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Development of new chiral ligand exchange capillary electrophoresis system with amino acid ionic liquids ligands and its application in studying the kinetics of L-amino acid oxidase

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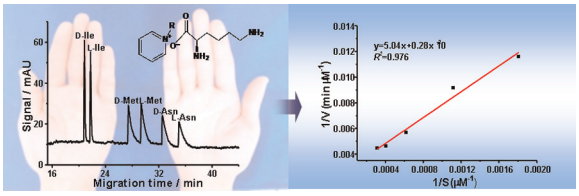
HIGHLIGHTS

- Novel amino acid ionic liquids with pyridinium as cations and L-lysine as anion were synthesized.
- These synthesized AAILs have been explored as the ligands coordinated with Zn(II) in CLE-CE system.
- The developed CLE-CE method could be used for the enantioseparation of Dns-D, L-amino acids.
- The kinetic contents of L-amino acid oxidase were investigated with the proposed CLE-CE system.

GRAPHICAL ABSTRACT



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ABSTRACT

New kinds of amino acid ionic liquids (AAILs) with pyridinium as cations and L-lysine (L-Lys) as anion have been developed as the available chiral ligands coordinated with Zn(II) in chiral ligand-exchange capillary electrophoresis (CLE-CE). Four kinds of AAILs, including [1-ethylpyridinium][L-lysine], 1-butylpyridinium][L-lysine], [1-hexylpyridinium][L-lysine] and 1-[octylpyridinium][L-lysine], were successfully synthesized and characterized by nuclear magnetic resonance and mass spectrometry. Compared with other AAILs, the best chiral separation of Dns-D, L-amino acids could be achieved when [1-ethylpyridinium][L-lysine] was chosen as the chiral ligand. It has been found that after investigating the influence of key factors on the separation efficiency, such as pH of buffer solution, the ratio of Zn(II) to ligand and complex concentration, eight pairs of Dns-D, L-AAs enantiomers could be baseline separated and three pairs were partly separated under the optimum conditions. The proposed CLE-CE method also exhibited favorable quantitative analysis property of Dns-D, L-Met with good linearity ($r^2 = 0.998$) and favorable repeatability (RSD $\leq$ 1.5%). Furthermore, the CLE-CE system was applied in investigating the kinetic contents of L-amino acid oxidase, which implied that the proposed system has the potential in studying the enzymatic reaction mechanism.

Abbreviations: AA, amino acid; CE, capillary electrophoresis; CLE, chiral ligand exchange; IL, ionic liquid; AAIL, amino acid ionic liquid; LAAO, L-amino acid oxidase; L-Lys, L-lysine; Epy, 1-ethylpyridinium; Bpy, 1-butylpyridinium; Hpy, 1-hexylpyridinium; Opy, 1-octylpyridinium; Dns-AA, dansylated amino acid; Dns-Cl, dansyl chloride; *Rs*, resolution.

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1. Introduction

Chirality is a pervasive characteristic in life process, because the chemical processes during different life phenomena are widely completed in highly asymmetric environment [1–4]. It has been established that the enantiomers have different biological and pharmacological activity in life science [5]. Thus, chiral recognition has drawn considerable attention over the past decades. Amino acids (AAs) are one of the foremost types of chiral molecules for life sciences and the enantioseparation of AAs attracts much interest. So far, several protocols have been developed for AAs enantioseparation, including gas chromatography (GC) [6], high-performance liquid chromatography (HPLC) [7,8] and capillary electrophoresis (CE) [9,10]. Compared with other techniques, CE exhibits excellent chiral separation power based on its advantages of high chiral separation efficiency, low sample loading and economical equipment [11–14]. As one of CE modes, chiral ligand exchange capillary electrophoresis (CLE-CE) has been widely applied in investigating the chiral analytes because of its outstanding advantages of easy manipulation and controllable migration order [15]. However, there are still some limitations for CLE-CE system, such as the limited chiral ligands, including AAs (such as L-Orn, L-Arg, L-Ala), organic acids (such as L-tartrate, D-saccharic acid) and some AAs derivatives (such as amino amides, L-4-hydroxyproline) which result in restricted numbers of separation objects and narrow application range. Therefore, it is highly pressing to explore more new chiral ligands for constructing efficient CLE-CE systems and broadening their application range.

Ionic liquids (ILs) are a group of organic salts with melting points close to or below room temperature. They possess several unique physical and chemical properties, including a negligibly low vapor pressure, high conductivity, and high thermal stability [16,17]. Due to these advantages of ILs, in recent years, researchers have paid more attention to design new kinds of ILs with controllable chemical and physical properties or even specific functions. Although it has reported that some kinds of chiral ILs have been synthesized and used as the chiral selectors in CE [18,19], few of them could be applied in CLE-CE system. Nevertheless, the advent of ILs can bring new ideas for researchers to explore for new chiral ligands and pave a new way for CLE-CE. As one kind of extremely representative task-specific ILs, which contains many advantages including convenient synthesis, low cost, good biocompatibility and chiral structure, amino acid ionic liquids (AAILs) have attracted much research interest. Recently, several kinds of AAILs based on imidazolium as the cations have been used for enantioseparation of D, L-AAs in CLE-CE and exhibited favorable enantioseparation efficiency [20–22]. However, the limited cations in AAILs restricted the variety of AAILs used as the chiral ligands in CLE-CE system. Thus, developing new AAILs as the chiral ligands becomes a significant and urgent assay to widen the ligand selection range and further extend their applications in CLE-CE systems.

L-amino acid oxidase (LAAO) is an enantio-selective enzyme which catalyzes the oxidative deamination of a wide range of L-AAs [23]. LAAO is usually purified from the venoms of various snake species and thought to be contributed to the toxicity of the venoms [24]. Moreover, LAAO also exhibits apoptosis inducing effects as well as antibacterial and anti-HIV activities [25]. Thus, the study for LAAO is of great significance in pharmacology for human being. It has been proved that CLE-CE was an efficient protocol in researching enzymatic reaction [26,27]. Therefore, the use of CLE-CE system to investigate the kinetics of LAAO is feasible and meaningful.

In this work, new AAILs with L-Lys as the anion and pyridinium as the cations were successfully synthesized and explored as new chiral ligands in CLE-CE for the enantioseparation of labeled AAs. As far as we know, it is the first time to introduce pyridinium as the cations of AAILs. Furthermore, the detailed characteristics of the AAILs have been performed. Among the AAILs, [1-ethylpyridinium][L-lysine] ([Epy][L-Lys]) was chosen as the optimal chiral ligand in this CLE-CE system. Moreover, the proposed CLE-CE method was applied in studying the enzyme kinetic constants of LAAO, which demonstrated the feasibility of the method in practical application.

2. Materials and methods

2.1. Chemicals

All D, L-AA enantiomers, LAAO (from crotalus atrox venom), anion exchange-resin (AMBERLITE IRA400CL), and dansyl chloride (Dns-Cl) were purchased from Sigma Chemical (St. Louis, MO, USA). 1-ethyl-pyridiniubromide ([Epy]Br), 1-butylpyridinium bromide ([Bpy]Br), 1-hexylpyridinium bromide ([Hpy]Br) and 1-octylpyridinium bromide ([Opy]Br) were obtained from Lanzhou Institute of Chemical Physics (Lanzhou Greenchem ILS, LICP, CAS, China). Lithium carbonate, Tris, boric acid, zinc sulfate, ammonium acetate, hydrochloric acid, sodium hydroxide, methanol, acetonitrile and other chemicals were purchased from Beijing Chemical Factory (Beijing, China). All the chemicals used in this work were of analytical reagent grade.

2.2. Derivatization of AAs

The dansylation of AAs was described in the previous literature [28]. Derivative solution was freshly prepared by dissolving 6.0 mg Dns-Cl in 4.0 mL acetone. Then 20 μL 40.0 mM lithiumcarbonate buffer, 20 μL Dns-Cl solution and 20 μL AA solution (2 mg mL^{-1}) were mixed in a 200 μL vial and kept at room temperature for 30 min. The derivatization reaction was terminated by addition of 5.0 mL 2% ethylamine. All Dns-AAs samples were kept at 4 °C before injection.

2.3. Synthesis of AAILs

AAILs were synthesized according to the reported literatures with proper modifications [20,29]. The synthesis process of [Epy][L-Lys] was shown as follows. Briefly, [Epy]OH aqueous solution was prepared through the anion exchange process of [Epy]Br with OH-form anion exchange resin. Then the obtained [Epy]OH aqueous solution was subsequently added drop-wise into a slightly excess equimolar L-Lys aqueous solution. The mixtures were reacted under vigorous agitation at 25 °C for 24 h and then evaporated at 55 °C in vacuo. Then, a solution of acetonitrile/methanol (9:1, v/v) was added into the solution to remove the excess L-Lys. After drying the filtrate under vacuum at 60 °C for 12 h, the final product [Epy][L-Lys] was obtained. Other AAILs were synthesized similarly as [Epy][L-Lys] except that Epy was replaced by equal mole of Bpy, Hpy and Opy. The waste solution of pyridinium and AAILs were treated by the standard operating procedures (GB8978-96, P.R. China). The structures of AAILs were confirmed by nuclear magnetic resonance (NMR) (Bruker Avance 400, Switzerland) and the NMR spectra were recorded in D_2O on a 400 MHz instrument. Mass spectrometry data were obtained on ESI-MS (AB SCIEX QTRAP 4500, USA). Melting points were determined using X-4 apparatus (YuHua X-4, P.R. China).

2.4. D, L-AAs incubation with LAAO

The D, L-AAs and LAAO were dissolved in 50.0 mM Tris–HCl (pH 8.2). All enzymatic reactions were performed in 0.2 mL polypropylene tubes at 37 °C. The final concentration of LAAO was 0.1 U mL^{-1}. In the substrate specificity experiment, the concentration of D, L-isoleucine (D, L-Ile), D, L-methionine (D, L-Met) and D, L-