Talanta 142 (2015) 28–34

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A comprehensive study of the enantioseparation of chiral drugs by cyclodextrin using capillary electrophoresis combined with theoretical approaches

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ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form
7 April 2015
Accepted 12 April 2015
Available online 22 April 2015

Keywords:
Chiral drug
Capillary electrophoresis chiral separation
Isothermal titration calorimetric
Nuclear magnetic resonance spectroscopy
Molecular modeling

ABSTRACT

Four chiral drugs were enantioseparated by native beta-cyclodextrin (β-CD) and negatively charged carboxymethyl-beta-cyclodextrin (CM-β-CD) using capillary electrophoresis coupled with electrochemiluminescence detection (CE-ECL). Using 50 mM pH 5.5 Tris–H_3PO_4 with 10 mM CM-β-CD as a running buffer, high resolution efficiency could be obtained. With the help of isothermal titration calorimetry (ITC), nuclear magnetic resonance (NMR) and molecular modeling, the chiral recognition mechanism was comprehensively investigated. Thermodynamic parameters data from ITC revealed that CM-β-CD exhibited stronger binding affinity with analytes than β-CD, and that the driving forces of CM-β-CD responsible for chiral recognition were mainly electrostatic interactions between negatively charged CM-β-CD and positively charged analytes. In addition, from both a macroscopic and microscopic point of view, the results of NMR and molecular modeling investigation adequately confirm the conclusion by comparing the stereochemical structures of complexes. Combination of ITC, NMR and molecular modeling techniques not only can assist CE to investigate the chiral discrimination mechanism, but also can predict and guide CE enantioseparation efficiency conversely.

1. Introduction

Chiral separation and quantification of enantiomers are attracting considerable attention due to their different pharmacological, toxicological and pharmacokinetic properties. It is well known that usually one of the enantiomers possesses the desired effect while the other may lower potency, inactive or even have adverse effect [1]. Therefore, enantioseparation of chiral drugs is very important and becomes pressing issue in many fields [2]. Various analytical techniques with high resolution and rapid analysis have been developed for enantioseparation in recent years. Among them, high-performance liquid chromatography (HPLC) [3,4] and capillary electrophoresis (CE) [5,6] are considered as the most acceptable techniques in enantioselective analysis. Compared with HPLC, CE offers specific advantages of higher separation efficiency, lower sample and chiral selector consumption as well as shorter analysis time. Unlike HPLC, chiral stationary phase is not always required in CE, as the chiral selectors can be directly added into the background electrolyte to provide a chiral environment and form enantiomer-chiral selector complexes with analytes. The different charge-to-mass ratio of these diastereoisomeric complexes will lead to an electrophoretic mobility shift, and finally enantioseparation can be obtained [7,8]. As its type and concentration directly affect the enantioseparation efficiency [9], it is important to choose chiral selectors carefully.

Up to now, a remarkable number of novel chiral selectors [10–14] have been widely applied in CE enantioseparation. Among them, cyclodextrins (CDs) and their derivatives are still the most frequently used [15–20] due to their good solubility, availability, low toxicity and low UV absorbance. Recently, the interaction between natural or derivative CDs and various analytes has been studied using experimental and theoretical methods. Combination of NMR and molecular modeling has been performed successfully to understand the inclusion mechanism of CDs with analytes

Abbreviations: β-CD, beta-cyclodextrin; CM-β-CD, carboxymethyl-beta-cyclodextrin; ITC, isothermal titration calorimetry; NMR, nuclear magnetic resonance; CE-ECL, capillary electrophoresis-electrochemiluminescence; AP, 2-amino-1-phenyl-ethanol; MME, 1-(4-methoxyphenyl)-2-(methylamine) ethanol; SAL, salbutamol sulfate; SOT, sotalol hydrochloride; Tris, Tris (Hydroxymethyl)aminomethane

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http://dx.doi.org/10.1016/j.talanta.2015.04.039
0039-9140/

[21,22]. In general, inclusion complexes are found to be formed by taking up whole or part of the drug molecule into the hydrophobic cavity of the host, accompanying with other interaction such as hydrogen bonds, electrostatic interaction or Van der Waals interactions, etc. [23]. In order to gain an insight into the chiral recognition process and the nature of the intermolecular forces responsible for guest–host interactions, isothermal titration calorimetry (ITC) technique has been utilized as a powerful tool to characterize the thermodynamic properties of the interaction. Combination of ITC with molecular modeling and NMR [24–27] could be comprehensively and systematically investigate the interaction between CDs and analytes. Over the past two decades, although CD-mediated chiral CE has been developed rapidly, detailed mechanism of this enantioseparation mode needs to be studied. Systemic and detailed investigation of the related mechanism is still lacking due to the limitation of the experimental techniques. Suliman et al. [28] separated baclofen enantiomers by β-CD-mediated CE and determined the structure of the inclusion complexes by NMR. Simultaneously, molecular modeling was performed to rationalize the experimental results and to explain the mechanism of the enantioseparation. Li et al. [29] conducted similar and deep work to study the effect of structural features of analytes on enantiomers separation efficiency. Furthermore, the established mathematical equation exhibited good capability in predicting the resolution of enantioseparation. However, there are few works on combining of ITC, NMR and molecular modeling together to investigate the enantioseparation mechanism of CD-mediated chiral CE.

In the present work, we take advantages of combining ITC, NMR and molecular modeling techniques together to provide a deeper understanding of the chiral recognition processes from a thermodynamic, macroscopic and microscopic point of view, respectively. We discussed possible chiral recognition mechanism, which was coincident well with CE results. The established mode not only can assist CE to understand the enantioseparation mechanism well, but also be helpful to predict the separation resolution of chiral CE using CDs or their derivatives as chiral selectors.

2. Material and methods

2.1. Reagents and chemicals

(2, 2′-bipyridyl) ruthenium (II) dichloridehexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$), 2-amino-1-phenyl-ethanol (AP) and D_2O were purchased from Sigma-Aldrich Chemical Co. (Wisconsin, USA). 1-(4-methoxyphenyl)-2-(methylamine) ethanol (MME), salbutamol sulfate (SAL) were provided by Alfa-Aesar Chemical Co. (Massachusetts, USA), while sotalol hydrochloride (SOT) was obtained from Jilin Institute of Drug Control (Jilin, China). CM-β-CD and β-CD were supplied by Binzhou Zhiyuan Tech Co., Ltd (Shandong, China). Na_2HPO_4, NaH_2PO_4, NaOH, H_3PO_4 and Tris(Hydroxymethyl) aminomethane (Tris) were offered by Beijing Chemical Reagent Factory (Beijing, China). All of the reagents were of analytical grade and used as received without further purification. Double-distilled water was prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA), and all the solution was filtered through a 0.22 μm cellulose acetate membrane prior to use.

2.2. Apparatus and instruments

2.2.1. Capillary electrophoresis (CE)

The CE experiments were performed on a MPI-A model CE system (Remax Electronic, Xi'an, China), equipped with an on-line electrochemiluminescence (ECL) detector. The enantioseparation was carried out with a 45 cm length fused silica uncoated capillary (50 μm i.d. × 360 μm o.d.) from Yongnian Optical Fabric Factory (Hebei, China). Before the first use, the new capillary was activated using 0.1 mol/L NaOH overnight. In order to maintain an active and reproducible inner surface, the capillary was rinsed with doubly distilled water, 0.1 mol/L NaOH, doubly distilled water and corresponding running buffer for 2 min, respectively between runs.

End-column ECL detection was employed using a traditional three electrodes configuration consisting of a 500 μm diameter Pt disk as a working electrode, a Pt wire as a counter electrode and a Ag/AgCl (saturated with KCl) as a reference electrode. The photomultiplier tube positioned under the detection cell was set at +800 V. Prior to use, the electrode was scanned in 1.0 mol/L H_2SO_4 from −0.2 to 1.35 V (*vs.* Ag/AgCl) until a characteristic cyclic voltammogram of a clean Pt electrode was obtained. The detection cell was filled with 0.1 mol/L phosphate buffer (PBS) at pH 8.0 containing 5 mmol/L $Ru(bpy)_3^{2+}$, which was refreshed every 2 h in order to maintain the reproducibility of the detection results.

2.2.2. Isothermal titration calorimetry (ITC)

ITC experiments were conducted using a NANO ITC system provided by TA instruments microcalorimetry (New Castle, Delaware, USA) to determine the thermodynamic parameters of the studied complexes. All titration were performed at 25 °C using an external water bath with a stir rate of 350 rpm. The titration protocol was: 300 μL 0.5 mmol/L CM-β-CD (or β-CD) degassed solution (50 mmol/L Tris–H_3PO_4 buffer at pH 5.5) was titrated with 30 mmol/L degassed guest solution (same buffer) in a 50 μL syringe. Each titration consisted of 25 steps, 2 μL each step, and the time interval between two consecutive injections was 360 s. The heat data were processed and fitted using the ITC data Nanoanalyze software.

2.2.3. Nuclear magnetic resonance spectroscopy (NMR)

NMR spectroscopy experiments were carried out on a Bruker Avance II 400 and Bruker Avance III 600 spectrometer operating at 400.16 MHz and 600.13 MHz, respectively, equipped with a BBO probe and a variable temperature unit (VTU). Spectra were measured at 298 K in D_2O. The inclusion complex was prepared by mixing CM-β-CD (or β-CD) and investigated drugs at a 1:1 mol ratio in water, and then stirred for 2 h at 70 °C and for 48 h at room temperature. Next, the inclusion complex was dried by vacuum under low pressure after being placed in the refrigerator overnight and finally was re-dissolved in D_2O prior to NMR measurements.

2.2.4. Molecular modeling

The initial structures of (R/S)-AP, (R/S)-MME, (R/S)-SAL and (R/S)-SOT enantiomers were constructed by Gaussview5.0 and then further optimized at the B3LYP/6-31 G levels using GAUSSIAN03 program package [30]. The geometry of CM-β-CD was constructed from the crystallographic parameters of β-CD provided by the Cambridge Structural Database (CSD). Automated docking simulations were conducted with AutoDock 4.2 to explore the interaction modes of CM-β-CD and β-CD with these derivatives [31]. After the Gasteiger partial charges were assigned to CM-β-CD [32] and each enantiomer, nonpolar hydrogen atoms were merged and rotatable bonds were defined. A grid box dimension 80 × 68 × 34 $Å^3$ was created to ensure an appropriate size of each enantiomer-accessible space. Using the Lamarckian genetic algorithm, 100 runs were carried out with the following settings: 2,500,000 evaluations, 27,000 generations, and 150 population size. Finally, cluster analysis was performed on the results according to the default root mean square deviation (RMSD) criteria and the dominating configurations of the docked compounds with minimum binding free energy (DG) were obtained [33,34].