Biochemical Engineering Journal 114 (2016) 262–267

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Complex of hydrogel with magnetic immobilized GOD for temperature controlling fiber optic glucose sensor

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ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form 4 July 2016
Accepted 20 July 2016
Available online 21 July 2016

Keywords:
P(NIPAAm-co-AAm)
PMIGC
Temperature controlling
Fiber optic glucose sensor

ABSTRACT

A fiber optic glucose biosensor based on Poly(N-isopropylacrylamide-co-acrylamide) (P(NIPAAm-co-AAm))-magnetic-immobilized glucose oxidase (GOD) complex (PMIGC)was fabricated to perform the controllable detection of glucose by changing temperature. The complex was prepared by combining P(NIPAAm-co-AAm) with GOD immobilized on Fe_3O_4@SiO_2(F)@meso-SiO_2 nanoparticles using in-situ complex method. Since P(NIPAAm-co-AAm) has the lower critical solution temperature (LCST) of 36 °C, at the temperature above LCST, P(NIPAAm-co-AAm) shrank and PMIGC could not catalyze the oxidation of glucose. At the temperature below LCST, P(NIPAAm-co-AAm) swelled and catalysis occurred. Because of this temperature sensing characteristics of PMIGC, the temperature controllable detection of glucose can be carried out by the biosensor. The optimal detection conditions for this biosensor were achieved with pH6.5, 35 °C and 12 mg of GOD amount. At 25 °C, a good linear relationship between ϕ, the phase delay change of the sensor head, and the glucose concentration in the range of 50–700 mg/dL (2.78–38.89 mmol/L) was observed, the detection limit was 8.33 mg/dL (0.46 mmol/L) (S/N = 3). This linear graph can be defined by the equation of $y = 0.01463 + 0.0003313x$, $R^2 = 0.9914$. The biosensor has the characteristics of good repeatability and selectivity, and can be used for the glucose detection in practical samples.

1. Introduction

Glucose is very important for the life cycles but the high level of glucose in blood could cause diabetes. In recent years, more and more people around the world are suffering from the long-term risks of diabetes complications, such as heart disease, cardiovascular diseases and blindness [1,2]. Therefore, it is very urgent to develop a fast, reliable and economic method for the determination of blood glucose concentration.

Over the last few decades, many methods have been developed to detect glucose, including high performance liquid chromatography (HPLC) [3,4], colorimetry [5], spectrophotometry [6], chemiluminescence [7,8], electrode [9–11], electrochemical sensor [12,13], quantum dots sensing [14–16] and optical sensing [17–19]. Fiber optic biosensors have many advantages compared with electrical sensors and electrochemical sensors [20]. As a branch of fiber optic sensors, the enzyme based fiber optic biosensors have great application potential in many fields and will be an effective way to detect glucose. If the enzyme performance can be controlled, the detection of glucose by the enzyme based fiber optic biosensors can be controlled, which will have important applications in many cases.

Stimuli-sensitive hydrogels have tunable three-dimensional physical network structures and good biocompatibility [21]. Their volumes will change with a slight variation of external stimuli, such as temperature, light, chemical environment, etc [22]. Thermo-sensitive hydrogels have a lower critical solution temperature (LCST), and will swell at the temperature lower than LCST and shrink at the temperature higher than LCST. Because of this characteristic, hydrogels have been extensively used as the carriers for the temperature controlled release of macromolecular drugs [23–25]. Poly(N-isopropylacrylamide) (PNIPAAm) is a well known temperature sensitive polymer with a LCST of ~32 °C in aqueous solution [26]. The introduction of segment acrylamide (AAm) to the PNIPAAm could form Poly(N-isopropylacrylamide-co-acrylamide) (P(NIPAAm-co-AAm)), which could slightly increase its LCST [23] and made the LCST close to human body temperature. If P(NIPAAm-

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http://dx.doi.org/10.1016/j.bej.2016.07.012
1369-703X/

co-AAm) is used to form a complex with enzyme, the hydrogel will shrink at the temperature higher than its LCST and the enzyme is isolated from the substrate, which will cause the enzymatic catalysis reaction to stop. On the other hand, when the temperature is lower than its LCST, the hydrogel will swell, which allows the enzyme to have contact with the substrate and cause the enzymatic catalysis reaction to take place. Therefore, the enzyme performance can be controlled by changing the temperature. This kind of complex materials can be used for the fiber optic biosensor to perform controllable detection, and also for multi parameters fiber optic biosensors which can detect different species such as glucose and cholesterol at different temperature.

In our previous work [27], the magnetic fluorescent core-shell structured Fe_3O_4@SiO_2(F)-@meso-SiO_2 nanoparticles were prepared. Cholesterol oxidase (COD) was immobilized on their surface and immobilization conditions were investigated and optimized. In this work, Glucose oxidase (GOD) was immobilized on Fe_3O_4@SiO_2(F)@meso-SiO_2 nanoparticles using the similar method [27]. P(NIPAAm-co-AAm) was combined with magnetic immobilized GOD to form P(NIPAAm-co-AAm)-magnetic immobilized GOD complex (PMIGC) using in-situ complex method. The immobilization of GOD will improve the enzyme stability and the nanoparticles with the magnetic core will be beneficial to the isolation of the immobilized enzyme from the reaction mixture. By changing temperature, the oxidation of glucose was controlled using PMIGC as the catalyzer. Using lock-in technology, a fiber optic glucose sensor based on PMIGC was fabricated to perform the controllable detection of glucose and the sensor properties were studied. The sensor can detect glucose effectively, indicating a promising prospect of practical application. To the best of our knowledge, there have been no publications on this temperature controlling fiber optic glucose biosensor based on PMIGC.

2. Experimental

2.1. Materials

Glucose oxidase (GOD) (E.C. 1.1.3.4, 100 U mg^{-1}) was obtained from Aspergillusniger. Glucose, $Ru(bpy)_3Cl_2$ (99.0%) were purchased from Aldrich–Sigma. N-isopropylacrylamide (NIPAAm) was purchased from Aldrich Chemical Co. Inc. (USA), and was recrystallized from benzene/n-hexane. Acrylamide (AAm), Ammonium persulfate (APS), N,N'-Methylenebisacrylamide (BIS), N,N,N',N'-Tetramethylethylenediamine (TEMED) were purchased from Sinopharm Chemical Reagent Co. All reagents were of analytical grade and used without further purification. Double-distilled water was used throughout the experiments. The oxygen sensing membrane was prepared according to our previously work [28].

2.2. Preparation of PMIGC

Fe_3O_4@SiO_2(F)@meso-SiO_2 nanoparticles were prepared according to our previous method [27]. GOD was immobilized on the nanoparticles using the similar chemical crosslinking method [27] to form the magnetic immobilized GOD (Fe_3O_4@SiO_2(F)@meso$-SiO_2$@GOD). The preparation procedure of PMIGC was depicted as follows. 150 mg NIPAAm, 18 mg AAm, 7 mg BIS were dissolved in 3 mL of immobilized enzyme solution containing 90 mg nanoparticles and 12 mg GOD. The mixture was stirred at room temperature for 30 min under the protection of nitrogen. 30 μL of TEMED and 70 μL of APS (5 wt%) were added in it. The mixture was kept at −20 °C for 12 h and PMIGC was formed. The product was immersed in distilled water for 48 h and the water was refreshed every several hours in order to allow the unreacted chemicals to leach out. PMIGC was stored in distilled water at 4 °C. The LCST of PMIGC was determined according to the reported method [29].

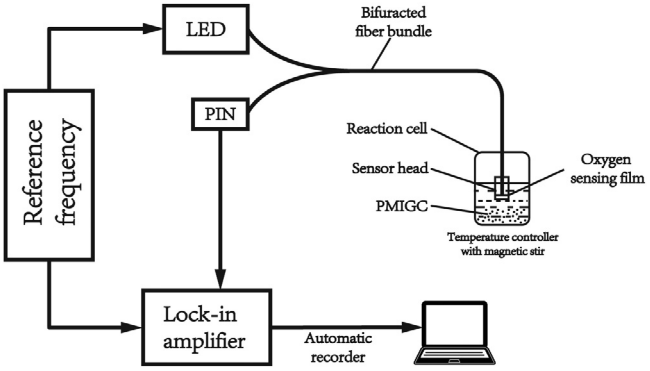

Fig. 1. Schematic diagram of the detecting system.

2.3. Preparation and principle of temperature controlling fiber optic glucose sensor

The detecting system is shown in Fig. 1. It consists of a lock-in amplifier, a light source of light emitting diode (LED) with an excitation wavelength of 416 nm, a sensor head with an oxygen sensing membrane, a temperature controller, and a computer for data processing.

This sensor was based on the fluorescence quenching and oxygen consumption. GOD catalyzed the oxidation of glucose and the oxygen in the solution would be consumed. By detecting the fluorescence of $Ru(bpy)_3Cl_2$ quenched by oxygen the oxygen concentration change was detected. Since a lock-in amplifier is used, the quenching could be described as

$$\frac{\tan\phi_0}{\tan\phi} = 1 + K_{sv}[Q] \quad (1)$$

Where ϕ_0 and ϕ are the phase delay change of the sensor in the absence and presence of the oxygen, respectively, and K_{sv} is the Stern-Volmer constant. [Q] is the oxygen concentration. The quantification of glucose is achieved by detecting the data of phase delay change ϕ.

2.4. Measurements

The detection of glucose concentration was performed with the setup shown schematically in Fig. 1. The sensor head was placed into a tiny reaction cell containing glucose buffer solution and PMIGC. In order to eliminate the interference of oxygen from the open air, an entire airtight reaction cell was introduced. A temperature controller was used to control the temperature of the reaction cell. The fluorescence signal was collected by positive intrinsic negative (PIN) and guided to the lock-in amplifier through the output bundle, and then transferred to phase-delay which was collected by the computer. After a simple washing of the sensor head and PMIGC with buffer solution, the following measurement could be performed. The magnetic core of immobilized GOD could make the isolation of PMIGC from the reaction solution and buffer faster and easier. All the measurements were performed in triplicate.

2.5. Characterizations

The morphology of P(NIPAAm-co-AAm) and PMIGC was observed using a field emission scanning electron microscope (FESEM) (JSM-5610LV, JEOLLtd., Japan) operated at 10 kV. Before FESEM test, the swollen hydrogel samples were quickly frozen in liquid nitrogen and then lyophilized in a freeze drier (ALPHA

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