Electrochemistry Communications 82 (2017) 43–46

Contents lists available at ScienceDirect

Electrochemistry Communications

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

An origami paper device for complete elimination of interferents in enzymatic electrochemical biosensors

Bo Liang[a], Qin Zhu[a], Lu Fang[b], Qingpeng Cao[a], Xiao Liang[c], Xuesong Ye[a,*]

[a] Biosensor National Special Laboratory, Key Laboratory of Biomedical Engineering of Ministry of Education, College of Biomedical Engineering and Instrument Science, Zhejiang University, Hangzhou 310027, PR China
[b] College of Life Information Science and Instrument Engineering, Hangzhou Dianzi University, Hangzhou 310018, PR China
[c] Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou 310016, PR China

ARTICLE INFO

Keywords:
Origami paper devices
Interference elimination
Electrochemical biosensors
Coulometry

ABSTRACT

Many efforts have been made to prevent interferences in enzymatic electrochemical biosensors by permselective membranes or mediators with low redox potential. However, it is difficult to completely eliminate interferents without compromised sensitivity by these traditional procedures. We propose here a method based on an origami paper device that separates the electrochemical reactions of interferents and substrates for complete depletion of interferents and precise analysis of substrates. Interferents such as ascorbate, urate and paracetamol were completely consumed by a simple electrolysis step, while substrates were quantitatively analyzed by coulometry. With GOx as a model enzyme, an interference-free and calibration-free coulometric glucose biosensor has been demonstrated successfully. The proposed origami paper device provides a facile and easy-controlled approach to eliminate the electroactive interferents completely for enzymatic electrochemical biosensors.

1. Introduction

Electrochemical biosensors have received increasing attention in the field of clinical analysis, food industry, and environmental monitoring, due to their remarkable features such as high sensitivity, simple instrumentation, and low production cost [1–3]. A key performance for electrochemical biosensors is their selective response to a particular substrate. Enzymes are widely used as bio-recognition elements in biosensing devices with high bio-catalytic activity and specificity [4]. Many enzymatic electrochemical biosensors have been developed for the detection of glucose, lactate, uric acid, and cholesterol [5–8]. Although, the oxidation of substrates like glucose by means of the catalytic activity of enzyme is highly specific, the detection principle of electrochemical biosensors, usually by amperometric detection of the formed products like hydrogen peroxide or reduced mediator, is unspecific. Therefore, electroactive interferents such as ascorbate, urate, and paracetamol, frequently found in biological samples will contribute to the sensor response [9,10].

A considerable number of studies have aimed to improve the selectivity of enzymatic electrochemical biosensors [11]. Coating a permselective membrane onto the electrode is a widely adopted approach in biosensor fabrications [12,13]. Polymers like Nafion, poly (phenylenediamine), or polyphenol eliminate the interferents by electrostatic repulsion or size [14–16]. However, coating the electrodes with permselective membranes also inhibits the diffusion of substrates, which increases the response time but reduces the sensitivity of the electrodes. What's more, it's very difficult to control the pore size, uniformity and the thickness of the membranes in fabrication procedures. The other strategy used to prevent interference in enzymatic electrochemical biosensors is lowering the operating potential by incorporating electron-carrying mediators or by direct electron transfer (DET) between enzyme and electrode [17,18]. In a mediator-based biosensor system, mediators like metal complexes, organic dyes, conducting organic salts are carefully selected according to enzyme types to provide low redox potential [19–21]. Although mediator based biosensors can effectively avoid most electroactive interfering species by lowering the electrode potential, oxygen still can compete with the mediators in the oxidation of the reduced enzyme thus decrease the current response. Moreover, interferents such as ascorbic acid can also reduce mediators, resulting in a compromised biosensor sensitivity [22]. DET avoids intermediate electron transfer steps arising from self-exchange reactions [23]. However, DET from enzyme to electrode is difficult to realize, since the prosthetic groups of most enzymes are deeply embedded within a protective protein shell [24–26]. Another

* Corresponding author.
E-mail address: yexuesong@zju.edu.cn (X. Ye).

http://dx.doi.org/10.1016/j.elecom.2017.07.001
Received 25 May 2017; Received in revised form 22 June 2017; Accepted 3 July 2017
Available online 04 July 2017
1388-2481/

feasible method to improved selectivity is removal of electroactive interferents in diffusion layer by electrochemical depletion [27,28]. However, selectively electrolysis of interferents without depletion of target substrates is a new problem because the electroactive products generated by enzyme-catalyzed reaction co-exist with interferents [29]. In summary, it is still a challenge to completely prevent interferences in enzymatic electrochemical biosensors.

Here, we proposed a facile and easy-controlled approach to completely eliminate interferents based on an origami paper device. Origami paper devices have been developed for colorimetric and fluorescence assays, enzyme-linked immunosorbent assays, and potentiometric biosensors with advantages of three-dimensional flow control, separated reagents location, and time-controlled reaction [30–33]. To the best of our knowledge, an origami paper device for interference prevention has not been reported. The aim of this work is to eliminate the electroactive interferents completely by a simple approach.

2. Experimental

Whatman No.1 paper was cut into a 1 cm × 2 cm piece and folded lengthwise to form an origami structure with two tabs (a base tab and a cover tab). A 7 mm hole in the center of the base tab was made by a punch. Wax pellets were put in a beaker and heated until they melted using a hotplate with the temperature kept in the range of 120–130 °C. The origami device was dipped into the chamber of melted wax for 5 s and cooled to room temperature in air. Then, the base tab was pasted onto a screen printed electrode (DS550, purchased from Dropsens), forming an electrochemical cell chamber (7 mm diameter, 180 μm height). The diameter of the working electrode is 4 mm. Enzymes were loaded on the cover tab by dropping 6 μL enzyme solution (5 U GOx, EC 1.1.3.4, from *Aspergillus niger* and 5 U mutarotase, EC 5.1.3.3, from porcine kidney) on the center position of the cover tab and dried in air.

All electrochemical experiments were performed in an electrochemical workstation (μAutolab III, Metrohm, Switzerland). In experiments, 6 μL buffer solution of 0.01 M PBS was dropped in the reservoir formed by the base tab on the screen printed electrode, covering three electrodes. An operating potential of 0.7 V vs. Ag/AgCl was applied to the working electrode. Then, 0.5 μL sample solution was syringed into the buffer solution, the catalytic current attributed to the oxidation of interferents could be observed directly. For glucose determination, the cover tab was folded onto the reservoir, enzymes preloaded on the tab were dissolved into electrolyte solution, catalyzing glucose to form H_2O_2 for detection.

3. Results and discussion

3.1. Operation principle

As shown in Scheme 1A, the origami paper device is comprised of a base tab and a cover tab. Both tabs were dipped with wax to provide hydrophobic surface [34,35]. The base tab was pasted onto a screen printed electrode. Here, a commercial screen printed electrode was used to simplify and unify the demonstration. For low-cost applications, paper-based electrochemical electrodes could be adopted as substitutes [36–38]. Enzymes were loaded on the cover tab rather than on the working electrode, making it possible to control the successive electrochemical reactions of interferents and substrates by a simple folding operation.

There are only two steps for precise analysis of substrates with complete elimination of electroactive interferents. First step, with applying an operating potential to the working electrode, electroactive interferents are depleted by electrolysis on the working electrode (Scheme 1B). Substrates like glucose will not be consumed at this step because enzymes are not preloaded on the working electrode and substrates cannot be electrolyzed on electrode directly at the applied potential. Second step, the cover tab is folded onto the reservoir, enzymes preloaded on the tab are dissolved into electrolyte solution and catalyze substrates to form the electroactive products for detection (Scheme 1C). It should be noted that, if some interferents won't be depleted in the first step, they won't contribute to the detection current in the second step either. That is to say, all electroactive interferents that will contribute to the sensor response can be completely eliminated. Here, glucose oxidase (GOx), a widely used analytical enzyme in electrochemical biosensors [39], was selected as a model enzyme for demonstration. GOx catalyzes the oxidation of glucose to gluconolactone and generates hydrogen peroxide (H_2O_2) simultaneously, while H_2O_2 is oxidized directly on the working electrode.

3.2. Electrochemical depletion of electroactive interferents

As shown in Fig. 1A, when 0.5 μL of a mixed solution of 4 mM glucose and interferents (0.5 mM ascorbate (AA), urate (UA) and paracetamol (PA)) was syringed into a 6 μL buffer solution in reservoir, a current response was observed immediately according to electrolysis of interferents. After that, the current decreased back to the baseline as the electrolysis reaction came to a completion. When the cover tab folded onto the reservoir, a second current response according to bioelectrocatalytic oxidation of glucose was observed. After a reaction time, the current decreased back to the baseline again as the glucose was consumed. The integrated charge vs. time curve of baseline-corrected current response was illustrated in Fig. 1A with dashed line. The integrated charges were 0.142 ± 0.006 mC (n = 5, n: number of repetition) and 0.381 ± 0.012 mC (n = 5) with the electrolysis efficiency 98% ± 4% (n = 5) and 99% ± 3% (n = 5) respectively for interferents and glucose, illustrating that interferents were completely consumed in the first step, while glucose was completely oxidized in the second step. The electric charge was obtained by integrating the current response. The electrolysis efficiency was obtained by dividing the integrated charge quantity by the theoretical value, which is calculated as $Q = nFN$, according to Faraday's law, where n, F, and N are the number of electrons (in case of this study, $n = 2$), the Faraday constant, and the amount of interferents or substrates, respectively [40]. Here, mutarotase was preloaded on the cover tab with GOx to accelerate the mutarotation reaction of α-D-glucose to β-D-glucose, since that GOx is specifically to β-D-glucose but does not act on α-D-glucose [41,42].

Fig. 1B shows the electrochemical depletion of individual interferents of AA, UA, and PA. 0.5 μL sample solutions of 0.5 mM AA, UA, and PA was syringed successively into 6 μL buffer solution in reservoir. As shown, AA, UA, and PA were completely consumed within 150 s, 120 s, and 200 s, while the electrolysis efficiencies were 98% ± 3% (n = 5), 97% ± 4% (n = 5) and 99% ± 3% (n = 5) respectively. The difference of times required for complete oxidation of AA, UA, and PA should be attributed to their electroactive properties and electrode reaction mechanisms. What's more, when glucose solution was added to the buffer solution, no current response was observed, indicating that glucose would not be electrolyzed on working electrode directly.

3.3. Quantitative determination of glucose by coulometry

The results above also indicate that the proposed device can be used for interference-free detection of glucose by coulometry. Coulometry is proposed as a method of absolute quantitative analysis, converting all the measured substance into electric charge and thus inherently needs no calibration procedure [43]. Furthermore, coulometric technology is not affected by the kinetics of the enzymatic reaction and mass-transfer processes [40]. This allowed coulometry to be an adequate methodology for calibration-free analysis in aqueous systems [44,45].

Fig. 2A shows the dependence of glucose concentration on the current response (dashed lines, left axis) and the charge (solid lines, right axis). 0.5 μL sample solutions of glucose with different concentration with interferents (0.5 mM AA, UA and PA) were syringed