Talanta ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at SciVerse ScienceDirect

Talanta

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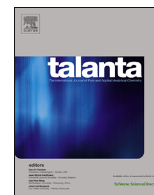

On-line sample preconcentration by sweeping and poly(ethylene oxide)-mediated stacking for simultaneous analysis of nine pairs of amino acid enantiomers in capillary electrophoresis

En-Ping Lin [a], Kai-Cheng Lin [b], Chia-Wei Chang [a], Ming-Mu Hsieh [a,*]

[a] Department of Chemistry, National Kaohsiung Normal University, Taiwan
[b] Department of Emergency Medicine, Division of Traumatology, Kaohsiung Veterans General Hospital, Taiwan

ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form
15 May 2013
Accepted 16 May 2013

Keywords:
Capillary electrophoresis
Amino acid
Chiral separation
9-fluorenylmethylchlorofrormate
Stacking
Polyethylene oxide

ABSTRACT

This study proposes a sensitive method for the simultaneous separation and concentration of 9 pairs of amino acid enantiomers by combining poly(ethylene oxide) (PEO)-based stacking, β-cyclodextrin (β-CD)-mediated micellar electrokinetic chromatography (MEKC), and 9-fluoroenylmethyl chloroformate (FMOC) derivatization. The 9 pairs of FMOC-derivatized amino acid enantiomers were baseline separated using a discontinuous system, and the buffer vials contained a solution of 150 mM Tris-borate (TB), 12.5% (v/v) isopropanol (IPA), 0.5% (w/v) PEO, 35 mM sodium taurodeoxycholate (STDC), and 35 mM β-CD, and the capillary was filled with a solution of 1.5 M TB, 12.5% (v/v) IPA, 35 mM STDC, and 35 mM β-CD. Based on the difference in viscosity between the sample zone and PEO solution and because of the STDC sweeping, the discontinuous system effectively stacked 670 nL of the 9 pairs of FMOC-derivatized amino acid enantiomers without losing chiral resolution. Consequently, the limits of detection for the 9 pairs of FMOC-derivatized amino acid enantiomers were reduced to 40–60 nM. This method was successfully used to determine D-Tryptophan (Trp), L-Trp, D-Phenylalanine (Phe), L-Phe, D-Glutamic acid (Glu), and L-Glu in various types of beers.

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1. Introduction

Amino acids are one of the most important biological compounds because they are ubiquitous in biological fluids, food product, and plant. All amino acids are chiral molecules except for glycine. Recently, D-amino acids are found to play an important role in fermented foods [1–3], such as beer, wine, vinegar, cheese, yogurt, fermented milk and fish sauce. For example, in fermented foods, D-alanine (D-Ala), D-glutamic acid (Glu), D-phenylalanine (Phe), and D-aspartic acid (Asp) provide important information about adulteration and quality of food products [4]. Also, the level of D-Glu, D-serine (ser), and D-Asp in cerebrospinal fluid were highly implicated with mental diseases, such Alzheimer's disease and Parkinson's disease [5,6]. Due to their biological and clinical significance, a rapid, convenient, and sensitive method is required for routine analysis of D-amino acids in food and human body fluids.

Current methods for the determination of chiral amino acids in biological samples include gas chromatography [7,8], high performance liquid chromatography [9,10], and supercritical fluid chromatography [11,12]. However, the high cost chiral stationary phase is a prerequisite of these separation techniques. Capillary electrophoresis (CE) is an alternative method for determining amino acid enantiomers that has the advantages of small injection volumes of the sample, low solvent consumption, rapid analysis, and high separation efficiency [13]. Two major modes of CE, including capillary zone electrophoresis (CZE) [14,15] and micellar electrokinetic chromatography (MEKC) [16,17] are widely used for the separation of chiral amino acids. However, amino acids lack chromophores and fluorophores, except for 3 aromatic amino acids (tryptophan (Trp), tyrosine, and Phe). Various reagents, such as o-phthaldialdehyde (OPA) [18], naphthalene-2,3-dicarboxaldehyde (NDA), [19] fluorescein isothiocyanate (FITC) [20], dansyl chloride [21], and 9-fluoroenylmethyl chloroformate (FMOC) [22], have been synthesized for the derivatization of amino acids. Among these organic dyes, FMOC has distinct advantages, including low cost of chemicals, a short reaction time, high stable derivatives, and high yield derivatives [23]. Furthermore, Chen et al. achieved the simultaneous baseline separation of 9 pairs of FMOC-derivatized amino acid enantiomers using a mixture of β-cyclodextrin (β-CD) and sodium taurodeoxycholate (STDC) [24]. Han and Chen used β-CD-mediated MEKC to separate a mixture of 8 pairs of FMOC-derivatized amino acid enantiomers [22]. Although FMOC derivatization is beneficial to the chiral separation of amino acid enantiomers, the detection of FMOC-derivatized amino acids using CE with UV absorbance results in poor sensitivity.

Numerous concentration methods, including field-amplified sample stacking (FASS) [19,25], acetonitrile stacking [26], large volume sample stacking (LVSS) [27,28], single-drop microextraction [29], CD-

* Correspondence to: Department of Chemistry, National Kaohsiung, Normal University, No.62, Shenjhong Rd., Yanchao District, Kaohsiung City, Taiwan.
Tel.: +886 7 717 2930x7162; fax: +886 7 6051083.
E-mail address: t3644@nknucc.nknu.edu.tw (M.M. Hsieh).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.05.039

sweeping [30] and sweeping [31], have been used to enhance the sensitivity of amino acid enantiomers in the CE methods. For example, 3 pairs of DL-amino acids were baseline separated by combining LVSS-CD-mediated MEKC with FITC derivatization [27]. Kirschner, et al. baseline separated four pairs of NDA-derivatized amino acid enantiomers using sulfated β-CD as the chiral selector with a low pH and reverse polarity [30]. Recently, the use of poly (ethylene oxide) (PEO) as an additive in CE is well-suited for the on-line stacking and separation of amino acids in [32–36]. This stacking mechanism is chiefly enabled by the viscosity difference between the PEO and sample solution, and because of the local electric field difference between the TB buffer and sample solution. This stacking method provides an approximately 100 to 1000-fold improvement in sensitivity. The combination of PEO-based stacking and hydroxypropyl-β-CD-mediated MEKC was developed for the on-line concentration and separation of 3 pairs of amino acid enantiomers in various biological fluids [19]. Although the mentioned methods have high sensitivity toward amino acid enantiomers, the simultaneous separation and stacking of numerous amino acid enantiomers is challenging.

This study proposes a combined PEO-based stacking method, β-CD-mediated MEKC, and FMOC derivatization for the on-line concentration and separation of 9 pairs of amino acid enantiomers. The PEO, β-CD, STDC, and FMOC were the concentrating media, a chiral selector, a pseudostationary phase, and a resolution enhancer, respectively. We evaluated the effects of the derivatizing agent, β-CD, STDC, PEO, and injection volume on the separation and stacking of FMOC-derivatized amino acid enantiomers.

2. Experimental

2.1. Chemicals and preparation

The proteinogenic D-, L-Asparagine (Asn), D-, L- Histidine (His), D-, L-Valine (Val), D-, L-Leucine (Leu), D-, L-Trp, D-, L-Phe, D-, L- Glu, D-, L-Asp, D-, L-Lysine (Lys), isopropanol (IPA), 9-fluorenylmethylchloroformate (FMOC), sodium taurodeoxycholate (STDC), β-CD, poly(ethylene oxide) (PEO) (M_w 8,000,000 g/mol), NaOH, methanol, acetonitrile (ACN), O-phthalaldehyde (OPA) and FMOC were obtained from Sigma-Aldrich (St Louis, MO, USA). Naphthalene-2,3-dicarboxaldehyde (NDA) was purchased from Tokyo Chemical Industry (Tokyo, Japan) and prepared in ACN. Tris and isopropanol (IPA) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Boric acid was purchased from Riedel-deHaën (Buchs, Switzerland). A stock solution of 2000 or 400 mM Tris-borate (TB) buffer was prepared by dissolving 121.15 or 24.23 g of Tris in 500-mL aqueous solution that was adjusted with suitable amounts of boric acid range from pH 8.5 to 10.0. Unless otherwise noted, the molarity of Tris represents that of TB buffer. The PEO (0.1−1.0% w/v) was gradually added to each of prepared 150 mM TB solutions at pH 8.5. During the addition of PEO, a magnetic stirring rod was used to produce a well-homogeneous suspension. After the addition was completed, the solutions were stirred for at least 12 h. Prior to use for CE separation, the solutions were degassed with a vacuum system in an ultrasonic tank for 10 min [37]. All the prepared solutions were stored at 4 °C and used within a week. All other chemicals were of analytical grade.

2.2. Instrument

A commercial UV absorbance detector (ECOM, Germany) was performed at 260 nm for analytes. Electrophoresis was driven by a high-voltage power supply (Bertan, Hicksville, NY, USA). The high-voltage end of the separation system was put in a laboratory-made plexiglass box for safety. Data acquisition (10 Hz) and control were performed using DataApex Software (DataApex, Prague, Czech Republic). The fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 50 cm length (10-cm to detector) with 75 μm I.D. and 365 μm O.D.

2.3. Precolumn derivatization

The derivatization of amino acid with FMOC, OPA, and NDA was performed according to the previous study [19,22,38]. Briefly, FMOC (300 μL, 10 mM) reacted with amino acid (300 μL, 0.4 −200 μM) in 70 mM borate buffer (pH 9.0) at room temperature for 2 min. For OPA derivatization, a solution (15 μL) containing OPA (4 mM) and 2-ME (4 mM) reacted with amino acid (10 μL, 1 mM) in 75 μL sodium tetraborate buffer (100 mM, pH 9.3) at room temperature for 2 min. For NDA derivatization, a solution (500 μL) containing 10 mM $Na_2B_4O_7$ (50 μL), 100 μM NaCN (50 μL), and 25 mM NDA was incubated with amino acid (5 μL, 10 mM) at room temperature for 30 min. The derivatized amino acid was detected by the proposed method.

2.4. On-line concentration and separation

Before CE analysis, the capillaries (Polymicro Technologies, Phoenix, AZ, USA; 75 μm I.D. and 360 μm O.D.) were treated with 1 M NaOH solution overnight to obtain a high electroosmotic flow (EOF) [19]. The capillary was filled with a solution containing 150∼2000 mM TB, 10–50 mM STDC and/or 10–50 mM β-CD, while the sample was injected by raising the capillary inlet 20-cm height for a period of time up to 240 s. Subsequently, the ends of the capillary were immersed in the cathodic and anodic vials containing 150 mM TB, 10–50 mM STDC, 10–50 mM β-CD, and/or 0.5% w/v PEO solutions. After separation, PEO molecules adsorbed on the capillary wall were flushed out. The capillary was re-equilibrated with 1 M NaOH at 1 kV for 10 min for the next run. Chang's group has reported that the EOF and migration time of analyte is highly reproducible when the capillary have been treated with 0.5 M NaOH [35].

2.5. Analysis of DL-amino acids in biological samples

Three kinds of beer (Taiwan, Japan, Netherlands, USA) were purchased from local market. We spiked samples of beer with standard solution (4-12 μM) of DL-amino acids and derivatized the resulting solution with FMOC at room temperature for 2 min. Note that samples of beer were diluted to 10-fold through sample preparation procedure. The derivatized amino acid was detected by the proposed method.

3. Results and discussion

3.1. Role of derivatizing agent in β-CD-mediated MEKC

The resolution of D- and L-amino acids is highly dependent on the type of derivatizing agent [19,24,39]. We explored how 3 types of derivatizing agents, OPA, NDA, and FMOC, affected the resolution of D- and L-amino acids when β-CD-mediated MEKC was used for chiral separation in the presence of 0.5% w/v PEO. Fig. 1A shows that the chiral separation of a mixture of DL-Trp and DL-Phe was unsuccessful in the mode of the β-CD-mediated MEKC. A similar phenomenon was observed with the use of OPA for the derivatization of the same amino acids (Fig. 1B). The chiral separation of the DL-Trp and DL-Phe was accomplished when NDA and FMOC were used instead of OPA (Fig. 1C and D). Fig. 1 shows the following migration time trends: FMOC-derivatized amino acid > NDA-derivatized amino acid > OPA-derivatized amino acid > underivatized amino acid. This

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