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Short communication

Chiral resolution of the dansyl derivative of 2-methyltaurine by capillary electrophoresis

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Abstract

A cyclodextrin-modified micellar capillary electrophoretic method was developed for the chiral separation of 2-methyltaurine. The racemic mixture was separated after derivatization with dansyl chloride. The enantiomeric separation of the dansyl derivative was done using electrolyte solutions containing β-cyclodextrin (β-CD) or different ratios of β- and γ-CD. The mixture of β- and γ-CD optimally resolved the compound studied.

Keywords: Enantiomer separation; 2-Methyltaurine; Taurine

1. Introduction

Chiral drugs may interact differently with biological components able to discriminate between enantiomers. Hence asymmetric synthesis and chiral analysis are becoming increasingly important in drug research. Currently, the methods of choice for chiral separations are HPLC and GC. Supercritical fluid chromatography is a new technique that is still in the developmental phase. Capillary electrophoresis (CE) has been found to be a powerful alternative for chiral separations [1].

Taurine (2-aminoethanesulfonic acid) is one of the most abundant low-molecular-mass organic constituents in mammals [2]. Considerable insight into the enigmatic and many-sided biological role of this compound has been achieved, but its mechanisms are not yet understood.

As a part of an on-going program aimed at investigating the effects of systematic variations of substituents on taurine activity, some of us are synthesizing several taurine derivatives. Among these compounds, 2-methyltaurine (2-aminopropanesulfonic acid) was studied. Earlier work had shown that only the (*S*)-enantiomer mimicked the hypotensive effect of taurine when injected into cerebral ventricles in rats [3]. Therefore, chiral separation of the latter compound is important for an accurate pharmacological evaluation of this and other taurine analogues.

Enantiomeric separation of amino acids can be carried out by HPLC, TLC, GC and more recently by CE. In this study a CD-modified micellar capillary electrophoretic method was developed, which allows the chiral separation of 2-methyltaurine.

This principle was introduced by Terabe et al. [4] and called CD-modified micellar electrokinetic chromatography (CD-MEKC). These authors applied this approach to the separation of highly hydrophobic

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compounds using CD together with the surfactant, sodium dodecyl sulfate (SDS). In this technique, the analyte is partitioned among three phases; the aqueous bulk phase, the free CD and a CD–SDS mixed micelle, which are to be regarded as pseudophases. Recently, a new general treatment for such three-pseudophase systems has been given by Rundlett and Armstrong [5].

Terabe et al. [6] reported the resolution of dansyl derivatives of amino acids using this principle. Dansyl chloride, which is an established derivatization reagent for amino acids, increases the hydrophobicity of the compounds. This paper deals with the application of this technique to the resolution of the enantiomers of 2-methyltaurine after derivatization with dansyl chloride using β-CD, or different ratios of β-CD and γ-CD together with SDS, as additives to the electrolyte.

2. Experimental

2.1. Reagents and chemicals

2-Methyltaurine it not available commercially and was synthesized according to the method described by Lambert and Rose [7].

All reagents were of analytical grade if not otherwise indicated. Acetonitrile, methanol (additionally twice distilled) were purchased from Merck (Darmstadt, Germany). β-CD and γ-CD were obtained from Chinoin Pharmaceutical and Chemical Works (Budapest, Hungary). Sodium dodecyl sulfate (SDS), sodium hydroxide, dansyl chloride, sodium carbonate, boric acid, ammonia solution and dimethyl sulfoxide were obtained from Fluka (Buchs, Switzerland). Water was deionized and double-distilled.

Fused-silica capillaries were purchased from Supelco (Bellefonte, PA, USA).

2.2. Sample preparation

For the derivatization of 2-methyltaurine, 1 ml of the reactant solution of dansyl chloride in acetonitrile (1 mg/ml) was rapidly added to 2 ml of the aminosulfonic acid solution (0.3 mg/ml) in 40 m*M* sodium carbonate buffer (adjusted to pH 9.5 with 2 *M* HCl). The mixture was stirred for 2 min and then analyzed by CE after storing for 1 h at room temperature.

A 100-ml volume of 0.1 *M* borate buffer solution was prepared by dissolving 0.618 g of boric acid in water–methanol (80:20, v/v) and the pH was adjusted to 8.6 with 2 *M* NaOH. Samples and buffer solutions were filtered through a 0.45-μm pore size filter (Schleicher/Schuell, Dassel, Germany) and degassed with helium 5.0.

2.3. Equipment

All separations were carried out using a Prince capillary electrophoresis instrument (Lauerlabs, Netherlands), equipped with an on-column UV detector (Lambda 1000, Bischoff Analysentechnik, Leonberg, Germany).

Separations were carried out at room temperature in a fused-silica capillary tube (80 cm × 75 μm I.D., effective length was 71 cm). Samples were injected hydrodynamically (0.005 MPa) for 6 s from the anodic end.

An Axxiom 737 system (Moorpark, CA, USA) was used for data acquisition.

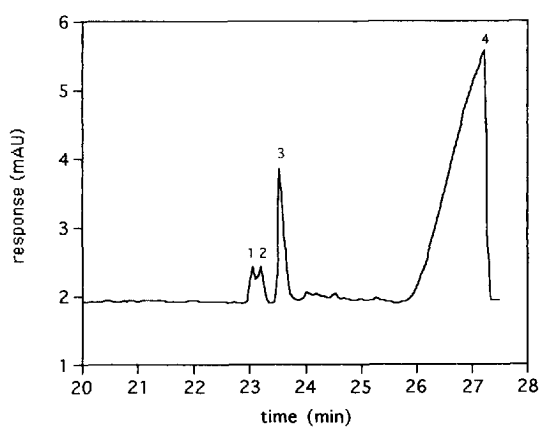

Fig. 1. Electropherogram of the partial chiral separation of the dansyl derivative of 2-methyltaurine. Electrolyte solution, 0.1 *M* SDS, 60 m*M* β-CD, 0.1 *M* borate buffer in water–methanol (80:20, v/v), pH 8.6; Applied voltage, 20 kV. Peaks: 1 and 2 = enantiomers of dansylated 2-methyltaurine; 3 and 4 = 5-dimethylaminonaphthalene-1-sulfonic acid and 5-dimethylaminonaphthalene-1-sulfonamide, respectively.

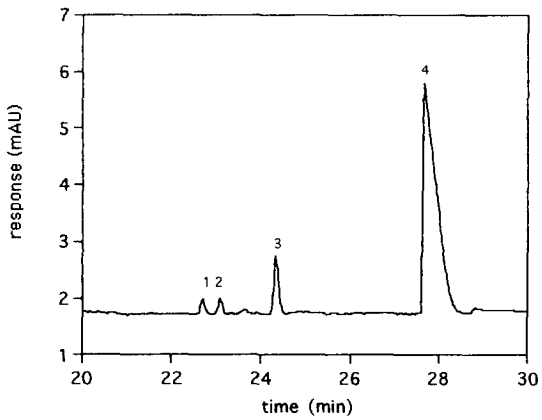

Fig. 2. Electropherogram of the enantiomeric separation of dansylated 2-methyltaurine. Electrolyte solution, 0.1 *M* SDS, 60 m*M* β-CD and 10 m*M* γ-CD, 0.1 *M* borate buffer in water–methanol (80:20, v/v), pH 8.6. Applied voltage, 20 kV. 1 and 2 = enantiomers of dansylated 2-methyltaurine; 3 and 4 = 5-dimethylaminonaphthalene-1-sulfonic acid and 5-dimethylaminonaphthalene-1-sulfonamide, respectively.

Table 1
Resolution factors (R_s) and separation factors ($\alpha = t_2/t_1$) of the dansyl derivative of 2-methyltaurine.

	α	R_s
0 m*M* γ-CD–60 m*M* β-CD	1.005	0.75
10 m*M* γ-CD–60 m*M* β-CD	1.020	1.75

2.4. Methods

The fused-silica capillary used in this study was conditioned by successive washings for 10 min each with 0.1 *M* sodium hydroxide, water and finally the electrolyte solution. After daily use, the capillary was washed for 10 min each with 0.1 *M* sodium hydroxide and water.

The potential during analysis was 20 kV (current 40 μA) and the substances were detected by UV absorption at 254 nm. The composition of the electrolyte solution was: 0.1 *M* SDS, varying ratios of β- and γ-CD, 0.1 *M* borate buffer in water–methanol (80:20, v/v), pH 8.6.

Electroosmotic flow (EOF) was determined by sampling a solution of dimethyl sulfoxide (1:10, v/v) in water. Effective electrophoretic mobilities were calculated by subtracting the EOF mobility from the measured mobilities.

3. Results and discussion

The tested β-CD–γ-CD ratios were 60:0; 60:5, 60:10 in a micellar solution of 0.1 *M* SDS, 0.1 *M* borate buffer (water–methanol, 80:20, v/v), pH 8.6.

An electrolyte solution containing only 60 m*M* β-CD resulted in partial resolution of the dansyl derivatives of 2-methyltaurine (Fig. 1), whereas complete resolution was obtained when a 60:10 mixture of β-CD and γ-CD was used (Fig. 2, Table 1).

The migration times of the first enantiomer were similar for all ratios of β- and γ-CD tested, while the migration time for the second enantiomer increased with increasing amounts of γ-CD (Table 2).

The electropherograms (Fig. 1 and Fig. 2) showed, in addition to the two peaks identified as the enantiomers of the dansyl derivatives of 2-methyltaurine, two other peaks which proved to correspond to the by-products 5-dimethylaminonaphthalene-1-sulfonamide and the corresponding acid, formed during the reaction [8].

This result underlines that β-CD has cavity sizes suitable for inclusion of this dansyl derivative, but γ-CD was shown to be more effective.

Table 2
Migration times (t), electrophoretic mobilities (μ) and effective electrophoretic mobilities ($\mu_{eff} = \mu - \mu_{EOF}$) of the first and of the second enantiomer in the absence and in the presence of γ-CD

	Enantiomer	t (min)	μ ($\times 10^{-2}$) ($cm^2/V \cdot min$)	μ_{eff} ($\times 10^{-2}$) ($cm^2/V \cdot min$)
0 m*M* γ-CD–60 m*M* β-CD	1	22.69	1.252	−0.148
	2	22.83	1.244	−0.156
10 m*M* γ-CD–60 m*M* β-CD	1	22.56	1.259	−0.141
	2	23.12	1.228	−0.172

$\mu_{EOF} = 1.40 \cdot 10^{-2}$ $cm^2/V \cdot min$.

These facts are in accordance with observations reported by Terabe et al. [6] who interpreted this phenomenon by co-inclusion of a monomeric surfactant molecule together with the analyte molecule.

Partition of the analyte between the bulk aqueous phase, the CD and the CD–SDS mixed micelle, which are pseudophases, is assumed to be the basic mechanism. The analyte is negatively charged and tends to migrate in the opposite direction to the EOF, however, upon complexation with CD it is transported faster to the cathode. As free CD is electrically neutral, it migrates at the same speed as the bulk solution. Since SDS is negatively charged, the velocity of the CD–SDS mixed micelle is lower than that of the EOF. Complexation of the analyte with CD therefore retards the migration of the analyte.

γ-CD showed a higher affinity for one enantiomer, probably due to a stronger inclusion complexation. Since the CD associates with the micelle and is therefore retarded, the stronger complexing enantiomer migrates slower.

The influence of organic modifiers was also investigated. Addition of 20% methanol resulted in a marked enhancement of enantioselectivity [9].

In conclusion, CD-MEKC proved to be very effective in the chiral separation of 2-methyltaurine dansyl derivative. The use of a mixture of β- and γ-CD gave optimal resolution of the enantiomers studied, which cannot be separated by using only β-CD.

The present method represents a simple and less expensive alternative to HPLC for the chiral separation of various aminosulfonic acids of pharmacological interest.

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