Biosensors and Bioelectronics 55 (2014) 307–312

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Enzymatic reactivity of glucose oxidase confined in nanochannels

Jiachao Yu, Yuanjian Zhang, Songqin Liu*

State Key Laboratory of Bioelectronics, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, PR China

ARTICLE INFO

Article history:
Received 17 October 2013
Received in revised form
18 December 2013
Accepted 18 December 2013
Available online 27 December 2013

Keywords:
Enzymatic reaction kinetics
Nanochannel
Electrochemical biosensor

ABSTRACT

The construction of nanodevices coupled with an integrated real-time detection system for evaluation of the function of biomolecules in biological processes, and enzymatic reaction kinetics occurring at the confined space or interface is a significant challenge. In this work, a nanochannel–enzyme system in which the enzymatic reaction could be investigated with an electrochemical method was constructed. The model system was established by covalently linking glucose oxidase (GOD) onto the inner wall of the nanochannels of the porous anodic alumina (PAA) membrane. An Au disc was attached at the end of the nanochannels of the PAA membrane as the working electrode for detection of H_2O_2 product of enzymatic reaction. The effects of ionic strength, amount of immobilized enzyme and pore diameter of the nanochannels on the enzymatic reaction kinetics were illustrated. The GOD confined in nanochannels showed high stability and reactivity. Upon addition of glucose to the nanochannel–enzyme system, the current response had a calibration range span from 0.005 to 2 mM of glucose concentration. The apparent Michaelis–Menten constant (K_m^{app}) of GOD confined in nanochannel was 0.4 mM. The presented work provided a platform for real-time monitoring of the enzyme reaction kinetics confined in nanospaces. Such a nanochannel–enzyme system could also help design future biosensors and enzyme reactors with high sensitivity and efficiency.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Recently, the construction of micro-/nanosystems has received promoted interest because of their various applications in evaluating the function of biomolecules in biological processes, and enzymatic reaction kinetics occurring at the confined space or interface (Cao et al., 2012a, 2012b; Vamvakaki and Chaniotakis, 2007; Moyano and Rotello, 2011; Nel et al., 2009; Pedone et al., 2011; Ressine et al., 2010; Lam et al., 2008). So far, four kinds of micro-/nanosystems have been applied in biological research. These include nanopores and micro-/nano-droplet formed in micro-/nano-channels using a micro-/nanofluidic technique (Chen et al., 2011; Wang et al., 2010; Woronoff et al., 2011); nanochannels or nanotubes (Chen et al., 2012; Li et al., 2010a, 2010b; Martin et al., 2001); two-dimensional nanostructures that are constructed by a layer-by-layer technique to incorporate a variety of biomolecules (Calvo et al., 2010; Hua et al., 2012; Smuleac et al., 2006); and the three-dimensionally ordered macroporous materials with highly ordered porous structure, large surface area, and tight pore size distributions (Qian et al., 2009a, 2009b; Wan et al., 2009; Wang et al., 2007, 2009). The recognition and detection of biomolecules such as DNA (Cherf et al., 2012; Min et al., 2011; Venkatesan and Bashir, 2011; Yang et al., 2011), proteins (Ali et al., 2008; Ding et al., 2009; Kowalczyk et al., 2011; Wei et al., 2012; Yusko et al., 2011) and biological molecular complexes (Dekker, 2007; Gao et al., 2009; Manrao et al., 2012) have been realized in nanochannel systems that had a one-dimensional confined cavity structure. For example, artificial nanochannels fabricated in track-etched polymer membranes and modified with β-cyclodextrin were used for highly selective recognition of histidine enantiomers through monitoring of ionic current signatures (Han et al., 2011). Ali et al. (2011) described the following nanochannel platform for detection of hydrogen peroxide. The nanochannels were fabricated in an ion tracked polymer membrane by the track-etching technique. The inner walls of the channel were decorated with horseradish peroxidase enzyme using carbodiimide coupling chemistry. The resultant enzyme–nanochannel system was capable of detecting nanomolar concentrations of hydrogen peroxide. Using polystyrene-block-poly(methyl methacrylate) copolymer, the nanochannels were vertically oriented cylindrical pores that spanned through the entire film and were prepared on the supporting membrane with a pore size of ~15 nm. The inside walls of nanochannels were covered with carboxylic acid, which served as highly sophisticated molecular recognition probes for DNA detection (Yang et al., 2011).

Aside from the polymer-based nanochannels, porous anodic alumina membrane was another significant nanochannel array material. The advantages of PAA, such as the tunable nanopore

* Corresponding author. Tel.: +86 25 52090613; fax: +86 25 52090618.
E-mail address: liusq@seu.edu.cn (S. Liu).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.12.042

diameter, high ordered nanochannel array, easy surface modification, commercial availability (in a wide range of pore diameters), good mechanical stability and biocompatibility, have allowed PAA to be integrated in biological devices with various applications (de la Escosura-Muñiz and Merkoçi, 2011; Chen et al., 2008; Jani et al., 2009, 2010; Lee et al., 2006, 2011; Li et al., 2010a, 2010b; Sah et al., 2004). For example, the PAA nanochannel arrays were employed as sensing platforms for label-free protein detection based on the blockage in the diffusion of indicator electroactive species through the nanochannels due to the immunoreactions (de la Escosura-Muñiz and Merkoçi, 2011). Li et al. (2010b) prepared a nano-enzyme array reactor by covalently linking glucose oxidase to the inner channels of PAA membrane to investigate the mass-transport-related enzymatic reaction kinetics. The activity and stability of the immobilized enzyme in the nanochannels were enhanced. The enzymatic reaction was controlled by mass transport in low flux rate, while the enzymatic reaction kinetics became the rate-determining step with an increase in the flux. Following this work, they provided a strategy for label-free oligonucleotide analysis by measuring the DNA-morpholino hybridization hindering diffusion flux of electroactive probe ions through nanochannels of PAA membrane (Li et al., 2010a).

Motivation for the present work also came from the very promising applications of nanochannels arrays in assembly of excellent biological devices for selective recognition and biosensing. To investigate the enzymatic reactivity of glucose oxidase confined in the porous anodic alumina membrane, the glucose oxidase was assembled onto the membrane and the inner wall of the nanochannels that were previously treated with silane to form epoxy groups modified surface. A homemade Au electrode that served as the working electrode was attached onto one side of the PAA membrane to detect the product H_2O_2 from the enzymatic reaction. The dependence of enzymatic reaction kinetics of the confined enzyme on the ionic strength, amount of enzyme immobilized and pore diameter of nanochannels, was also illustrated.

2. Experimental section

2.1. Covalent linking of enzymes to PAA nanochannels

The whole process for functionalization of PAA nanochannels with enzyme is illustrated in Scheme 1(A). PAA membranes were immersed into a 1 mL dried toluene solution containing 5% GPTMS and shaken gently for about 12 h (Gopishetty et al., 2008; Long et al., 2010; Szczepanski et al., 2006). Then, the PAA membranes were washed with toluene and ethanol thoroughly to remove any residual silane inside the nanochannels. Lastly, the PAA membranes were dried in nitrogen. Thus, PAA membranes with nanochannels modified using epoxy functional groups were obtained and ready for enzyme immobilization. The resultant PAA membrane was then immersed in a 0.5 mg mL^{-1} GOD solution in water and shaken gently at 4 °C for about 24 h (Li et al., 2010; Lin et al., 2008; Lu et al., 2007; Tang et al., 2010; Wu et al., 2009; Yang et al., 2009). The unbound GOD was removed by immersing in water, gently shook for 1 h, and rinsed with water thoroughly. The enzyme functionalized PAA membrane with GOD immobilized on the inner-wall of PAA nanochannels was thus obtained and restored in double distilled water at 4 °C. The amount of GOD immobilized in PAA nanochannels was determined by measuring absorption at 280 nm.

2.2. Electrochemical measurements

All the electrochemical measurements were performed with a homemade cell containing 2 mL of electrolytes, as shown in Scheme S1. To illustrate the electrostatic and steric effects of the assembled GOD in PAA nanochannels, 1 mM of $Fe(CN)_6^{3-}$ was introduced into the cell. The flux of $Fe(CN)_6^{3-}$ ions passing through the nanochannels was monitored by the current response at the working electrode at 0 V.

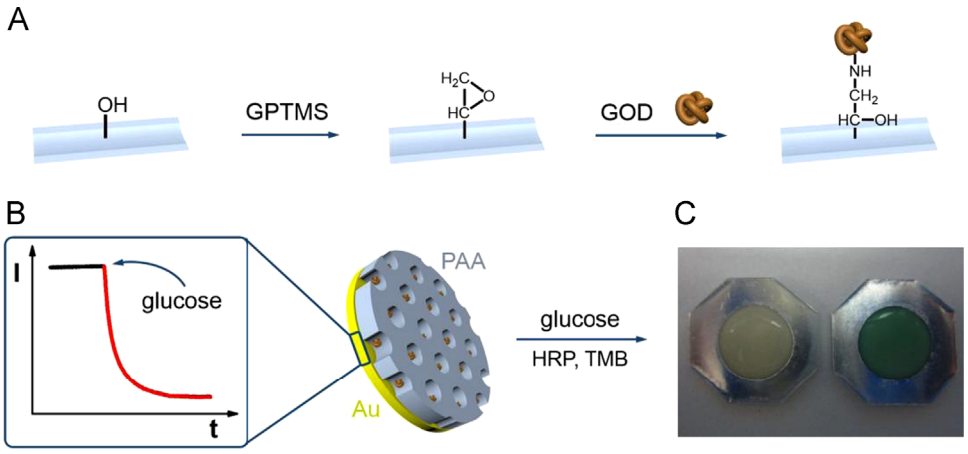
To measure the current response of H_2O_2 produced from the enzymatic reaction involving glucose catalyzed by GOD in PAA nanochannels, a potential of 0.7 V was applied to the working electrode. As shown in Scheme 1(B), after the steady-state background current is obtained, glucose with various concentrations is introduced into the cell, and the current change could be observed as H_2O_2 formed and transported through the nanochannels to the working electrode surface. The electrocatalytic response of free GOD in PBS was processed under the same conditions as control.

All other material and instrument details can be found in the Supporting information.

3. Results and discussion

3.1. Enzyme assembled in PAA nanochannels

For enzyme assembling, the PAA membranes were first treated with silane to form epoxy groups modified inner surface of PAA nanochannels. Then GOD was assembled onto the membrane and



Scheme 1. (A) The functionalization process of PAA nanochannels with GOD. (B) The electrochemical measurement for the enzymatic reaction. (C) Colorimetric images of the PAA membrane with (right) or without (left) GOD immobilized.

Download English Version:

https://daneshyari.com/en/article/866503

Download Persian Version:

https://daneshyari.com/article/866503

Daneshyari.com