Biosensors and Bioelectronics 51 (2014) 158–163

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Co-immobilization of glucoamylase and glucose oxidase for electrochemical sequential enzyme electrode for starch biosensor and biofuel cell

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ARTICLE INFO

Article history:
Received 19 April 2013
Received in revised form
9 July 2013
Accepted 10 July 2013
Available online 30 July 2013

Keywords:
Glucoamylase
Glucose oxidase
Sequential enzyme biosensor
Starch biosensor
Biofuel cell

ABSTRACT

A novel electrochemical sequential biosensor was constructed by co-immobilizing glucoamylase (GA) and glucose oxidase (GOD) on the multi-walled carbon nanotubes (MWNTs)-modified glassy carbon electrode (GCE) by chemical crosslinking method, where glutaraldehyde and bovine serum albumin was used as crosslinking and blocking agent, respectively. The proposed biosensor (GA/GOD/MWNTs/GCE) is capable of determining starch without using extra sensors such as Clark-type oxygen sensor or H_2O_2 sensor. The current linearly decreased with the increasing concentration of starch ranging from 0.005% to 0.7% (w/w) with the limit of detection of 0.003% (w/w) starch. The as-fabricated sequential biosensor can be applicable to the detection of the content of starch in real samples, which are in good accordance with traditional Fehling's titration. Finally, a stable starch/O_2 biofuel cell was assembled using the GA/GOD/MWNTs/GCE as bioanode and laccase/MWNTs/GCE as biocathode, which exhibited open circuit voltage of ca. 0.53 V and the maximum power density of 8.15 μW cm^{-2} at 0.31 V, comparable with the other glucose/O_2 based biofuel cells reported recently. Therefore, the proposed biosensor exhibited attractive features such as good stability in weak acidic buffer, good operational stability, wide linear range and capable of determination of starch in real samples as well as optimal bioanode for the biofuel cell.

1. Introduction

As one of the most general carbohydrates in crops, starch is usually used as food processing auxiliary to improve the taste and nutrition, and can also be used as filler of composite materials for the degradation of certain synthetic polymers due to its character of innocuity and easy degradation (Star et al., 2004). Due to its cost-effectivity, starch is also considered as a good fuel for biofuel cells. Desirable technological, organoleptic, and nutritional properties in the end products are all dependent on the addition of starch in the processes such as the baking of bread, the production of pasta products and starch-based snack foods, breakfast cereals, pregelatinized flour, baby foods, and parboiled cereals (Olkku and Rha, 1978; Lineback and Wongsrikasem, 1980; Lund and Lorenz, 1984). The level of starch content in food or pill is a vital parameter in quality inspection of brewing, food industry and pharmacy. Traditional ways to detect starch include polarimetric (Garcia and Wolf, 1972) and the Fehling titration method (Menyhert, 1908), however, they are complex and time-consuming in sample pretreatment. Especially, the results of Fehling titration method are greatly affected by the interference from other possible reduced sugars co-existed in the sample. On the other hand, the enzyme-based electrodes in combination with hydrogen peroxide sensors (Cordonnier et al., 1975; Mascini et al., 1983) or oxygen sensors (Coulet and Bertrand, 1979; Bardeletti and Coulet, 1987) were reported for starch measurement, which were based on the determination of the reduced saccharides, the hydrolytic products of starch, nevertheless, it is laborious. Sequential biosensors containing two or more enzymes which catalyze substrate in sequence, are mostly used in the determination of disaccharides (Zhang and Rechnitz, 1994; Zhang, 2000), starch (Abdul Hamid et al., 1990) and cholesterol (Motonaka and Faulkner, 1993). The performance of this kind of biosensors greatly depends on the amount, ratio, and distribution control of two enzymes as well as the immobilization methods (Zhou et al., 2001). However, the sensitivity and operational stability are usually not so satisfactory

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http://dx.doi.org/10.1016/j.bios.2013.07.021

compared with single enzyme biosensor, probably arising from the complexity in enzyme membrane preparation (Zhou et al., 2001). Electrochemical sequential electrode was also used in the surface-displaying enzyme microbial fuel cell (Bahartan et al., 2012).

Biofuel cell (BFC) which employs enzymes or/and microorganisms as the biocatalysts for the production of electricity from renewable organic matter, represents a new kind of green power sources and has attracted much attention in recent years (Bullen et al., 2006; Du et al., 2007; Cracknell et al., 2008). There are intensive studies on the design and characterization of enzyme-based BFCs, however, most of work has been focused on using monosaccharides such as glucose or xylose as fuels (Li et al., 2009; Gao et al., 2010; Wen et al., 2011; Zebda et al., 2011; Xia et al., 2013). In comparison with monosaccharides, starch represents an alternative energy source with lower cost and easier processing procedures. By far, starch has been used as energy resource in microbial fuel cells (Velasquez-Orta et al., 2011; Herrero-Hernandez et al., 2013). However, there are no reports on BFCs based on sequential enzyme bioelectrocatalysis of starch.

Glucoamylase (GA, α-1,4-glucan-gluco-hydrolase, EC. 3.2.1.3) is a starch hydrolyzing enzyme which catalyzes the hydrolysis of α-(1,4) glycosidic bonds at the non-reducing end of starch polymer to release free glucose (Marin-Navarro and Polaina, 2011). GA is an important enzyme extensively used in bio-industry for production of starch sugar, alcohol and single-cell protein (Velasquez-Orta et al., 2011; Yamakawa et al., 2012). As an essential polysaccharide hydrolase, GA is widely used in the hydrolysis of starch into glucose before their measurement with either Fehling's titration or electrochemical method (Abdul Hamid et al., 1990; Zhang and Rechnitz, 1994; Zhang, 2000).

In the present study, we constructed a sequential biosensor based on the co-immobilization of GA and glucose oxidase (GOD) for the determination of starch. With the incorporation of carbon nanotubes, which could facilitate the direct electron transfer between electrode and GOD, the redox center (flavin adenine dinucleotide, FAD) of GOD presented direct electrochemistry. The reduction peak current decreased with the increasing of glucose in solution based on the oxygen consumption (Wang et al., 2009). The proposed biosensor enabled to determine starch without the measurement of H_2O_2, thus simplified starch biosensor and biofuel cell. To the best of our knowledge, this is the first report on the construction of starch biosensor without extra sensors such as Clark-type oxygen sensor or H_2O_2 sensor. Finally, a starch/O_2 biofuel cell was assembled using the GA/GOD/MWNTs/GCE electrode as bioanode and laccase/MWNTs/GCE as biocathode, which exhibited open circuit voltage up to ca. 0.53 V and the maximum power density of 8.15 μW cm^{-2} at 0.31 V, comparable with the other glucose/O_2 based biofuel cells reported recently.

2. Materials and Methods

2.1. Chemicals and reagents

Glucose oxidase (GOD), laccase and bovine serum albumin (BSA) were purchased from F. Hoffmann-La Roche, Ltd. GA, starch and glutaraldehyde were purchased from Sinopharm Chemical Reagent Co., Ltd. Starch solution was prepared by suspending suitable amount of starch powder into 0.1 M phosphate buffer under heat to boiling in microwave oven, which was cooled down at room temperature before use.

The specific enzymatic activity of GA is defined as the amount of glucose (in μmol) generated by 1 mg GA per minute in the excess of starch, while the specific enzymatic activity of GOD is defined as the amount of glucose (in μmol) consumed by 1 mg GOD per minute in the excess of glucose. Their activities were measured separately by spectrophotometry method, showing that 1 mg GA could generate 118 μmol glucose from starch per minute while 1 mg GOD could consume 297 μmol glucose per minute at the same condition.

2.2. Preparation of sequential biosensor

The sequential biosensor was fabricated on a glassy carbon electrode (GCE, diameter of 3 mm) which was polished to a mirror finish using 0.3 and 0.05 μm alumina slurry, followed by rinsing thoroughly with deionized water. After ultrasonic processing in anhydrous ethanol and ultrapure water, respectively, the electrode was rinsed with ultrapure water and dried at room temperature.

In preparation of sequential biosensor, 5 μL of multiwalled carbon nanotubes (MWNTs) suspension (2 mg MWNTs dispersed in 1 ml ultrapure water with ultrasonic processing) was dripped on the inverted GCE surface and dried in air. Next, different volumes of GOD solution (3000 U/ml) and GA solution (1200 U/ml), 2 μL of BSA (1% w/w) and 5 μL of glutaraldehyde (1% w/w) were mixed together on the inverted GCE to fabricate various modified electrodes and dried overnight at 4 °C in refrigerator. A glucose biosensor was also constructed with the similar method in which 6 μL of GOD solution (3000 U/ml), 2 μL of BSA and 5 μL of glutaraldehyde were applied.

2.3. Apparatus and electrochemical measurements

Electrochemical measurements were performed using a CHI660D potentiostat (CH Instruments, Chenhua, Shanghai, China). The electrochemical response was measured in a conventional three-electrode system using a chemically modified GCE as working electrode, a Pt wire auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. All potentials were reported in this context with respect to this reference. All measurements were performed at room temperature (~23 °C).

2.4. Preparation of biofuel

The one-compartment biofuel cell contained GA/GOD/MWNTs/GCE employed as the bioanode and the laccase/MWNTs/GCE as biocathode, which were assembled together in 5 ml of 0.5% (w/w) starch (pH 5.0) solution. In the biocathode fabrication, laccase was used as biocatalyst to catalyze oxygen reduction to water. To improve the bioelectrocatalysis efficiency of the laccase based biocathode towards O_2 reduction, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was used as a redox mediator.

3. Results and discussions

3.1. Construction of sequential biosensor

The sequential biosensor was constructed by co-immobilizing GA and GOD on the MWNTs-modified GCE by chemical crosslinking method, where glutaraldehyde and BSA was used as crosslinking and blocking agent, respectively (Fig. 1A). The thus-prepared bioelectrode is denoted as GA/GOD/MWNTs/GCE. Cyclic voltammograms (CVs) of different modified electrodes are shown in Fig. 1B. No redox peaks could be found for both GA/GCE and GA/GOD/GCE in the presence of starch (Fig. 1B, curves a, b). Only increased background current was observed at GA/MWNTs/GCE in the presence starch solution (Fig. 1B, curve c). A pair of well-defined redox peaks were clearly observed at GA/GOD/MWNTs/GCE in bare phosphate buffer (Fig. 1B, curve f), which meant the direct electron transfer between enzyme (GOD) and electrode was facilitated by MWNTs through the redox center FAD/$FADH_2$