ELSEVIER

Journal of Chromatography A, 680 (1994) 317–320

JOURNAL OF CHROMATOGRAPHY A

Optical resolution of amino acid derivatives by micellar electrokinetic chromatography with N-dodecanoyl-L-serine

Koji Otsuka[a,*], Kaoru Karuhaka[a], Mitsuo Higashimori[a], Shigeru Terabe[b]

[a]*Department of Industrial Chemistry, Osaka Prefectural College of Technology, Saiwai-cho, Neyagawa, Osaka 572, Japan*

[b]*Department of Material Science, Faculty of Science, Himeji Institute of Technology, Kamigori, Hyogo 678-12, Japan*

Abstract

Optical resolution by micellar electrokinetic chromatography with N-dodecanoyl-L-serine (DSer) was investigated. Similarly to the use of sodium N-dodecanoyl-L-valinate or sodium N-dodecanoyl-L-glutamate, addition of sodium dodecyl sulfate (SDS), urea and organic modifiers such as methanol and 2-propanol (IPA) to DSer micellar solutions could give improved peak shapes and enhance the enantioselectivity. With a DSer–SDS–urea–methanol solution, six phenylthiohydantoin (PTH)DL-amino acids were separated and each enantiomeric pair was optically resolved. By using a DSer–SDS–urea–IPA solution, the same PTH–DL-amino acids were partially resolved, while PTH-DL-Thr was resolved only in this system. N-Dodecanoyl-L-aspartic acid and sodium N-tetradecanoyl-L-glutamate were also investigated, but satisfactory results were not obtained.

1. Introduction

Recently, optical resolution has become one of major applications of high-performance capillary electrophoresis (HPCE) [1–3], especially in pharmaceutical fields. To achieve direct enantiomeric separation by HPCE, cyclodextrin capillary zone electrophoresis (CD-CZE), micellar electrokinetic chromatography (MEKC) with chiral surfactants and cyclodextrin-modified MEKC (CD-MEKC) are usually employed. MEKC is also a popular technique for separating small neutral molecules in addition to charged solutes.

In optical resolution by MEKC with chiral micelles, sodium N-dodecanoyl-L-valinate (SDVal) [4–8], sodium N-dodecanoyl-L-glutamate (SDGlu) [9], various bile salts [10–13], digitonin [6,9] and saponins [14] have been used as chiral selectors. In the present investigation, we used N-dodecanoyl-L-serine (DSer) instead of SDVal and SDGlu. To dissolve this surfactant, basic (pH 11.0–12.0) buffer solutions were employed and sodium dodecyl sulfate (SDS), urea and organic modifiers, such as methanol and 2-propanol (IPA), were also added to DSer solutions to enhance the enantioselectivity of aqueous buffers. Some phenylthiohydantoin-DL-amino acids (PTH-DL-AAs) were successfully resolved with DSer–SDS–urea–methanol and DSer–SDS–urea–IPA solutions. Although the enantioselectivities in these micellar systems were not substantially different from the SDVal or SDGlu systems, PTH-DL-Thr was optically resolved only with the DSer–IPA solution. As other chiral surfactants, N-dodecanoyl-L-aspartic acid (DAsp) and sodium N-tetradecanoyl-L-

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00481-N

glutamate (STGlu) were also examined, but successful results were not obtained.

2. Experimental

DSer, DAsp and STGlu (Fig. 1) were obtained from Ajinomoto (Tokyo, Japan), SDS and methanol from Nacalai Tesque (Kyoto, Japan) and urea, IPA and PTH-DL-AAs from Wako (Osaka, Japan). Micellar solutions were prepared by dissolving surfactants and urea in 50 m*M* borate buffer adjusted to an appropriate pH with 100 m*M* sodium hydroxide solution. Organic modifiers were added to the micellar solutions when required. Sample solutions were prepared by dissolving enantiomers in acetonitrile. 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 HCZE-30PN0.25-LDS regulated high-voltage power supply (Kusatsu, Shiga, Japan), a Shimadzu (Kyoto, Japan) SPD-6A UV spectrophotometric detector and a Shimadzu Chromatopac C-R6A data processor. An untreated fused-silia tube purchased from Polymicro Technologies (Phoenix, AZ, USA), 252 or 300 mm (effective length) × 52 μm I.D. was used as a separation capillary and on-column UV detection was employed.

Sample injection was carried out by the hydrodynamic method. Separation was performed at constant voltage and ambient temperature.

3. Results and discussion

As the solubility of DSer in neutral aqueous solutions is low, owing to its free acid form, basic buffer solutions were employed and/or organic modifiers were added to the DSer solutions. By using buffers of pH 7.0–9.0 without organic modifiers, sufficient solubility of DSer was not attained. On the other hand, by using buffers of pH 11.0–12.0, DSer was dissolved completely even without organic solvents. However, at pH 12.0, chiral separation could not be achieved for any enantiomers. In this instance, unstable baselines were always observed, mainly owing to the degradation of the inside wall of the capillary by high-pH solutions, and also no reproducible result was obtained. At pH 11.0, although stable baselines and reproducible separation were obtained, still no enantiomeric resolution was observed.

Addition of organic modifiers, such as methanol and IPA, could enhance the solubility of DSer: even at pH 7.0, a buffer containing 20% (v/v) IPA or methanol could dissolve DSer completely. However, no optical resolution could be obtained, and moreover, the separation of some conventional test solutes [15], a mixture of some neutral compounds, was not successful. These results suggested that sufficient micellar formation did not occur in such a buffer solution. By using a buffer of pH 11.0, addition of IPA could give a partial enantiomeric separation of some PTH-DL-AAs, but the efficiency was still very low.

As reported previously [8,9], addition of SDS,

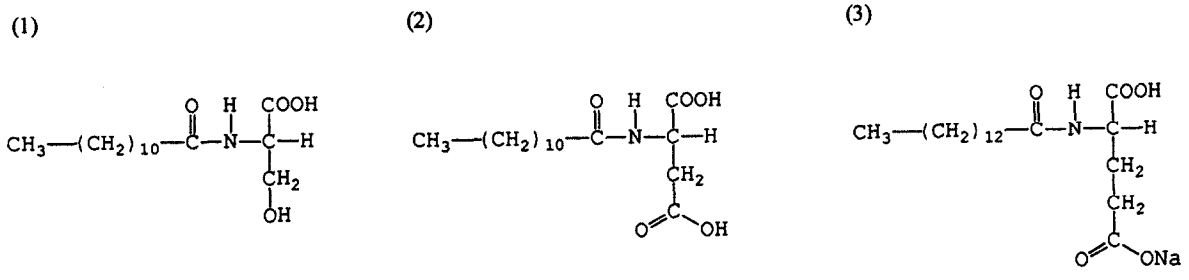

Fig. 1. Structures of (1) DSer, (2) DAsp and (3) STGlu.

urea and methanol to SDVal or SDGlu micellar solutions could give efficient enantiomeric resolution of PTH-DL-AAs and some other enantiomers. Similarly, we used DSer–SDS mixed micellar solutions (pH 11.0) containing urea and methanol or IPA to obtain good peak shapes and enhanced enantioselectivity. These electrolyte systems were stable enough to obtain a good baseline and reproducible separation, although they contained urea in a high-pH region. By using a 75 m*M* DSer–75 m*M* SDS–1 *M* urea (pH 11.0) solution containing 20% (v/v) methanol, six PTH-DL-AAs were separated and each enantiomer was optically resolved, as shown in Fig. 2. Although the order of the migration and the separation characteristic in the DSer–methanol system were very similar to those obtained with SDVal and SDGlu solutions [8,9], PTH derivatives of DL-Met and DL-Val were successfully separated from each other by this DSer–methanol system, whereas these two PTH-DL-AAs could not be separated with SDVal and SDGlu solutions.

By using IPA instead of methanol as an organic modifier, similar results for the optical resolution of the six PTH-DL-AAs were obtained, as shown in Fig. 3, although the efficiency was not comparable to that in the DSer–methanol system. However, as for PTH-DL-Thr, only the DSer–IPA system could give an enantiomeric separation, as shown in Fig. 4: in our previous studies, PTH-DL-Thr has never been optically resolved with any other N-alkanoyl-L-amino acid, i.e., SDVal and SDGlu and DSer–methanol systems. This implies that IPA is effective in enhancing the enantioselectivity of the enantiomeric pair of Thr. In Fig. 4, the peaks are not confirmed as to which corresponds to which enantiomeric form, as we have no authentic compounds of PTH-D- and -L-Thr. However, the DSer–IPA system gave two peaks for PTH-DL-

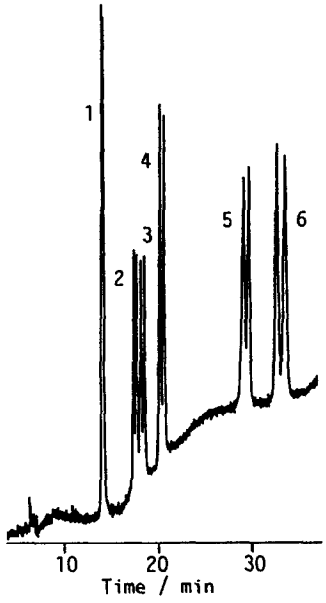

Fig. 2. Chiral separation of six PTH-DL-AAs by MEKC with DSer–methanol. Corresponding AAs: 1 = Aba; 2 = Met; 3 = Val; 4 = Nva; 5 = Trp; 6 = Nle. Micellar solution, 75 m*M* DSer–75 m*M* SDS–1 *M* urea (pH 11.0) containing 20% (v/v) methanol; separation capillary, 300 mm (effective length) × 52 μm I.D.; total applied voltage, 15 kV; electric field strength, 300 V cm^{-1}; detection wavelength, 260 nm; temperature, ambient.

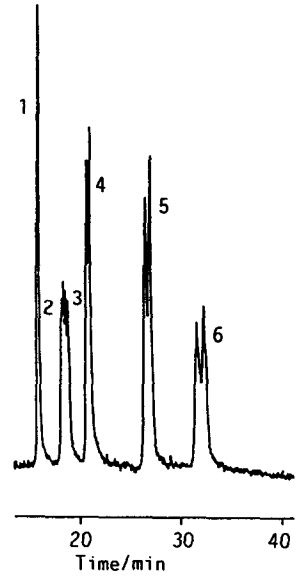

Fig. 3. Chiral separation of six PTH-DL-AAs by MEKC with DSer–IPA. Micellar solution, 75 m*M* DSer–75 m*M* SDS–1 *M* urea (pH 11.0) containing 20% (v/v) IPA; effective length of the separation capillary, 252 mm; electric field strength; 332 V cm^{-1}. Other conditions and peak numbers as in Fig. 2.

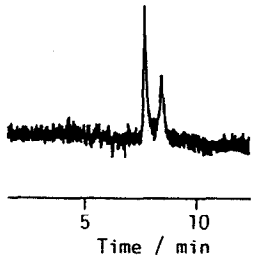

Fig. 4. Optical resolution of PTH-DL-Thr by MEKC with DSer–IPA. Conditions as in Fig. 3.

Thr, whereas only one peak was obtained with DSer–methanol for the same enantiomer.

As for other chiral surfactants, DAsp and STGlu were also examined for enantiomeric separation. The former showed some resolution but the efficiency was always low, and the latter did not show any resolution.

In conclusion, the use of DSer–SDS–urea–methanol solutions could provide a good optical resolution of PTH-DL-AAs, although the resolution was not very different from that with SDVal–SDS–urea–methanol solutions. On the other hand, DSer–SDS–urea–IPA systems were effective only for the enantiomeric separation of PTH-DL-Thr. Further investigations on chiral separations by MEKC with N-alkanoyl-L-amino acids and some other surfactants and additives are in progress.

Acknowledgements

The authors are grateful to Dr. Akio Ishiwata of Ajinomoto for kindly providing various N-alkanoyl-L-amino acids. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 05750729) from the Ministry of Education, Science and Culture, Japan.

References

[1] K. Otsuka and S. Terabe, *Trends Anal. Chem.*, 12 (1993) 125–130.
[2] K. Otsuka and S. Terabe, in N.A. Guzman (Editor), *Capillary Electrophoresis Technology*, Marcel Dekker, New York, 1993, Ch. 20.
[3] S. Terabe, K. Otsuka and H. Nishi, *J. Chromatogr. A*, 666 (1994) 295–319.
[4] A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, *Anal. Chem.*, 61 (1989) 1984–1986.
[5] A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, *J. Chromatogr.*, 480 (1989) 413–420.
[6] K. Otsuka and S. Terabe, *J. Chromatogr.*, 515 (1990) 221–226.
[7] K. Otsuka and S. Terabe, *Electrophoresis*, 11 (1990) 982–984.
[8] K. Otsuka, J. Kawahara, K. Tatekawa and S. Terabe, *J. Chromatogr.*, 559 (1991) 209–214.
[9] K. Otsuka, M. Kashihara, Y. Kawaguchi, R. Koike, T. Hisamitsu and S. Terabe, *J. Chromatogr. A*, 652 (1993) 253–257.
[10] S. Terabe, M. Shibata and Y. Miyashita, *J. Chromatogr.*, 480 (1989) 403–411.
[11] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Microcol. Sep.*, 1 (1989) 234–241.
[12] H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr.*, 515 (1990) 233–243.
[13] R.O. Cole, M.J. Sepaniak and W.L. Hinze, *J. High Resolut. Chromatogr.*, 13 (1990) 579–582.
[14] Y. Ishihama and S. Terabe, *J. Liq. Chromatogr.*, 16 (1993) 933–944.
[15] S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834–841.