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Enantioselective capillary electrophoresis-mass spectrometry of amino acids in cerebrospinal fluid using a chiral derivatizing agent and volatile surfactant

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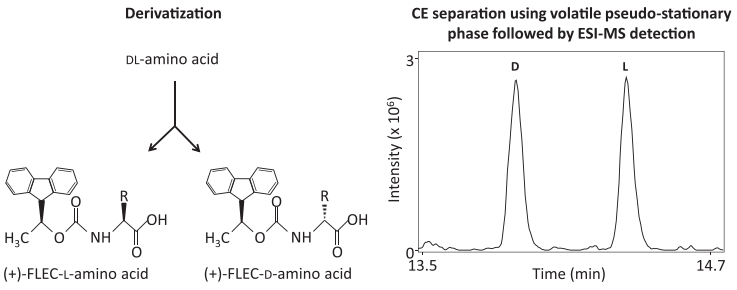
HIGHLIGHTS

- New chiral CE-MS method providing enhanced compatibility and sensitivity is presented.
- Enantioseparation and selective detection of 14 proteinogenic DL-AAs is achieved.
- Aspartic acid and glutamic acid are detected but not enantioseparated.
- Endogenous levels of D-amino acids in cerebrospinal fluid can be detected.

GRAPHICAL ABSTRACT



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ABSTRACT

The sensitivity of coupled enantioselective capillary electrophoresis-mass spectrometry (CE-MS) of amino acids (AAs) is often hampered by the chiral selectors in the background electrolyte (BGE). A new method is presented in which the use of a chiral selector is circumvented by employing (+)-1-(9-fluorenyl)ethyl chloroformate (FLEC) as chiral AA derivatizing agent and ammonium perfluorooctanoate (APFO) as a volatile pseudostationary phase for separation of the formed diastereomers. Efficient AA derivatization with FLEC was completed within 10 min. Infusion experiments showed that the APFO concentration hardly affects the MS response of FLEC-AAs and presents significantly less ion suppression than equal concentrations of ammonium acetate. The effect of the pH and APFO concentration of the BGE and the capillary temperature were studied in order to achieve optimized enantioseparation. Optimization of CE-MS parameters, such as sheath-liquid composition and flow rate, ESI and MS settings was performed in order to prevent analyte fragmentation and achieve sensitive detection. Selective detection and quantification of 14 chiral proteinogenic AAs was achieved with chiral resolution between 1.2 and 8.6, and limits of detection ranging from 130 to 630 nM injected concentration. Aspartic acid and glutamic acid were detected, but not enantioseparated. The optimized method was applied to the analysis of chiral AAs in cerebrospinal fluid (CSF). Good linearity ($R^2 > 0.99$) and acceptable peak area and electrophoretic mobility repeatability (RSDs below 21% and 2.4%, respectively) were achieved for the

Abbreviations: AA, amino acid; Ala, alanine; APFO, ammonium perfluorooctanoate; Arg, arginine; Asn, asparagine; Asp, aspartic acid; CSF, cerebrospinal fluid; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Ser, serine; SL, sheath liquid; Thr, threonine; Trp, tryptophan; Val, valine.

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chiral proteinogenic AAs, with sensitivity and chiral resolution mostly similar to obtained for standard solutions. Next to L-AAs, endogenous levels of D-serine and D-glutamine could be measured in CSF revealing enantiomeric ratios of 4.8%–8.0% and 0.34%–0.74%, respectively, and indicating the method's potential for the analysis of low concentrations of D-AAs in presence of abundant L-AAs.

1. Introduction

Recently, the chiral analysis of endogenous compounds, and in particular of amino acids (AAs) in human biofluids, has gained increasing attention. Initially, D-AAs were considered to origin from intestinal bacteria, but later studies showed that D-AAs may be naturally occurring and physiologically active substances in mammals [1–4], and their biological functions have been studied [5–9]. Aberrant D-AAs concentrations in human tissues and biofluids were reported to be related with diseases, such as chronic renal failure [2], schizophrenia [10] and Alzheimer [11]. The full involvement of D-AAs in physiopathological processes is not yet well understood. Research in this field relies on the availability of analytical methodologies exhibiting adequate enantioselectivity, chemical selectivity and sensitivity for the detection of the commonly very low levels of D-AAs next to abundant L-AAs in complex mixtures, such as biofluids.

Several electrophoretic and chromatographic techniques for chiral separation of AAs have been published, as reviewed by Waldhier et al. [12]. Among these, capillary electrophoresis (CE) has the advantages of high peak efficiency and resolution, relatively fast separation, and low sample and reagent consumption. For the chiral analysis of AAs in complex mixtures, such as body fluids [13–15], hyphenation of the enantioselective technique to electrospray ionization-mass spectrometry (ESI-MS) is indicated in order to reliably assign signals from enantiomers. Enantioseparation by CE can be achieved by using chiral selectors, such as cyclodextrins (CDs), in the background electrolyte (BGE). The different affinities of the respective enantiomers towards the chiral selector will allow their separation. Chiral CE-MS employing CDs in the BGE have been reported for the enantioselective analysis of AAs in soy, vinegar, orange juice, fertilizers and CSF [16–20]. However, chiral selectors such as CDs and vancomycin are often not well compatible with MS analysis, as they may cause suppression of ESI. As reviewed by Shamsi [21], several procedures have been developed in order to prevent chiral selectors from entering the ion source, such as partial-filling techniques [22–27] or use of reverse-migrating selectors [28–35]. With these adapted CE systems AA-specific optimization is often needed and overall chiral separation may be compromised.

In an alternative approach for achieving chiral separation by CE, analyte enantiomers are first derivatized with a chiral agent, enabling the separation of the formed diastereomers under non-chiral conditions. As no chiral selectors are needed, such an enantioselective method potentially renders more favorable conditions for the hyphenation to MS. The chiral agents (+)- or (−)-1-(9-fluorenyl)ethyl chloroformate (FLEC) can be used for the derivatization of AAs enantiomers to form diastereomers. FLEC reacts quickly at room temperature with primary and secondary amines to form highly stable derivatives. CE analysis of FLEC-derivatized carnitine, a non-proteinogenic AA, has been reported previously [36,37] showing its chiral separation with LODs in the μM range. As FLEC diastereomers of an AA have the same charge and mass, their CE resolution commonly will require the addition of a surfactant to the BGE above its critical micelle concentration (CMC), allowing separation by micellar electrokinetic chromatography (MEKC). Chan et al. [38] reported the chiral analysis of seven (+)-FLEC-derivatized proteinogenic AAs using BGEs of sodium phosphate or sodium borate containing 25 mM sodium dodecyl sulfate (SDS) as pseudo-stationary phase, achieving LODs in the nM range using laser-induced fluorescence detection. Recently, we reported a CE-UV method employing in-capillary derivatization with (−)-FLEC for the chiral separation of 13 proteinogenic AAs using a BGE comprising 40 mM sodium borate and 21 mM SDS [39]. However, in order to achieve unambiguous detection of AAs in biological samples, MS detection is highly preferable.

MEKC is commonly performed with nonvolatile surfactants, such as SDS, which would cause serious suppression of analyte signals and ion-source contamination during ESI-MS detection [40,41]. Compatibility of MEKC and MS can be improved by employing alternative ionization techniques, such as atmospheric pressure photoionization or chemical ionization [40,42], or by the use of (semi)volatile surfactants, such as ammonium perfluorooctansulfonate or ammonium perfluorooctanoate (APFO). The feasibility of the latter approach has been demonstrated for the analysis of drugs [43] and pesticides [44,45], showing good analyte resolution and sensitivity in CE-MS. Recently, Moreno-Gonzalez et al. [46] showed the feasibility of using APFO as a MS-compatible surfactant for the non-chiral MEKC-MS analysis of AAs.

In the present work, we studied the combination of (+)-FLEC derivatization of AAs and use of APFO in order to achieve good compatibility between chiral CE and ESI-MS. CE was coupled to positive-mode ESI-MS using sheath-liquid interfacing. The effect of APFO on FLEC-AA MS signal intensities was studied by infusion experiments. CE separation conditions, interface settings, and MS parameters were optimized, and linearity, LODs, and electrophoretic mobility and peak area precision were assessed. The applicability of the proposed method for the selective determination of D-amino acids in biofluids was evaluated by the analysis of human cerebrospinal fluid (CSF).

2. Materials and methods

2.1. Reagents and materials

All reagents were of analytical grade. Isopropanol, ammonium acetate, formic acid and acetonitrile were supplied by Fluka (Steinheim, Germany). Perfluorooctanoic acid (96%), (+)-1-(9-fluorenyl)ethyl chloroformate solution (18 mM), ammonium hydroxide (28%, w/v), sodium tetraborate and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was deionized and purified with a Milli-Q purification system (Millipore, Belford, NJ, USA). *N*-Acetyl-DL-tryptophan, the DL-AAs tyrosine, alanine, valine, phenylalanine, tryptophan, histidine, arginine, lysine, aspartic acid, isoleucine, methionine, leucine, asparagine, serine, glutamic acid, glutamine, cysteine, glycine, proline and threonine were supplied by Sigma-Aldrich.

Aqueous solutions of perfluorooctanoic acid were adjusted to the desired pH with 14.2 M ammonium hydroxide. Stock DL-AA solutions of 1 mg mL^{-1} were prepared in deionized water. Working