.*, **474**, 441–446 (1989)
3. R. Kuhn, F. Stoecklin, F. Erni, *Chromatographia*, **33**, 32–36 (1992)
4. A. S. Cohen, A. Paulus, B. L. Karger, *Chromatographia*, **24**, 15–24 (1987)
5. A. Dobashi, T. Ono, S. Hara, and J. Yamaguchi, *Anal. Chem.*, **61**, 1984–1986 (1989)
6. S. Terabe, M. Shibata, Y. Miyashita, *J. Chromatogr.*, **480**, 403–411 (1989)
7. H. Nishi, T. Fukuyama, M. Matsuo, S. Terabe, *J. Microcol. Sep.*, **1**, 234–241 (1989)
8. K. Otsuka, S. Terabe, *J. Chromatogr.*, **515**, 221–226 (1990)
9. S. Terabe, *Trends Anal. Chem.*, **8**, 129–134 (1989)
10. H. Nishi, T. Fukuyama, S. Terabe, *J. Chromatogr.*, **553**, 503–516 (1991)
11. T. Ueda, F. Kitamura, R. Mitchel, T. Metcalf, T. Kuwana, A. Nakamoto, *Anal. Chem.*, **63**, 2979–2981 (1991)
12. J. Vindevogel and P. Sandra, *Introduction to Micellar Electrokinetic Chromatography*, Huthig, Heidelberg (1992)
13. K. Otsuka, J. Kawahara, K. Takekawa, and S. Terabe, *J. Chromatogr.* **559**, 209–214 (1991)
14. R. O. Cole, M. J. Sepaniak, and W. L. Hinze, *J. High Resolut. Chromatogr.* **13**, 579–582 (1990)

15. R. O. Cole, M. J. Sepaniak, W. L. Hinze, J. Gorse, and K. Oldiges, *J. Chromatogr.* **557**, 113–123 (1991)

16. A. Dobashi, T. Ono, S. Hara, and J. Yamaguchi, *J. Chromatogr.*, **480**, 413–420 (1989)

17. Y. Kurosu, T. Sasaki, and M. Saito, *J. High Resol. Chromatogr.*, **14**, 186–190 (1991)

18. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, and T. Ando, *Anal. Chem.*, **56**, 111–113 (1984)

19. K. Ghowsi, J. P. Foley, and R. J. Gale, *Anal. Chem.*, **62**, 2714–2721 (1990)

20. S. Terabe, K. Otsuka, and T. Ando, *Anal. Chem.*, **57**, 834–841 (1985)

21. K. Otsuka and S. Terabe, *J. Microcol. Sep.*, **1**, 150 (1989)