Biosensors and Bioelectronics 87 (2017) 278–284

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Functionalized poly (ionic liquid) as the support to construct a ratiometric electrochemical biosensor for the selective determination of copper ions in AD rats

Yanyan Yu [a,1], Chao Yu [a,1], Tianxiao Yin [a], Shanshan Ou [a], Xiaoyu Sun [a], Xiangru Wen [b], Lin Zhang [c], Daoquan Tang [a,**], Xiaoxing Yin [a,*]

[a] Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical University, 209 Tongshan Road, Xuzhou, Jiangsu 221004, PR China
[b] College of Biomedical Sciences, Xuzhou Medical University, 209 Tongshan Road, Xuzhou 221004, PR China
[c] Jiangsu Vcare Pharmatech Company, 15 Wanshou Road, Nanjing, Jiangsu 210000, PR China

ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form
11 August 2016
Accepted 19 August 2016
Available online 20 August 2016

Keywords:
Functionalized PILs
Neurokinin B
Cu^{2+}
Electrochemistry
AD

ABSTRACT

An efficient ratiometric electrochemical biosensor for Cu^{2+} determination was constructed using dual hydroxyl-functionalized poly (ionic liquid) (DHF-PIL) as the catalyst support. The DHF-PIL exhibited typical macroporous structure, which provided a high surface area of 39.31 m^2/g for the sufficient loading of biomolecules. The specific recognition of Cu^{2+} was accomplished by employing neurokinin B (NKB) for the first time, which could bind to Cu^{2+} to form a $[Cu^{II}(NKB)_2]$ complex with high specificity. Meanwhile, a common redox mediator, 2, 2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) was modified into DHF-PIL by electrostatic interactions to act as an inner reference molecule, which provided a built-in correction for environmental effects and improving the detection accuracy. With this strategy, the developed electrochemical biosensor was capable of determining Cu^{2+} with a linear range between 0.9 and 36.1 μM and low detection limit (LOD) and quantification limit (LOQ) of 0.24 and 0.6 μM, respectively. The sensor also displayed a satisfactory selectivity against a series of interferences in the brain, including metal ions, amino acids and other endogenous compounds. Accordingly, the present biosensor was successfully applied to evaluate Cu^{2+} levels in normal and AD rats.

1. Introduction

It has been widely believed that copper ions (Cu^{2+}) play crucial roles in living systems as cofactors of numerous metalloenzymes that generate cellular energy, reduce molecular oxygen, and induce signal transduction (Bull et al., 1993; Gaggelli et al., 2006; Kang et al., 2014). However, if unregulated, the disturbance of the cellular Cu^{2+} homeostasis may lead to cell death and carious neurodegenerative diseases, including Alzheimer's disease (AD), Wilson's disease and Parkinson's disease (Taki et al., 2010; Valentine and Hart, 2003). The concentration of Cu^{2+} is found to be more than doubled in the cerebral amyloid deposits of AD brains compared with the neuropil of normal age-matched brains (Lovell et al., 1998; Smith et al., 1997), which might be responsible for inducing and enhancing the formation of the α-helix conformation of the alanine-based peptides by complexation with them (Zou and Sugimoto, 2000a, 2000b). It is, therefore, of considerable significance to establish highly sensitive and selective approaches for Cu^{2+} determination.

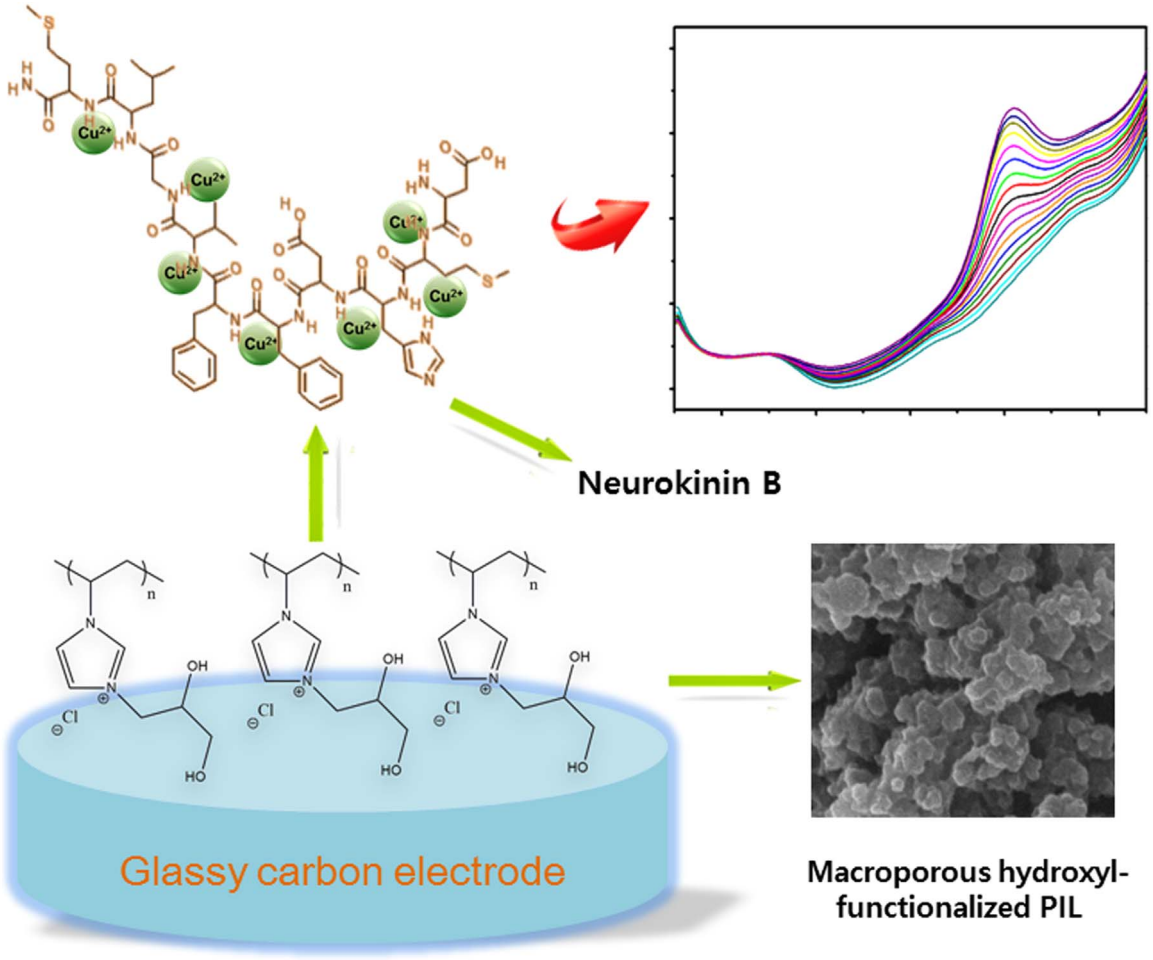
Up to now, a variety of strategies have been put forwarded for this purpose, including atomic absorption spectrometry (AAS) (Lin and Huang, 2001), inductively coupled plasma mass spectroscopy (ICP-MS) (Becker et al., 2007) and atomic emission spectrometry (ICP-AES) (Liu et al., 2005), fluorescence (Liu et al., 2016; Qing et al., 2015; Qu et al., 2012; Su et al., 2013; Vedamalai et al., 2014; Wang et al., 2010; Xiong et al., 2016; Zhu et al., 2012), immunoassay (Liu et al., 2013), colorimetric (Ding et al., 2016) and electrochemical approaches (Chai et al., 2013; Kimmel et al., 2012). However, those methods are usually complicated, time-consuming and costly. Fluorescence, although high sensitivity and specificity can be obtained with a number of well-designed fluorescent probes, including small organic molecules (Xiong et al., 2016; Zhu et al., 2012), metal nanoparticles and nanoclusters (Qing et al., 2015; Su et al., 2013; Vedamalai et al., 2014) and quantum dots (Liu et al., 2016; Qu et al., 2012), the susceptibility to the detection

* Corresponding author.
** Corresponding author.
E-mail addresses: tdq993@126.com (D. Tang), yinxx@xzmc.edu.cn (X. Yin).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2016.08.066
0956-5663/

conditions often influences its fluorescence intensity and thus limits its wide application. Alternatively, electrochemical biosensors have been considered a promising technique because of their, simplicity, rapid response, and potential ability for real-time and on-site analysis (Yu et al., 2015), which are typically labeled with an electroactive species to generate corresponding electrochemical signals. For this application, specific recognition elements for Cu^{2+} should be designed for selectively capturing Cu^{2+} in the complicated in vivo environment. For instance, Tian's group has prepared a series of organic molecules, such as N-(2-aminoethyl)-N,N,N'tris(pyridin-2-ylmethyl)ethane-1,2-diamine and 2,2′,2″-(2,2′,2″-nitrilotris(ethane-2,1-diyl)-tris((pyridin-2-ylmethyl)azanediyl)triethanethiol, as well as the native Cu-free derivative of bovine erythrocyte copper-zinc superoxide dismutase (SOD-E_2Zn_2SOD) for specific recognitions of Cu^{2+} (Chai et al., 2013; Zhang et al., 2015; Zhu et al., 2012). However, the complicated synthesis procedure limits their further practical applications and in this regard, it is still of great necessity to look for a new and natural recognition biomolecule to construct an electrochemical biosensor, which is not only able to recognize Cu^{2+} with high selectivity, but also can fulfill the requirements of other analytical performances, particularly sensitivity and accuracy.

Porous polymers have received expanding interest, due to their low bulk density, extraordinary mechanical properties, variability of their monomer building blocks and the intrinsically charged characters (Li et al., 2010; Yang and Luo, 2014; Zhao et al., 2012). In particular, these polymers can serve as an immobilizing matrix for biomolecules in the biosensor system, which not only provide a suitable environment for firmly immobilizing biomolecules onto sensors through covalent binding at the functional sites, but also improve the loading capacity due to the existence of multiple pores (Rahman et al., 2005), enabling the realization of the signal amplification effect. Polymeric/polymerized ionic liquids (PILs) refer to a special type of polymers synthesized via polymerization of their ionic liquid monomers (IL) and differ from conventional polyelectrolytes in that the cations and anions are usually organic and weakly coordinated (Zhang et al., 2011). In comparison with common polyelectrolytes, the physical properties of these PILs (e.g., solubility, polarity, etc.) can be altered more broadly and flexibly by counterion exchange (Zhao et al., 2012). The potential utility of PILs lies in the established tunability of the molecular structure of IL, leading to exquisite control over their physicochemical properties, in combination with the advantages of polymers: stability, processability and durability (Lee et al., 2012; Yuan and Antonietti, 2011). These characters make PILs versatile polymers for a multitude applications, including catalytic membranes, ionic conductive materials, carbon dioxide absorbents, microwave absorbing materials, stabilizing agent, and carbon precursors (Mao et al., 2015; Marcilla et al., 2006; Yuan and Antonietti, 2011; Yuan et al., 2010). It is worth mentioning that nanostructured PILs have received special interests due to their wide and potential range of applications, e.g., nanoparticles, nanosphere, nanogels, etc. For instance, Yuan's group has reported a highly ordered and tunable inner structure of PIL nanoparticles by precipitation polymerization from water with a much smaller diameter (20–40 nm) in the absence of external stabilizers (Yuan et al., 2011). Vancso et al.



Scheme 1. Electrochemical sensing for Cu^{2+} based on the ratiometric DHF-PIL-ABTS/NKB/Glu biosensor.