Biosensors and Bioelectronics 41 (2013) 511–518

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Size-tunable Pt nanoparticles assembled on functionalized ordered mesoporous carbon for the simultaneous and on-line detection of glucose and L-lactate in brain microdialysate

Yanyan Yu [a,b], Yan Yang [a], Hui Gu [a], Tianshu Zhou [c], Guoyue Shi [a,*]

[a] Department of Chemistry and Shanghai Key Laboratory of Green Chemistry and Chemical Process, East China Normal University, 500 Dongchuan Road, Shanghai 200241, PR China

[b] School of Pharmacy, Xuzhou Medical University, 209 Tongshan Road, Xuzhou, Jiangsu 221004, PR China

[c] Department of Environmental Science, East China Normal University, 500 Dongchuan Road, Shanghai 200241, PR China

ARTICLE INFO

Article history:
Received 12 July 2012
Received in revised form
30 August 2012
Accepted 10 September 2012
Available online 5 October 2012

Keywords:
On-line microdialysis system
Pt/PDDA-CMM hybrid nanocomposite
Glucose
L-lactate
In vivo

ABSTRACT

This study presents a facile electrochemical method for simultaneous and selective on-line detection of glucose and L-lactate in the striatum of anesthetic rats through the integration of selective electrochemical detection with in vivo microdialysis system. A positively-charged polyelectrolyte, (diallyldimethylammonium chloride) (PDDA), was attached onto carbon mesoporous material (CMM) through non-covalent interaction, which provided an ideal environment for the assembling and dispersion of nanoparticle electrocatalysts. Platinum nanoparticles with wide loadings from 5 to 50 wt% were successfully self-assembled on PDDA-functionalized CMM via electrostatic interaction. TEM results showed that with the increase in the Pt loadings, both the size and interconnectivity between particles increased, with particle sizes ranging from 3.2 ± 0.4 to 6.8 ± 1.4 nm. Moreover, the electrocatalytic activities of the as-prepared six Pt/PDDA-CMM hybrid nanocomposites were also observed to show an inverted-V-shaped profile as a function of loading amount of Pt NPs. Integrated with glucose oxidase (GOx), L-lactate oxidase (LOD) and the in vivo microdialysis system, the constructed dual oxidase/Pt/PDDA-CMM/Nafion biosensors were successfully applied for the simultaneous and on-line detection of glucose and L-lactate. After post-calibration, the basal level of glucose and L-lactate in the striatum of anesthetic rats was calculated to be 0.27 ± 0.03 and 0.71 ± 0.05 mM (mean $\pm$ s.d., $n=3$), respectively. What is more important, the dual oxidase biosensors almost suffered from little cross-talk, which is characteristic of an excellent sensor with high performance. This property, along with the good linearity and a high stability substantially enables this method promising application in physiology and pathology.

1. Introduction

Glucose is a main nutrient in the brain and the well-documented effects of circulating glucose on cognitive function has suggested that glucose might act on brain systems important for memory formation (Dong et al., 2003). Nowadays, an increasing body of evidence shows that glucose might not the only energy source in cerebral energy metabolism. L-lactate by itself is sufficient to support synaptic function in rat hippocampal slices in the absence of glucose, further emphasizing the ability of neurons to use L-lactate for energy (Izumi et al., 1997; Izumi et al., 1994; McIlwain, 1953; Schurr et al., 1988). Glucose and L-lactate monitoring in the brain of stroke, head trauma or subarachnoid hemorrhage patients has become a diagnostic tool to detect ischemic events and monitor therapeutic interventions (Persson et al., 1996; Hutchinson et al., 2000; Schulz et al., 2000; Jan et al., 2003). Consequently, simultaneous detection of glucose and L-lactate in the central nervous system is of great physiological and pathological importance. Among various detection protocols, electrochemical methods to continuously monitor chemical species involved in brain activities have been extensively developed, which allows on-line (near) real-time measurements (Guntermann et al., 1996; Perdomo et al., 2000; Yamazaki et al., 2011). What is the most important, the integration of microdialysis with a fast and sensitive biosensor based electrochemical detection system to construct an effective on-line analytical system, without any sample separation or pretreatment, has been developed to track the dynamic chemical process in living system (Lin et al., 2009). With this on-line system, our group has successfully realized the in vivo monitoring of glutamate level both in normal and stimulated rats (Yu et al., 2011a, 2011b).

* Corresponding author Tel.: +86 021 54340043.
E-mail address: gyshi@chem.ecnu.edu.cn (G. Shi).

0956-5663/$ - see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bios.2012.09.055

It has been well-known that the performance of metal NPs catalysts largely depends on their morphology, size and composition. To date, various physical or chemical techniques have been used to tailor monodispersed metal nano-spheres, cubes, rods, wires, etc, including vapor deposition, laser ablation, metal salt reduction, sol–gel process and micelles (Park et al., 2007; Yanagisawa et al., 1990; Kresge et al., 1992). The emergence of ordered mesoporous materials, such as mesoporous silica, mesoporous carbon and mesoporous tungsten oxide, provides great opportunities in separation, catalysis, sensors, and synthesis of novel nanostructured materials (Zhao et al., 1998; Fukuoka et al., 2003; Liang et al., 2008). In particular, the use of carbon mesoporous material (CMM) as catalysts has attracted great attention, since they exhibit intriguing structural properties that are favorable for the electrocatalytical application, including high surface area for preparation of highly dispersed catalytic nanoparticles, highly interconnected mesopores for facile diffusion and transport of reactants and byproducts, and graphitic framework microstructures for pathways of electrons (Sang et al., 2009). As a consequence, CMM would be a particularly promising support material for the preparation of high metal loading supported catalyst and much effort has been directed toward the hybridization of nanometer-sized metals and CMM (Choi et al., 2005; Liu et al., 2008; Orilall et al., 2009; Wen et al., 2008; Bo et al., 2011). Moreover, due to their tunable and consistent pore system, functionalizable surfaces, CMM becomes more suitable for enzyme immobilization (Yu et al., 2010), on which improved immobilization ability and enhanced electron transfer of electrons of biomolecules could be efficiently obtained (Vinu et al., 2003; Feng et al., 2007).

This study described a physiologically relevant on-line electrochemical method for simultaneous monitoring of glucose and L-lactate in vivo with high sensitivity and selectivity. The analytical system was actually based on a novel and effective CMM based dual-enzyme biosensor coupled with the on-line microdialysis system, which made the continuous and simultaneous monitoring of glucose and L-lactate in rat brain possible. Firstly, we introduced a positive charged polyelectrolyte, (diallyldimethylammonium chloride) (PDDA), to functionalize carbon mesoporous material (CMM). Pt nanoparticles with precise loading amount and size control were grown on PDDA-CMM using a novel self-assembling technique. The relationship between Pt loading amount and the particle size as well as the interconnectivity was systematically discussed. It was also found that Pt loading amount also exerted a significant influence on the electrocatalytic activity of the six Pt/PDDA-CMM films. Then, a dual glucose and L-lactate biosensor based upon Pt/PDDA-CMM film were constructed with glucose and L-lactate oxidase as the biological recognition elements, respectively. The mediatorless simultaneous and on-line detection of glucose and L-lactate in the striatum of rat was realized with on-line microdialysis system. To the best of our knowledge, few works till date have been done on the in vivo simultaneous detection of glucose and L-lactate combined with CMM–Pt hybrid.

2. Experimental

2.1. Reagents

Glucose oxidase (GOx, EC 1.1.3.4. from *Aspergillus niger*), L-lactate oxidase (LOD, EC 1.1.3.2, from *Pediococcus* species), glucose, L-lactate, ascorbic acid (AA), dopamine (DA), uric acid (UA), 3, 4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), Nafion (5 wt%) and poly (diallyldimethylammonium) (PDDA) were all purchased from Sigma Co. and used as supplied. Hexachloroplatinate ($H_2PtCl_6 \cdot 6H_2O$, 99.9%, 1 g/5 mL), glutaraldehyde solution (25%) were bought from Shanghai Chemical Reagent Company (Shanghai, China). 30% H_2O_2 was from Beijing Chemical Co (Beijing, China). Artificial cerebrospinal fluid (aCSF: 126 mM NaCl, 2.4 mM KCl, 0.5 mM KH_2PO_4, 0.85 mM $MgCl_2$, 27.5 mM $NaHCO_3$, 0.5 mM Na_2SO_4, 1.1 mM $CaCl_2$, pH 7.4) was prepared with doubly distilled water, filtered (filter pore size 0.22 μm, Millipore, Bedford, MA) and used both as electrolyte for off-line experiment and perfusion solution for on-line measurement.

2.2. Preparation of Pt/PDDA-CMM nanocomposites with different Pt loadings from 5 to 50% (wt)

The preparation procedure of Pt/PDDA-CMM nanocomposites with different Pt loadings from 5 to 50% (wt) was provided in Supplementary Information.

2.3. Fabrication of Pt/PDDA-CMM/GOx/Nafion and Pt/PDDA-CMM/LOD/Nafion biosensors

Dual glassy carbon (3 mm diameter, CHI Company, China) electrodes (GC) used in both off-line and on-line electrochemical experiments were polished with alumina paste (0.05 μm) on a micro-cloth and subsequently ultrasonically cleaned thoroughly with acetone, NaOH (1:1), HNO_3 (1:1) and doubly distilled water and then dried at room temperature. Before Pt/PDDA-CMM film casting, both parts of the dual GC electrodes were electrochemically activated from −0.5 V to 1.2 V in 0.1 M KCl solution buffered with 0.05 M phosphate buffer (pH 7.4) until a stable cyclic voltammetry response was obtained. 2 mg of Pt/PDDA-CMM was dispersed in 1 mL of N, N-dimethylformamide (DMF) with the aid of the ultrasonic agitation to form a stable black suspension solution. Then, 5 μL of the mixture was respectively dropped onto each part of the cleaned bare electrodes, being allowed to dry at ambient temperature.

For immobilization of GOx and LOD onto each part of the dual GC electrodes, 5 μL of GOx (10 mg/mL) or 5 μL of LOD (20 mg/mL) in 0.1 M phosphate buffer solution (pH 7.4) was mixed with 0.4% polyethyleneimide (PEI) aqueous solution and 5 μL of the mixture was applied to each part of the Pt/PDDA-CMM modified dual GC electrodes. PEI was employed here to stabilize the enzymes. After being air-dried for 3 h, the prepared electrodes were cross-linked in a closed vessel containing 25% glutaraldehyde and water vapor for 30 min. Finally, 1 μL of 2.5% Nafion was dropped onto the electrodes. The dual modified GC electrodes were dried at room temperature for 2 h, and stored at 4 °C when not in use.

2.4. Instrumentation

The morphology of the prepared Pt/PDDA-CMM nanocomposite was studied on a scanning electron microscope (SEM) (Hitachi Co. Ltd., Tokyo, Japan) and a JEOL 2010 transmission electron microscope (TEM) operating at 200 KV. All the electrochemical experiments were performed on CHI 832 electrochemical workstation (CHI Company, China). The thin-layer electrochemical flow cell for the on-line experiments, with an inner volume of 0.085–0.34 μL, consisted of a thin-layer flow block with a 25 μm gasket, GC electrode (3 mm diameter) as working electrode, stainless steel as counter electrode, and saturated calomel electrode as reference electrode and was integrated with microdialysis to form an on-line analytical system for continuous measurement of brain microdialysate of anesthetized rats. Standard solutions or brain microdialysates were delivered from gas-impermeable syringes (BAS) and pumped through a liquid switch (CMA 110, Solna, Sweden) by a