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Review

Recent advances in chiral separation of amino acids using capillary electromigration techniques

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ABSTRACT

This review highlights recent progresses in the chiral recognition and separation of amino acid enantiomers obtained by capillary electromigration techniques, using different chiral selectors and especially cyclodextrins, covering the literature published from January 2010 to March 2014. Sections are dedicated to the use of derivatization reagents and to the possibility to enantioseparate underivatized amino acids by using either ligand exchange capillary electrophoresis (LECE) and capillary electrophoresis (CE) coupled on line with mass spectrometry. A short insight on frontier nanomaterials is also given.

Contents

1. Introduction

Chiral molecules in nature exist almost exclusively as single enantiomers, a property that is critical for molecular recognition and replication processes and would thus seem to be a prerequisite for the origin of life [1]. Amino acids (AAs) are also chiral and until 50 years ago, scientists believed that only their L forms are of relevance in living organisms [2]. However studies in the last decades [3,4] have shown that D-AAs are widely present in the tissues of higher organisms, including humans, either as products of the vital activities of endogenous flora or during spontaneous racemization of L-AAs in the structure of polypeptides during ageing [5,6], as well as endogenous active substances and biomarkers [7,8]. On the other hand, alterations in D-amino acid concentrations may be related to different pathological states, as in Alzheimer's disease [9,10].

Foodstuffs are the most significant sources of non-natural D-amino acids. In fact, modern food industry technology applies a different range of procedures to modify the characteristics of proteins to improve the flavour, consistency, and nonperishability of food and thus D-AAs generated during technological processing are found in commercial foodstuffs and alcoholic beverages. The

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presence of free D-AAs in foodstuff is an indication of microbial contamination, making these compounds as indicators of food quality [11]. In fact, even a minor degree of racemization on the proteins' AAs is the cause of a reduced digestion of such proteins impairing the nutritional quality of an edible product. Detection of D-AAs in foodstuff also allows to assess authenticity and adulteration of foods and beverages [12], either as regard distinction between wild and transgenic materials [13,14].

Thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and gas chromatography (GC) have been extensively used for the enantiomeric separation of single L-amino acids and their corresponding D-enantiomers, giving rise to high resolution values and detectability of amino acids in picomoles and femtomoles [15]. However nowadays there is no doubt that Capillary Electrophoresis (CE) has evolved as an attractive and powerful separation technique providing high separation efficiency and short migration times and needing low sample volumes [16,17]. In this field the use of single isomer chiral selectors [18] and the deep knowledge of solution equilibria involved in the separation processes have made possible a fine tuning of the experimental conditions used, allowing significant improvements in the chiral analysis of amino acids, especially if chiral separations exploit the formation of metal complexes, as in ligand exchange capillary electrophoresis (LECE), where the solution equilibria involved are well known in numerous cases [19].

This review focuses on recent results in the chiral separation of amino acids by capillary electromigration techniques, covering the literature published from January 2010 to March 2014. To make easier the discussion of the literature data we have divided them in four different sections dedicated to derivatized amino acids, ligand exchange capillary electrophoresis, CE coupled with mass spectrometry (MS) and nanomaterials in capillary electromigration techniques, respectively. We have summarized the different experimental conditions and performances in Table 1, which describes the different developments of the chiral separation of amino acids that were published in the investigated time interval.

2. Derivatized amino acids

Enantiorecognition of amino acids by capillary electromigration techniques is accomplished in most cases on their derivatives. In fact, amino acids lack chromophores and fluorophores, except for the aromatic amino acids tryptophan, tyrosine and phenylalanine and in less extent histidine. Several reagents, such as o-phthaldialdehyde (OPA), benzoylchloride, naphthalene-2,3-dicarboxaldehyde (NDA), fluorescein isothiocyanate (FITC), dansylchloride (Dns), and 9-fluoroenylmethylchloroformate (FMOC) have been synthesized for the derivatization of amino acids. This introduces an additional analytical step, but offer interesting advantages during the analysis of these molecules. Besides the sensitivity enhancement achieved by using adequate probes together with fluorescence detection, derivatization frequently facilitates the chiral separation of amino acids. In fact, enantiorecognition based on macrocyclic receptors involves multimodal interactions via hydrogen bonding, π–π interactions, steric hindrance, hydrophobic interactions and so on. For derivatized amino acids the inclusion in and the interaction with the chiral selector becomes more discriminating not only due to the increase in size but also due to the new interactions involving the label.

Several derivatization labels and techniques have been employed. Jiang et al. [20] exploited the inclusion properties of a class of macrocyclic antibiotics achieving the enantioseparations of a series of N-benzoylated derivatives of four amino acids using a CE method, which combined the partial filling technique with the dynamic coating technique and the co-EOF

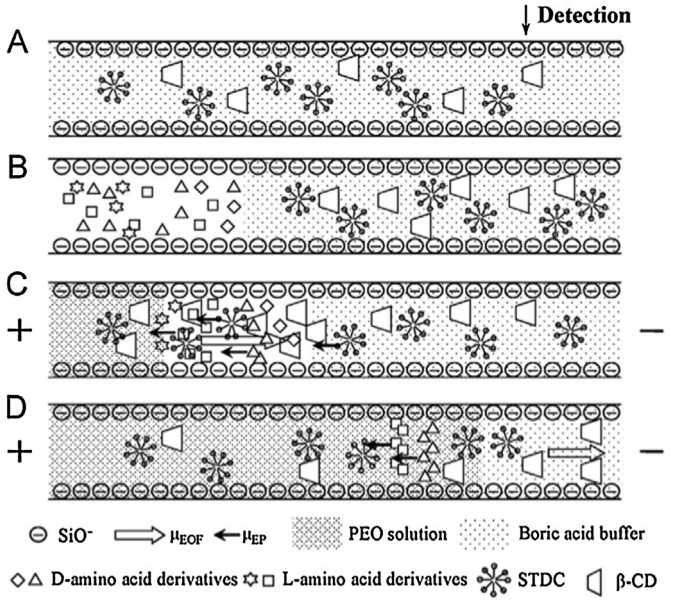

Fig. 1. Evolution of analyte zone in the separation and stacking of neuro-chemicals. (A) Filling of capillary with 1.5 MTB (pH 10) containing 12.5% (v/v) IPA; (B) injection of a large volume of anlaytes solution; (C) stacking of 16 analytes by PEO solution (150 mM TB pH 8.5, 35 mM STDC, 35 mM β-CD and 12.5% (v/v) IPA); (D) separation of the stacking CBI-DL-amino acids PEO. The μEOF and μEP represent the EOF mobility and the electrophoretic mobilities of cationic and anionic neurochemicals, respectively. The detection wavelength was set at 260 nm [23].

electrophoresis technique, highlighting the important role of non covalent interactions in the enatiorecognition process. Enantioseparation of 28 N-benzoylated amino acids was also investigated using the partial filling technique (PFT) on both polycationic polymer hexadimethrine bromide (HDB) modified capillary and eCAP neutral capillary, respectively and bromobalhimycin as CE additive [21]. In the eCAP neutral capillaries 26 of 28 tested racemic amino acid derivatives were almost baseline resolved without observing any interference from the front of the bromobalhimycin plug.

Due to low cost of chemicals, short reaction times, high stable derivatives, and high yield derivatives, the FMOC group is one of the most useful labelling groups for α-amino acids, providing also the advantage of high sensitivity in fluorescence detection. Wang et al. [22] developed a highly sensitive method for enantioseparation of trace fenoprofen and amino acid derivatives by capillary electrophoresis with vancomycin as the chiral selector. By combining different techniques, i.e. large-volume sample stacking using EOF pump with anion-selective exhaustive injection (LVSEP–ASEI), they obtained more than 1000-fold enhancement in detection sensitivity compared with the normal injection mode. FMOC derivatization was also successfully used 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) [23]. The simultaneous use of the on-line sample preconcentration (Fig. 1) and the FMOC derivatization enabled detection at the nanomolar level (40–60 nM), as well as the determination of FMOC-derivatized DL-Trp, DL-Phe and DL-Glu in three different types of beer, demonstrating the potential of this method for food analysis.

Fluorescein isothiocyanate (FITC) is a commonly used fluorescent labelling reagent for high sensitive detection by the application of laser induced fluorescence (LIF). This derivatization has been successfully used to test the performances of microfluidic glass chips with durable modification of the channel surface with the neutral hydrophilic-coating material poly(ethylene glycol) PEG-1M-100 in microchip electrophoresis (MCE) [24]. The results showed that both