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Optical resolution by high-performance capillary electrophoresis

Micellar electrokinetic chromatography with sodium N-dodecanoyl-L-glutamate and digitonin

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ABSTRACT

Optical resolution by micellar electrokinetic chromatography with sodium N-dodecanoyl-L-glutamate (SDGlu) and with digitonin–sodium taurodeoxycholate (STDC) mixed micelles was investigated. Addition of sodium dodecyl sulphate, urea and methanol to SDGlu micellar solutions could give improved peak shapes and resolution. With SDGlu, phenylthiohydantoin (PTH) derivatives of five DL-amino acids (α-aminobutyric acid, methionine, norvaline, tryptophan and norleucine) were separated from each other and each pair of enantiomers was optically resolved. On the other hand, three PTH–DL-amino acids (norvaline, valine and α-aminobutyric acid) were also successfully resolved with a digitonin–STDC–urea solution (pH 2.5).

INTRODUCTION

High-performance capillary electrophoresis (HPCE) [1–3], which is a highly efficient separation technique, has become popular owing to the development of fully automated instruments. Among some modes of HPCE, capillary zone electrophoresis (CZE) is the most popular method because of the ease of operation, especially in terms of preparation of capillaries and separation solutions. Micellar electrokinetic chromatography (MEKC) [4–7], which is a branch of HPCE and uses an ionic micellar solution, has also become a well-known technique to separate small neutral molecules as well as charged solutes.

Optical resolution is one of the major objectives of HPCE, especially in the pharmaceutical field. Since Zare and co-workers [8,9] first reported enantiomeric separation by CZE using formation of copper (II) complexes, some papers on chiral separation have appeared. To achieve direct optical resolution by HPCE, the following three modes are usually employed: (1) CZE with chelating reagents or with cyclodextrins (CDs); (2) electrokinetic chromatography (EKC), which includes MEKC with chiral surfactants, cyclodextrin–modified MEKC (CD–MEKC), cyclodextrin EKC (CDEKC) and microemulsion

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EKC (MEEKC); and (3) capillary gel electrophoresis with immobilized CDs. The direct enantiomeric separation by CZE using CD (CD–CZE) was first reported by Fanali [10]. This system can be applied to the optical resolution of charged enantiomers. We have also demonstrated the enantiomeric separation of *RS*-chlorpheniramine by CD–CZE [11]. In MEKC, sodium N-dodecanoyl-L-valinate (SDVal) [12–16], various bile salts [17–20], digitonin [14] and saponins [21] have been used as chiral surfactants. Optical resolution by CD–MEKC, in which achiral micelles such as sodium dodecyl sulphate (SDS) are normally used, has also been reported [11,22–24].

In the present investigation, we first used sodium N-dodecanoyl-L-glutamate (SDGlu) instead of SDVal. Some phenylthiohydantoin (PTH)–DL-amino acids were successfully resolved with a SDGlu–SDS–urea–methanol solution, although the selectivity difference between SDGlu and SDVal was not remarkable. Then, a digitonin–sodium taurodeoxycholate (STDC) comicellar system was employed. In the previous study [14], we found that a digitonin–SDS system was effective in enantiomeric resolution of PTH-DL-amino acids, but a long separation time was required. In this study, we changed some conditions so that a reduced separation time could be achieved.

EXPERIMENTAL

SDGlu was received from Ajinomoto (Tokyo, Japan), SDS and methanol from Nacalai Tesque (Kyoto, Japan), digitonin, urea, PTH–DL-amino acids and benzoin from Wako (Osaka, Japan) and STDC from Sigma (St. Louis, MO, USA). Separation solutions were prepared by dissolving surfactants and urea in a 50 m*M* phosphate buffer adjusted to an appropriate pH. Then, methanol was added to the micellar solutions when required. Sample solutions were made by dissolving solutes in a water–acetonitrile (1:1) solution. All the chemicals were of analytical reagent grade and used as received.

Capillary electrophoresis was performed with a laboratory-built system consisting of a Matsusada HepLL–30P0.08-LS or HCZE–30PN0.25-LDS regulated high-voltage power supply (Kusatsu, Shiga, Japan), a Shimadzu SPD-6A UV spectrophotometric detector (Kyoto, Japan) and a Shimadzu Chromatopac C-R6A data processor. An untreated fused-silica tube purchased from Polymicro Technologies (Phoenix, AZ, USA), 550 mm × 50 μm I.D. (effective length was 350 mm) was used as a separation capillary and on-column UV detection was employed.

Sample injection was carried out by the manual or hydrodynamic method. Separation was performed under the constant voltage and ambient temperature conditions.

RESULTS AND DISCUSSION

MEKC with SDGlu

As reported previously [16], by using a SDVal–SDS mixed micellar solution containing urea and methanol, six PTH–DL-amino acids were successfully separated from each other and each enantiomeric pair was resolved. Here, urea addition resulted in improved peak shapes compared with those obtained in the absence of urea, probably because of the adsorption of urea to the inside wall of the fused-silica capillary, which might prevent the irreversible adsorption of solutes.

In this study, SDGlu was employed to examine the possibility of optical resolution, instead of SDVal. As shown in Fig. 1, the structure of SDGlu is similar to that of SDVal: both have an N-dodecanoyl group and consist of an L-amino acid with five carbon atoms. SDGlu has an *n*-propyl and two carboxyl groups, whereas SDVal has isopropyl and carboxyl groups. Hence, resolution characteristics were expected to be similar in SDGlu and SDVal micellar

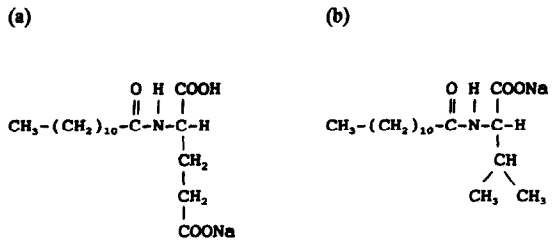

Fig. 1. Structures of (a) SDGlu and (b) SDVal.

systems. The critical micelle concentrations (CMCs) in an aqueous solution are reported to be 10.6 and 6.4 m*M* for SDGlu [25] and SDVal [26], respectively, at 40°C, and were measured by the conductivity method.

Similar to the case of SDVal, we used SDGlu–SDS mixed micellar solutions containing urea and methanol to obtain good peak shapes and enhanced selectivity. Three PTH–DL-amino acids, such as Nva, Val and Trp, were separated from each other and each pair was optically resolved, as shown in Fig. 2. Here, because the migration time window was not wide enough and also the capacity factor of the each pair was small, sufficient resolution could not be achieved.

The capacity factor (k') is represented with the concentration of the surfactant (C_{sf}) as [6]:

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

where K and $\bar{v}$ are the distribution coefficient and partial specific volume of the micelle, respectively. The term (C_{sf} – CMC) reveals the net concentration of the micelle. Therefore, an increase in C_{sf} causes an increase in k'. We then tried to use higher SDGlu or SDS concentrations than in Fig. 2, keeping the other conditions constant. However, higher SDGlu concentrations were not effective in improving resolution, but higher SDS concentrations led to improved results.

We could obtain improved resolution by using

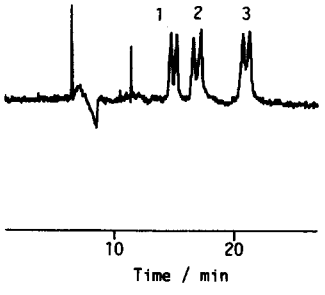

Fig. 2. Chiral separation of three PTH–DL-amino acids by MEKC with SDGlu. Corresponding amino acids: 1 = Nva; 2 = Val; 3 = Trp. Micellar solution, 75 m*M* SDGlu– 30 m*M* SDS–1 *M* urea (pH 9.0) containing 10% (v/v) methanol; separation capillary, 550 mm × 50 μm I.D.; effective length, 350 mm; total applied voltage, 12.5 kV (227 V cm^{-1}); current, 38 μA; detection wavelength, 260 nm; temperature, ambient.

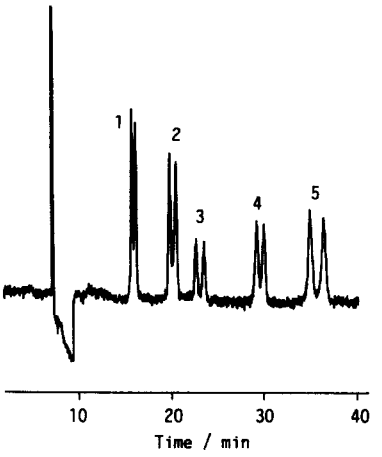

Fig. 3. Chiral separation of five PTH–DL-amino acids by MEKC with SDGlu. Corresponding amino acids: 1 = Aba; 2 = Met; 3 = Nva; 4 = Trp; 5 = Nle. Micellar solution, 75 m*M* SDGlu–50 m*M* SDS–1 *M* urea (pH 9.0) containing 10% (v/v) methanol; current, 32 μA. Other conditions are the same as in Fig. 2.

a 75 m*M* SDGlu–50 m*M* SDS–1 M urea (pH 9.0) solution containing 10% (v/v) methanol, as shown in Fig. 3. In this case, five PTH–DL-amino acids, α-aminobutyric acid (Aba), Met, Nva, Trp and Nle, were successfully separated and each enantiomeric pair was optically resolved. The separation characteristic was similar to that obtained with SDVal [16].

For each pair of the five PTH–DL-amino acids in Fig. 3, k' and the separation factor (α) are calculated according to the equations reported previously [6]. The results are listed in Table I. Here, the migration times of an unretained

TABLE I

CAPACITY FACTORS (k') AND SEPARATION FACTORS (α) OF FIVE PTH–DL-AMINO ACIDS IN THE SDGlu SOLUTION

Conditions as in Fig. 3

Solute	k'_1	k'_2	α
Aba	1.7	1.7	1.06
Met	2.7	2.9	1.07
Nva	3.8	3.9	1.01
Trp	6.2	6.6	1.08
Nle	9.7	11.0	1.14

solute (t_0) and the micelle (t_{mc}) were measured by acetonitrile and Sudan IV, respectively. Note that Sudan IV was assumed not to exist in the aqueous phase even containing 10% (v/v) methanol. For all the solutes, very similar k' values were observed compared with the case in SDVal, regardless of the difference in the micellar concentrations; in the SDVal solution, 50 m*M* SDVal–30 m*M* SDS–0.5 *M* urea (pH 9.0) containing 10% (v/v) methanol was employed. These results imply that SDGlu and SDVal have almost the same characteristics in terms of enantiomeric resolution of PTH–DL-amino acids. In the SDGlu solution, the value of t_0/t_{mc} was 0.12, and this value was smaller than that in the SDVal solution, 0.16, reported previously [16]. This reveals that a wider migration time window can be attained in the SDGlu system than in the SDVal system.

By using the same SDGlu–SDS–urea–methanol solution as in Fig. 3, optical resolution of benzoin was successfully resolved as shown in Fig. 4. This was the same result as in the SDVal system [16]. However, warfarin, which could be resolved with SDVal [16], was only partially resolved with the SDGlu solution.

Although the results are preliminary and SDGlu might have no advantage over SDVal, comparison of the resolution characteristics between the SDGlu and SDVal systems will provide useful information to investigate the chiral recognition mechanism of these N-acylamino acid micelles.

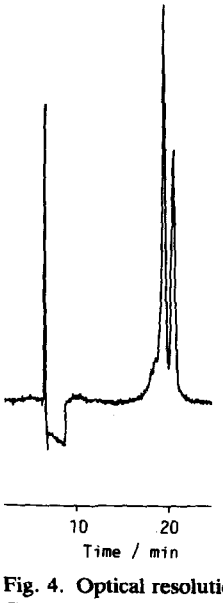

Fig. 4. Optical resolution of benzoin by MEKC with SDGlu. Current, 33 μA. Other conditions as in Fig. 3.

MEKC with digitonin

Digitonin is a natural surfactant with optical activity; it is a glycoside of digitogenin and is used for the determination of cholesterol. Since digitonin is electrically neutral, it is essential to add an ionic micelle to the digitonin solution to form charged mixed micelles that can be used as chiral carriers in MEKC. As reported previously [14], we used the digitonin–SDS co-micellar system for optical resolution of some PTH–DL-amino acids. Although good resolution could be achieved, a long separation time was required, *e.g.*, 90 min for Ala.

In the present investigation, we used a shorter column than the previous one and changed the co-surfactant from SDS to STDC. Although STDC alone has found to be capable of enantiomeric separation of dansylated DL-amino acids (Dns–DL-amino acids), it is not effective for optical resolution of PTH–DL-amino acids, which is similar to the case in other bile salts [27]. However, by using a 50 m*M* digitonin–50 m*M* STDC solution (pH 2.5) containing 1 *M* urea, three PTH–DL-amino acids such as Nva, Val and Aba were separated from each other and each

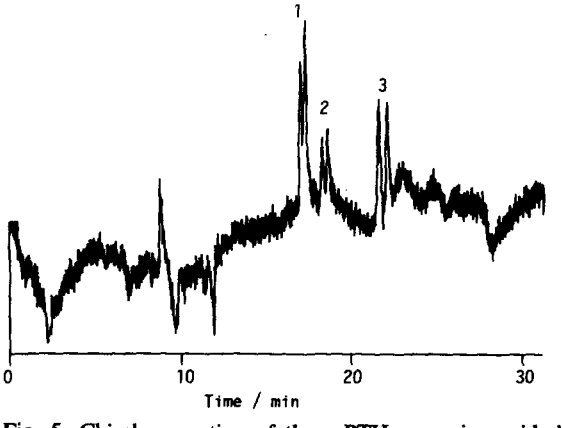

Fig. 5. Chiral separation of three PTH–DL-amino acids by MEKC with digitonin. 1 = Nva; 2 = Val; 3 = Aba. Micellar solution, 50 m*M* digitonin–50 m*M* STDC–1 *M* urea (pH 2.5); capillary, 540 mm × 50 μm I.D.; effective length, 340 mm; total applied voltage, 17.5 kV (324 V cm^{-1}); current, 46 μA. Other conditions as in Fig. 2.

pair was optically resolved, as shown in Fig. 5. In this instance, the electroosmotic velocity was sufficiently suppressed, and the migration direction of the mixed micelle was towards the positive electrode or opposite to the electroosmotic flow, as reported previously [14]: if the solutes are well incorporated into the micelle, they will migrate toward the positive electrode, while the solutes less incorporated into the micelle will migrate toward the negative electrode or the same direction as the electroosmosis [28]. Therefore, as long as the solute migrates toward the positive electrode, the larger the capacity factor, the shorter the migration time.

Although this was also a preliminary result and not optimized, a remarkably reduced separation time was achieved, *e.g.* for Aba *ca.* 22 min in Fig. 5, compared with *ca.* 43 min with the digitonin–SDS system in the previous study [14]. It should be noted that optical resolution of any Dns–DL-amino acids with the digitonin–STDC solution could not be achieved. This suggests that digitonin plays a major role in optical resolution in the digitonin–STDC system.

CONCLUSION

The use of SDGlu–SDS–urea–methanol solutions could give good results in the optical resolution of PTH–DL-amino acids, although the resolution characteristic was not very different from that in SDVal–SDS micellar solutions. The digitonin–STDC mixed micellar system was also effective for enantiomeric resolution of PTH–DL-amino acids, even using a shorter capillary than before. At present, chiral separation by MEKC and by CZE has not been fully investigated, especially compared with HPLC. Further applications on chiral separations of other compounds are being investigated with some other surfactants and additives.

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REFERENCES

1 F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, *J. Chromatogr.*, 169 (1979) 11–20.
2 J.W. Jorgenson and K.D. Lukacs, *Anal. Chem.*, 53 (1981) 1298–1302.
3 S. Hjertén, *J. Chromatogr.*, 270 (1983) 1–6.
4 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129–134.
5 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111–113.
6 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834–841.
7 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 61 (1989) 251–260.
8 E. Gassmann, J.E. Kuo and R.N. Zare, *Science*, 230 (1985) 813–814.
9 P. Gozel, E. Gassmann, H. Michelsen and R.N. Zare, *Anal. Chem.*, 59 (1987) 44–49.
10 S. Fanali, *J. Chromatogr.*, 474 (1989) 441–446.
11 K. Otsuka and S. Terabe, *J. Liq. Chromatogr.*, 16 (1993) 945–953.
12 A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, *Anal. Chem.*, 61 (1989) 1984–1986.
13 A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, *J. Chromatogr.*, 480 (1989) 413–420.
14 K. Otsuka and S. Terabe, *J. Chromatogr.*, 515 (1990) 221–226.
15 K. Otsuka and S. Terabe, *Electrophoresis*, 11 (1990) 982–984.
16 K. Otsuka, J. Kawahara, K. Tatekawa and S. Terabe, *J. Chromatogr.* 559 (1991) 209–214.
17 S. Terabe, M. Shibata and Y. Miyashita, *J. Chromatogr.*, 480 (1989) 403–411.
18 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Microcol. Sep.*, 1 (1989) 234–241.
19 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr.*, 515 (1990) 233–243.
20 R.O. Cole, M.J. Sepaniak and W.L. Hinze, *J. High Resolut. Chromatogr.*, 13 (1990) 579–582.
21 Y. Ishihama and S. Terabe, *J. Liq. Chromatogr.*, 16 (1993) 933–944.
22 Y. Miyashita and S. Terabe, *Application Data*, High Performance Capillary Electrophoresis, DS–767, Beckman, Palo Alto, CA, 1990.
23 H. Nishi, T. Fukuyama and S. Terabe, *J. Chromatogr.*, 553 (1991) 503–516.
24 T. Ueda, F. Kitamura, R. Mitchel, T. Metcalf, T. Kuwana and A. Nakamoto, *Anal. Chem.*, 63 (1991) 2979–2981.
25 M. Takehara, K. Sakamoto and R. Yoshida, *Yukagaku*, 25 (1976) 789–792.
26 R. Yoshida, M. Takehara and K. Sakamoto, *Yukagaku*, 24 (1975) 538–541.
27 K. Otsuka, Y. Kawaguchi and M. Kashihara, unpublished results (1992).
28 K. Otsuka and S. Terabe, *J. Microcol. Sep.*, 1 (1989) 150–154.