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Chiral separations by micellar electrokinetic chromatography with sodium N-dodecanoyl-L-valinate

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ABSTRACT

Chiral separations by micellar electrokinetic chromatography with sodium N-dodecanoyl-L-valinate (SDVal) were investigated. Addition of sodium dodecyl sulphate (SDS), urea and methanol to SDVal micellar solutions improved the peak shapes and resolution and also changed the selectivity. Phenylthiohydantoin (PTH) derivatives of six DL-amino acids (serine, α-aminobutyric acid, norvaline, valine, tryptophan and norleucine) were separated from each other and each pair of enantiomers was optically resolved with a 50 m*M* SDVal–30 m*M* SDS–0.5 *M* urea (pH 9.0)–10% (v/v) methanol solution. Some other enantiomers were also resolved.

INTRODUCTION

Recently, high-performance capillary electrophoresis (HPCE) [1–3], an instrumental version of electrophoresis, has been widely used in various fields because of its high efficiency. Most commercial HPCE instruments are operated automatically with microcomputer systems so that they can be used for routine work. Electrokinetic chromatography (EKC) [4], which is one branch of HPCE and based on chromatographic principles using homogeneous solutions, has also become a well known high-resolution separation technique. One of the unique characteristics of EKC is that it can separate both neutral analytes and charged solutes electrophoretically. Among various modes of EKC, micellar EKC (MEKC) [5,6], also called micellar electrokinetic capillary chromatography (MECC) [7], has become the most popular method for separating small neutral compounds. MEKC uses an ionic micelle as a "carrier" in EKC [4].

Chiral separations will be one of the major applications of both HPCE and EKC. Zare and co-workers [8,9] first reported optical resolution by EKC using copper(II) complexes. In MEKC, optical resolution of racemic compounds can be

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achieved by using chiral surfactants. Recently, some papers have appeared on chiral separations by MEKC without any additives such as metal ions or complexation reagents. As chiral surfactants L-amino acid derivatives, *e.g.*, sodium N-dodecanoyl-L-valinate (SDVal) [10,11], or bile salts [12–14] were used. We have reported the enantiomeric resolution of some phenylthiohydantoin-DL-amino acids (PTH-DL-AA) by MEKC with chiral surfactants, digitonin and SDVal [15]. Some PTH-DL-AA were optically resolved with SDVal alone, although seriously tailed peaks were observed. By adding methanol and urea to SDVal solutions, the worst peak shapes could be improved and the selectivity changed, but sufficient resolution was not attained because of the small capacity factors of the solutes and/or narrow migration-time window [16].

In this investigation, we used SDVal–sodium dodecyl sulphate (SDS) co-micellar solutions containing methanol and urea to improve resolution through increased capacity factors and extended migration-time windows. Changes in the selectivity were also observed. Some PTH-DL-AA were successfully optically resolved and separated from each other. Some other enantiomers were also resolved.

EXPERIMENTAL

SDVal was obtained from Ajinomoto (Tokyo, Japan), SDS and methanol from Nacalai Tesque (Kyoto, Japan), urea and PTH-DL-AA from Wako (Osaka, Japan), benzoin from Tokyo Kasei (Tokyo, Japan) and warfarin from Tanabe Seiyaku (Osaka, Japan). All chemicals were used as received.

Micellar solutions were prepared by dissolving SDVal, SDS and urea in 50 m*M* borate buffers (pH 9.0) or 50 m*M* phosphate buffers (pH 7.0) and then mixed with appropriate amounts of methanol. PTH-DL-AA were injected as acetonitrile solutions and benzoin and warfarin as methanol solutions.

An untreated fused-silica capillary tube (Scientific Glass Engineering, Ringwood, Australia), 650 mm × 0.05 mm I.D., was used as a separation column, and on-column UV detection was carried out at a position 500 mm from the injection end with an SPD-6A spectrophotometric detector (Shimadzu, Kyoto, Japan). A Chromatopac C-R6A (Shimadzu) was used for data processing. Samples were injected manually by siphoning [5]. An HepLL-30P0.08-LS (Matsusada, Kusatsu, Shiga, Japan) was used for a regulated high-voltage power supply operated in the constant-voltage mode. All experiments were carried out at ambient temperature.

RESULTS AND DISCUSSION

Optical resolution of PTH-DL-AA

As reported previously [16], the addition of 5 *M* urea to a 25 m*M* SDVal solution (pH 7.0) containing 10% (v/v) methanol led to a significant improvement in peak shapes. PTH derivatives of three DL-amino acids, norvaline, tryptophan and norleucine, were separated from each other and each pair of enantiomers was resolved. However, reduced capacity factors ($\tilde{k}'$) of the solutes compared with those in the absence of urea were observed and sufficient resolution was not attained although the migration-time window was not narrower. The effects of urea addition will be reported elsewhere [17]: $\log \tilde{k}'$ decreases with increasing concentration of urea (C_{urea}).

The value of t_0/t_{mc}, which is related to the migration-time window [6], also decreases with increasing C_{urea}, where t_0 and t_{mc} are the migration times of an insolubilized solute and of the micelle, respectively, that is, the migration-time window increases with increasing C_{urea}. Although the reason for the improvement in peak shapes on addition of urea is not clear, the adsorption of urea on the inside wall of the fused-silica capillary might prevent the irreversible adsorption of solutes, hence improving the peak shapes.

The capacity factor is related to the concentration of the surfactant (C_{sf}) by [6]

$$\tilde{k}' = K\bar{v}(C_{sf} - \text{CMC}) \quad (1)$$

where K, $\bar{v}$ and CMC are the distribution coefficient, partial specific volume of the micelle and critical micelle concentration, respectively. The term $C_{sf} - \text{CMC}$ represents the net concentration of the micelle. Therefore, larger $\tilde{k}'$ can be obtained with higher C_{sf}. To increase the capacity factors and improve the resolution, we tried to use a higher SDVal concentration, 50 mM, and a lower concentration of urea, 0.5 M, keeping the amount of methanol constant. Here, the pH of micellar solutions was selected as 9.0 to increase the solubility of SDVal. In the pH range 7–9, both the electroosmotic velocity and electrophoretic velocity of the micelle were found to be almost constant [18,19]. With the SDVal–urea–methanol solution, however, improvements in both resolution and separation were not achieved.

We then examined the addition of SDS to the SDVal–urea–methanol solutions. In this instance, negatively charged SDVal–SDS mixed micelles were formed in the solution [10,11] and the selectivity was expected to be changed. By using 50 mM SDVal–30 mM SDS–0.5 M urea (pH 9.0)–10% (v/v) methanol, PTH derivatives of six DL-amino acids, serine (Ser), α-aminobutyric acid (Aba), norvaline (Nva), valine (Val), tryptophan (Trp) and norleucine (Nle), were separated from each other and each DL-pair was optically resolved as shown in Fig. 1. Although PTH-DL-Ser was partially resolved, this pair of enantiomers could not be resolved with SDVal alone, SDVal–methanol or SDVal–methanol–urea solutions. For another PTH derivative, methionine (Met) was successfuly resolved as shown in Fig. 2, but it could no be separated from above six PTH-DL-AA in Fig. 1.

For each pair of the six PTH-DL-AA in Fig. 1, $\tilde{k}'$, the separation factor (α) and resolution (R_s) were calculated using the equations reported previously [6]. The results are given in Table I. Here, t_0 and t_{mc} were obtained from the migration times of acetonitrile and Sudan IV, respectively. Note that Sudan IV was assumed not to exist even in the aqueous phase containing methanol. In this instance, $t_0/t_{mc} = 0.16$ and this value is smaller than that in 20 mM SDVal alone (0.20) [15], that is, an extended migration-time window was achieved. All the values of $\tilde{k}'$ for each solute in Table I are 3–4 times larger than those observed with 20 mM SDVal [15]. As the $\tilde{k}'$ values for Ser and Aba are smaller than the optimum value of 2–3, the resolution between each pair of enantiomers might be improved when these $\tilde{k}'$ are increased to the optimum value.

To examine the effect of urea addition, we used the same micellar solution as in Fig. 1 without urea, *i.e.*, 50 mM SDVal–30 mM SDS–10% (v/v) methanol, for separating the same PTH-DL-AA. In this instance, Nva and Val co-migrated and Ser was still only partially resolved although the migration order was the same as in Fig. 1.

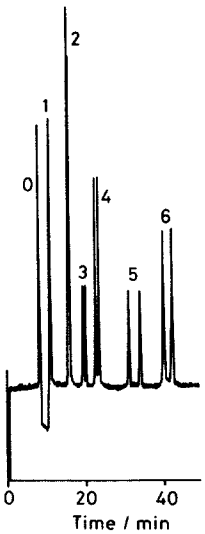

Fig. 1. Chiral separation of six PTH-DL-AA by MEKC. Corresponding amino acids: 1 = Ser; 2 = Aba; 3 = Nva; 4 = Val; 5 = Trp; 6 = Nle. 0 = acetonitrile. Micellar solution, 50 m*M* SDVal–30 m*M* SDS–0.5 *M* urea (pH 9.0) containing 10% (v/v) methanol; separation column, 650 mm × 0.05 mm I.D.; length of the tube used for separation, 500 mm; total applied voltage, 20 kV; current, 17 μA; detection wavelength, 260 nm; temperature, ambient.

Fig. 2. Chiral separation of PTH-DL-Met by MEKC. current, 19 μA; other conditions as in Fig. 1.

Resolution of other chiral compounds

The optical resolution of various chiral compounds was investigated with the same SDVal–SDS–urea–methanol solution as in Fig. 1. As a result, 2-hydroxy-1,2-diphenylethanone (benzoin) and 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one (warfarin) were successfully resolved, as shown in Fig. 3. These two compounds were not optically resolved with SDVal alone, SDVal–methanol or SDVal–methanol–urea solutions. Moreover, they could not be resolved with digitonin–SDS solutions.

TABLE I

CAPACITY FACTORS ($\tilde{k}'$), SEPARATION FACTORS (α) AND RESOLUTION (R_s) OF SOME PTH DERIVATIVES OF DL-AMINO ACIDS

Conditions as in Fig. 1.

Solute	$\tilde{k}'_1$	$\tilde{k}'_2$	α	R_s
Ser	0.5	0.5	1.00	
Aba	1.4	1.4	1.04	0.7
Nva	2.2	2.4	1.10	1.7
Val	3.2	3.4	1.09	1.8
Trp	7.0	9.2	1.32	3.6
Nle	16.8	22.2	1.32	3.0

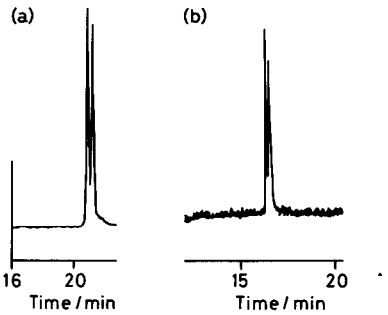

Fig. 3. Optical resolution of (a) benzoin and (b) warfarin by MEKC. current, (a) 20 μA and (b) 19 μA; other conditions as in Fig. 1.

CONCLUSIONS

The addition of SDS to SDVal–urea–methanol solutions caused a change in selectivity and provided a good enantiomeric resolution of PTH-DL-AA and some other enantiomers. The addition of urea to the micellar solution was also effective in improving the peak shapes and resolution. There remain many chiral compounds that cannot be resolved with the same SDVal–SDS–urea–methanol solution. At present, the possibilities for chiral separations of various kinds of enantiomers by MEKC are limited as long as only one type of chiral surfactant is used. Further applications to chiral separations of other compounds are being investigated with some other surfactants and additives.

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