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Optical resolution of amino acid derivatives by micellar electrokinetic chromatography with sodium N-tetradecanoyl-L-glutamate

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Abstract

Optical resolution by micellar electrokinetic chromatography with sodium N-tetradecanoyl-L-glutamate (STGlu) was investigated. Similar to the case when using sodium N-dodecanoyl-L-valinate (SDVal), sodium N-dodecanoyl-L-glutamate (SDGlu) and N-dodecanoyl-L-serine (DSer), addition of sodium dodecyl sulfate (SDS), urea and methanol to STGlu micellar solutions gave improved peak shapes and enhanced the enantioselectivity. With an STGlu–3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate–SDS–urea–methanol solution, five phenylthiohydantoin-DL-amino acids were separated and each enantiomeric pair was optically resolved. The resolution characteristic in the STGlu system, however, was not substantially different from those in SDVal, SDGlu and DSer systems.

1. Introduction

Micellar electrokinetic chromatography (MEKC) is capable of separating both neutral and charged compounds and has become a popular technique in capillary electrophoresis (CE) for separating small neutral compounds. In MEKC, an ionic micelle is used as a pseudo-stationary phase that migrates with a different velocity as an aqueous phase or the electro-osmotic velocity.

Optical resolution is one of important objectives in chromatography and also CE, especially in pharmaceutical and medical fields, and many reports on enantiomeric separations by CE have already appeared [1,2], including by MEKC with chiral micelles and cyclodextrin-modified MEKC (CD-MEKC). The use of amino acid derivatives as chiral selectors in MEKC was first reported by Dobashi et al. [3,4]. We have reported the enantiomeric resolution of phenylthiohydantoin derivatives of racemic amino acids by MEKC using chiral micelles of amino acid derivatives, such as sodium N-dodecanoyl-L-valinate (SDVal) [5–7], sodium N-dodecanoyl-L-glutamate (SDGlu) [8] and N-dodecanoyl-L-serine (DSer) [9], and using digitonin [5,8], which is a neutral glycoside. Phenylthiohydantoin-amino acids (PTH-AAs) are produced from the Edman degradation of peptides and proteins and important materials for determining amino acid sequences. Also, each PTH-AA has a strong UV absorbance, hence we have been using them as model compounds.

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In this study, another type of N-alkanoyl-L-amino acid, i.e., sodium N-tetradecanoyl-L-glutamate (STGlu), was used as a chiral surfactant. As additives to STGlu solutions, two surfactants, achiral and anionic sodium dodecyl sulfate (SDS) and chiral and neutral 3 - [(3 - cholamidopropyl)dimethylammonio] - 1 - propanesulfonate (CHAPS), were used. Urea and methanol or 2-propanol (IPA) were also added to micellar solutions. As with SDVal, SDGlu and DSer, some PTH-DL-AAs were optically resolved by using an STGlu–CHAPS–SDS–urea–methanol solution, and also with an STGlu–SDS–urea–methanol system. However, the enantioselectivity in the STGlu micellar system was not substantially different from those in the SDVal, SDGlu and DSer systems.

2. Experimental

STGlu, the structure of which was given in the previous paper [9], was obtained from Ajinomoto (Tokyo, Japan), SDS and methanol from Nacalai Tesque (Kyoto, Japan), urea and PTH-DL-AAs from Wako (Osaka, Japan) and CHAPS from Dojin (Osaka, Japan). Micellar solutions were prepared by dissolving the surfactants and urea in a 50 m*M* borate buffer (pH 11.0) followed by the addition of methanol to the micellar solutions. Sample solutions were made by dissolving enantiomers in methanol–water (1:1). Although the concentration of the sample was not determined precisely, it was around 1 mg ml^{-1}. All the chemicals were of analytical-reagent grade and used as received.

Capillary electrophoresis was performed with a Beckman (Fullerton, CA, USA) P/ACE System 2000 controlled by an IBM (Tokyo, Japan) PS/V personal computer. As a separation capillary, an untreated fused-silica tube purchased from Polymicro Technologies (Phoenix, AZ, USA), 300 mm (effective length) × 52 μm I.D., was used. Separation was performed in the constant voltage mode and the temperature was maintained at 30°C.

3. Results and discussion

Since the solubility of STGlu in an aqueous solution is low, a basic buffer (pH 11.0) was used and the content of methanol was increased to 30% (v/v). As reported previously [6–9], addition of SDS, urea and methanol to the SDVal, SDGlu or DSer micellar solutions could give good efficiency and resolution in the enantiomeric resolution of PTH-DL-AAs. Similarly to those cases, we used STGlu–SDS mixed micellar systems (pH 11.0) containing urea and methanol to obtain good peak shapes and enhanced enantioselectivity. The addition of CHAPS to the above-mentioned solutions was also examined. It should be noted that these micellar solutions were sufficiently stable to achieve reproducible separations even containing urea in high-pH buffer. By using a 100 m*M* STGlu–25 m*M* CHAPS–100 m*M* SDS–1 *M* urea (pH 11.0) solution containing 30% (v/v) methanol, five PTH-DL-AAs were separated from each other and each enantiomeric pair was optically resolved, as shown in Fig. 1. Here, the order of the migration and the separation characteristic in the STGlu system were almost similar to those

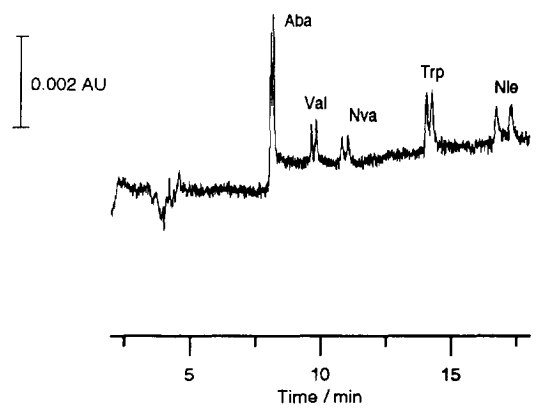

Fig. 1. Chiral separation of five PTH-DL-AAs by MEKC with STGlu–CHAPS. Corresponding AAs: Aba, Val, Nva, Trp and Nle as shown. Micellar solution, 100 m*M* STGlu–25 m*M* CHAPS–100 m*M* SDS–1 *M* urea (pH 11.0) containing 30% (v/v) methanol; separation capillary, 370 mm (effective length, 300 mm) × 52 μm I.D.; total applied voltage, 20 kV, electric field strength, 541 V cm^{-1}; detection wavelength, 254 nm; temperature, 30°C.

obtained with SDVal, SDGlu, and DSer solutions [7–9].

By using IPA instead of methanol as an organic modifier, no enantiomeric resolution of any PTH-DL-AAs was achieved. In general, IPA is not effective in enhancing the enantioselectivity and/or improving the resolution of PTH-DL-AAs with N-alkanoyl-L-amino acids, except for the resolution of PTH-DL-Thr by using a DSer system [9], while the addition of methanol is essential to obtain good resolution: when an STGlu–CHAPS–urea system without methanol was used, the PTH-DL-AAs were partially resolved.

By using an STGlu solution without CHAPS, or an STGlu–SDS–urea–methanol system, almost the same resolution of the five PTH-DL-AAs as in Fig. 1 was obtained, as shown in Fig. 2. This implies that the addition of CHAPS does not affect the resolution characteristic of the STGlu system. On the other hand, by using a CHAPS–SDS–methanol solution or without STGlu, only PTH-DL-Met was optically resolved, as shown in Fig. 3. Although insufficient resolution was attained, the CHAPS–SDS mixed micelle system should be useful for enantiomeric resolution by MEKC.

In conclusion, the use of STGlu–SDS–urea–methanol solutions could provide good results

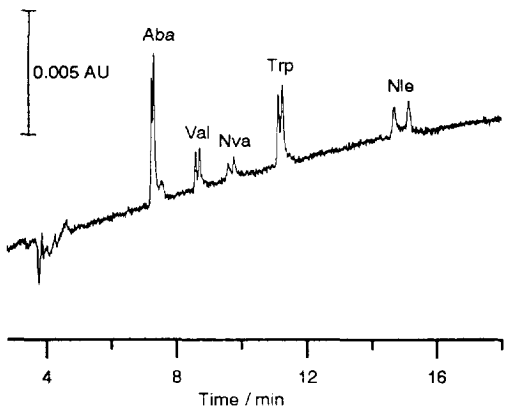

Fig. 2. Chiral separation of five PTH-DL-AAs by MEKC with STGlu without CHAPS. Micellar solution, 100 m*M* STGlu–100 m*M* SDS–1 *M* urea (pH 11.0) containing 30% (v/v) methanol. Other conditions as in Fig. 1.

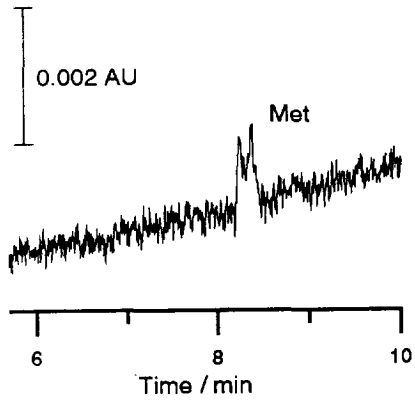

Fig. 3. Optical resolution of PTH-DL-Met by MEKC with CHAPS without STGlu. Micellar solution, 25 m*M* CHAPS–100 m*M* SDS–1 *M* urea (pH 11.0) containing 30% (v/v) methanol. Other conditions as in Fig. 2.

for the optical resolution of PTH-DL-AAs, although the resolution characteristic was not substantially different from those with SDVal, STGlu, and DSer systems. Further investigations on chiral separations by MEKC with N-alkanoyl-L-amino acids and some other surfactants and additives are in progress.

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