Sensors and Actuators B 248 (2017) 682–689

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Magnetism based electrochemical immunosensor for chiral separation of amlodipine

Lisha Zhang, Pei Song, Hao Long, Meng Meng, Yongmei Yin *, Rimo Xi *

State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Haihe Education Park, 38 Tongyan Road, Tianjin 300353, People's Republic of China

ARTICLE INFO

Article history:
Received 12 November 2016
Received in revised form 31 March 2017
Accepted 3 April 2017
Available online 8 April 2017

Keywords:
Electrochemical immunosensor
Magnetic electrode
Amlodipine
Enantioseparation

ABSTRACT

Amlodipine is one of the most important anti-hypertension agents and S-amlodipine is 1000 folds more efficient than R-amlodipine. However, chiral separation of amlodipine remains a challenge. Here we present an effective strategy for chiral resolution of amlodipine using anti-S-amlodipine antibody for ultra-specific capture and magnetic beads for convenient collecting. The collected enantiomers are ultra-pure based on the chiral HPLC identification. Simultaneously, the captured S-amlodipine could be quantitatively detected with a linear range from 0.1 to 1000 ng mL^{-1} (R^2 = 0.9937), along with a limit of detection of 0.04 ng mL^{-1}. The advantages of the developed magnetic electrochemical biosensor for amlodipine include specific chiral separation, sensitive quantitation, easy-to-operate procedures and time-saving protocols. This method is demonstrated as a novel tool for highly confident separation and quantification of amlodipine enantiomers, in this study. The magnetic biosensor may open up new avenues for intensive study of amlodipine enantiomers and thus be potential in clinical pharmacy and pharmacokinetics studies.

1. Introduction

Amlodipine has emerged as one of the latest generation of dihydropyridine type calcium channel blockers which are used for treatment of hypertension and angina pectoris [1,2]. Amlodipine possesses one chiral center and two enantiomers. It is reported that the pharmacological activity of S-amlodipine is 1000 folds higher than that of R-amlodipine [3]. In addition, R-amlodipine may lead to peripheral edema owing to nitric oxide release in peripheral blood vessels [4]. Therefore, it is significant to develop an effective method to separate and quantitatively detect its enantiomers, which would potentially promote the study of amlodipine enantiomers and monitor their clinical behaviors. However, simultaneous separation and determination of amlodipine enantiomers is challenging, because of their low abundance in clinical samples.

HPLC coupled with different detectors [5,6] and capillary electrophoresis (CE) [7] are employed for chiral amlodipine assay. But their sample pretreatments and enantiomer collection are laborious and time-consuming, and relatively large amount of samples are required. In the past decades, enantioselective immunoassay research has arisen in chiral compounds analysis, since it displays high sensitivity [8], simplicity [9], low cost [10], and high throughput [11]. At the same time, several enantioselective antibodies against small molecules have emerged [9–12]. However, the stratagem of immunogen design and enantioselective antibody preparation for amlodipine or other dihydropyridines has not been revealed.

Electrochemical immunoassay, which possesses the advantages of potential portability [13], low cost [14] and high sensitivity [15], has been well developed and widely applied to chemical analysis [16,17]. Moreover, magnetic beads (MBs) are extensively selected to fabricate biosensors, which benefit from their high agent immobilization due to their small-size effect, high large surface area [18], biological compatibility and various functional groups coating. Nevertheless, utilizing magnetic electrode and MBs to fabricate biosensors for chiral separation is rarely reported.

In the study, a novel dual-functional immunosensor has been proposed based on highly enantioselective antibody modified MBs and a magnetic electrode. The magnetic electrode was modified by an immunoconjugate which was prepared by coupling MB with antibody against S-amlodipine (MB-Ab) and used as the capture probe. The MB-Ab immunoconjugate could be modified to the magnetic electrode quickly and firmly by magnetic adsorption. It could separate S and R-amlodipine from racemic amlodipine, and the purity of the separated products was evaluated by HPLC. More-

* Corresponding authors.
E-mail addresses: yinyongmei@nankai.edu.cn (Y. Yin), xirimo@nankai.edu.cn (R. Xi).

http://dx.doi.org/10.1016/j.snb.2017.04.010
0925-4005/

over, the immunosensor could detect the content of S-amlodipine, simultaneously. The conditions influencing the effect of separation and determination were optimized. And the methodology of the immunosensor was also investigated. All results showed that the proposed method was simple and sensitive for separating and detecting racemic amlodipine.

2. Experimental

2.1. Materials and reagents

Amlodipine, S-amlodipine, R-amlodipine, potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$) and potassium chloride (KCl) were purchased from J&K Chemical Company (Beijing, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), N,N-dimethylformamide (DMF) and ethanolamine were provided by Aladdin Industrial Corporation (Shanghai China). Keyhole limpet hemocyanin (KLH) was from Sigma–Aldrich (St. Louis, MO, USA). NHS-activated MBs were provided by Thermo Fisher Scientific (New York, USA). The chromatographic grade n-hexane and ethanol were purchased from Mindray Technology Company (Beijing, China). The buffers applied in this study including sodium phosphate-buffered saline solution (PBS), washing buffer (PBST), coating buffer and blocking buffer were prepared according to a published report [19]. Other reagents were purchased from Sigma–Aldrich and were all of analytical grade.

2.2. Instruments and apparatus

All electrochemical measurements were conducted on a CHI 660E electrochemical workstation (Shanghai CH Instruments Co., China) with a conventional three-electrode system comprised of modified magnetic electrode as working electrode, saturated calomel electrode as reference electrode and platinum wire as auxiliary electrode. Cyclic voltammetry (CV) and square wave voltammetry (SWV) were performed in the solution of 0.1 mol L^{-1} KCl containing 5.0 mmol L^{-1} $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$. Dynamic light scattering (DLS) and zeta potential were performed on a Nicomp 380 zls Laser Particle Size Analyzer (Particle Sizing Systems, USA). HPLC detections were carried out using a Waters Alliance e2695 liquid chromatography system (Tokyo, Japan) with an UV detector at 220 nm. A Chiralpak AD-H column (250 mm × 4.6 mm, 5 μm) was purchased from Daicel (Tokyo, Japan).

2.3. Preparation of enantioselective antibody

An enantioselective immunogen of S-amlodipine was prepared firstly by coupling amino groups of S-amlodipine with carboxylic acid groups in KLH (Fig. S1). EDC (33.8 mg), NHS (10.2 mg) and KLH (10 mg) were mixed and S-amlodipine (10.0 mg mL^{-1}) was added subsequently. The mixture was stirred for 8 h at room temperature and purified by dialysis (MWCO = 8000). The produced enantioselective immunogen was hypodermically injected into multiple sites of New Zealand rabbits for immune. After five immunizations, antiserum was obtained [19].

2.4. Preparation of the magnetic electrode

The magnetic electrode was assembled as following. Firstly, essential materials were prepared, including: copper column, copper wire, cylindrical magnet with a pore, glass carbon and polytetrafluoroethylene (PTFE) pipe. The glass carbon was fabricated into a small cylinder which had a height of 5 mm and a diameter of 4 mm. One side of the glass carbon was polished by the metallographic sandpaper into mirror finish. Then, the glass carbon was placed into the top of a polytetrafluoroethylene (PTFE) pipe which had a length of 60–80 mm and a diameter of 5 mm. After that, it was sealed using epoxy resin. Following, the cylindrical magnet was placed into the PIFE pipe and the conductivity copper wire was put through it. One side of the copper wire was connected with the glass carbon using a gold plated conductive spring. The other side of the copper wire was linked with the copper column which installed on the bottom of the PTFE pipe by welding. All of the above procedures were sealed by epoxy resin.

After the magnetic electrode prepared, cyclic voltammetry (CV) was conducted into $K_3[Fe(CN)_6]$ solution (1×10^{-3} mol L^{-1}) to test the performance of the prepared electrode. The scanning rate was 50 mV s^{-1} and the scanning scope was −0.2 to 0.6 V. The difference of the peak potentials obtained by the CV response should be below 80 mV.

2.5. Preparation of antibody modified magnetic beads

NHS-activated magnetic beads (50 μL, 10 mg mL^{-1}) were mixed with 20 μL of the antibody. The solution was stirred for 8 h at room temperature and blocked by PBS containing 0.05% OVA. The product (MB-Ab) was stored at 4 °C before use.

2.6. Fabrication of electrochemical immunosensor

Before modification, the bare magnetic electrode (Φ = 4 mm) was firstly polished to a mirror finish using 1.0, 0.3 and 0.05 μm alumina powder and washed with ultrapure water for a few seconds. Then, the magnetic electrode was modified with pre-prepared MB-Ab (5 μL) by magnetic adsorption. Subsequently, 5 μL of different concentrations of S-amlodipine was added and the prepared immunosensor was incubated for 40 min at 37 °C. After washing, the electrode was ready to measure by CV or SWV. The standard curve was obtained by plotting the current change (μA) against the concentrations (c) of S-amlodipine. The exact amount of captured S-amlodipine in samples could be calculated from the standard curve.

2.7. Collection of S and R-amlodipine from the immunosensor

After the immunocomplex formed in the electrode, the supernatant containing R-amlodipine was collected. S-amlodipine which was combined with the antibody was eluted with sodium hydroxide solution (10 mM) and freeze-dried. The purity of the collected S and R-amlodipine was evaluated by HPLC.

3. Results and discussion

3.1. Principle of the proposed method for chiral separation of amlodipine

In this study, we demonstrate a novel multi-functional immunosensor based on magnetic electrode, MBs and highly enantioselective antibody. Magnetic electrode, which can be modified easily and rapidly with MBs by magnetism, has not been integrated with MBs and specific antibody for chiral resolution, enantiomer quantification and collection thus far. On the basis of specific immunogen design, the produced antibody is specific to S-amlodipine. The immunosensor provides a linear detection range from 0.1 to 1000 ng mL^{-1} for S-amlodipine and two collected enantiomers are highly pure. The above mentioned ability of the proposed immunosensor could provide an alternative for chiral amlodipine separating, collecting and quantifying in low