Available online at www.sciencedirect.com
ScienceDirect
Talanta 71 (2007) 391–396
Talanta
www.elsevier.com/locate/talanta

Development of a long-life capillary enzyme bioreactor for the determination of blood glucose

Ja-an Annie Ho [a,b,*], Li-chen Wu [b], Nien-Chu Fan [a], Ming-Shih Lee [c,d], Hung-Yi Kuo [b], Chung-Shi Yang [b]

[a] *Department of Chemistry, National Tsing Hua University, Hsinchu 300, Taiwan*
[b] *Department of Applied Chemistry, National Chi-Nan University, Puli, Nantou 545, Taiwan*
[c] *Department of Medical Laboratory, Taichung Veterans General Hospital, Taichung 407, Taiwan*
[d] *Department of Medical Technology, Chung Shan Medical University, Taichung 402, Taiwan*

Received 11 January 2006; received in revised form 11 April 2006; accepted 11 April 2006
Available online 5 June 2006

Abstract

A long-life capillary enzyme bioreactor was developed that determines glucose concentrations with high sensitivity and better stability than previous systems. The bioreactor was constructed by immobilizing glucose oxidase (GOx) onto the inner surface of a 0.53 mm i.d. fused-silica capillary that was part of a continuous-flow system. In the presence of oxygen, GOx converts glucose to gluconic acid and hydrogen peroxide (H_2O_2). Hydrogen peroxide detection was accomplished using an amperometric electrochemical detector. The integration of this capillary reactor into a flow-injection (FIA) system offered a larger surface-to-volume ratio, reduced band-broadening effects, and reduced reagent consumption compared to packed column in FIA or other settings. To obtain operational (at ambient temp) and storage (at 4 °C) stability for 20 weeks, the glucose biosensing system was prepared using an optimal GOx concentration (200 mg/mL). This exhibited an FIA peak response of 7 min and a detection limit of 10 μM (S/N = 3) with excellent reproducibility (coefficient of variation, CV < 0.75%). It also had a linear working range from 10^1 to 10^4 μM. The enzyme activity in this proposed capillary enzyme reactor was well maintained for 20 weeks. Furthermore, 20 serum samples were analyzed using this system, and these correlated favorably (correlation coefficient, $r^2 = 0.935$) with results for the same samples obtained using a routine clinical method. The resulting biosensing system exhibited characteristics that make it suitable for *in vivo* application.

Keywords: Continuous-flow system; Flow-injection analysis; FIA; Glucose; Blood glucose; Glucose oxidase

1. Introduction

Human body needs to maintain blood glucose within a very narrow range of 70–110 mg/dL. People who have diabetes or increased fasting levels of glucose have elevated blood glucose levels because of an inability to use insulin properly. This is often referred to as insulin resistance. Statistics show that diabetes has reached epidemic levels in the U.S. because of increased incidence among older Americans, as well as more obesity in the population. About 2200 people are diagnosed with diabetes each day, but about one-third of the individuals who have diabetes are not aware of it until one of its life-threatening complications has developed. Diabetes results in long-term health consequences, including cardiovascular disease, nephropathy, neuropathy, diabetic retinopathy and blindness. Recent research has indicated that hyperglycemia is common in critically ill patients, even in those without diabetes mellitus. It has been reported that aggressive glycemic control may reduce mortality in this population [1]. However, the relationship among mortality, the control of hyperglycemia, and the administration of exogenous insulin is still unclear. Therefore, it is very important to have a simpler, more-stable, and more-sensitive method that allows the monitoring of blood glucose in clinics and laboratories.

The glucose sensor reported by Clark and Lyons in 1962 [2] has generally been recognized as the first biosensor. Since then, many types of sensors have been developed for medical diagnosis applications. The use of glucose oxidase (GOx)-based electrodes is a well-established method of detection for *in vivo* levels of circulating glucose [3–5]. In this approach, glucose is converted to gluconic acid and easily detectable hydrogen perox-

* Corresponding author. Tel.: +886 3571 5131x31286; fax: +886 3 571 1082.
E-mail address: jaho@mx.nthu.edu.tw (J.-a.A. Ho).

0039-9140/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2006.04.023

ide (H_2O_2) by the enzyme glucose oxidase. The process requires oxygen as a cosubstrate. The produced H_2O_2 is then measured using a charged platinum electrode surface. Hydrogen peroxide has become by far the most widely used method of signal transduction in enzyme biosensors, and a majority of all biosensors (65%) use hydrogen peroxide detection [6]. According to Wilson and Thevenot [7], the construction of a hydrogen peroxide sensor usually involves a platinum anode and a silver/silver chloride cathode. When the anode is poised at +0.6–0.7 V [8], the plateau of oxidation of peroxide is reached at the anode. The enzyme employed in the construction of hydrogen peroxide-producing biosensors frequently involves the immobilization of oxido-reductases to the surface of the sensor by glutaraldehyde cross-linking [8]. Other methods have been reported for the immobilization of enzyme, such as physical deposition onto solid supports, covalent binding [9], and entrapment within a polyer matrix [10]. In recent years, sol–gel technology has been widely used to entrap enzyme for different uses [11–14], because it retains better enzyme activity compared to the free enzyme. Matrixes are usually prepared under ambient conditions and exhibit tunable porosity, high thermal stability, and chemical inertness [12]. However, the silica sol–gel matrixes have some drawbacks, including fragility, complicated preparation procedures, and a tendency to be hydrolyzed at high acidity, which often results in the loss of enzyme stability and also limits their application and feasibility in the development of electrochemical sensors [15,16].

Many methods have been developed in an effort to find a noninvasive detection system for circulating glucose at *in vivo* levels, including ultrasound-assisted transdermal monitoring, electromagnetic-based sensor, and fluorescence-affinity hollow-fiber sensors [17–27]. Other methods of glucose determination that have been reported include those based on a genetically engineered protein [28], on concanavalin A [29], and on a microcantilever [30].

In this work we develop a longer-life capillary enzyme bioreactor for the determination of glucose. The greatly improved activity and stability of this new enzyme bioreactor is facilitated by the direct attachment of GOx to the wall of a 530 μm i.d. fused-silica capillary. To the best of our knowledge this is the first demonstration of the capillary glucose oxidase bioreactor with improved enzyme stability and longer shelf life, which provides a higher surface-to-volume ratio, maximizing the interaction between glucose and GOx compared to bead-packed column. The results from blood sample analysis promised well for the use of this biosensing system in online blood glucose monitoring of critically ill patients before and after surgical operations. Reduced mortality can therefore be achieved by intensive glycemic control.

2. Materials and methods

2.1. Reagents and materials

D-(+)-Glucose, glucose oxidase (glucose: oxygen oxidoreductase E.C. 1.1.3.4, from *Aspergillus niger*, 181.6 U/mg), 3-glycidoxy propyltrimethoxysilane, potassium carbonate, sodium metaperiodate, sodium cyanborohydride and triethanolamine, glycine, and Trizma® Base tris[hydroxymethyl] aminomethane (Tris) were obtained from Sigma Chemicals Co. (St. Louis, MO). The fused-silica capillary (0.53 mm i.d.) was obtained from Alltech (Deerfield, IL). All other inorganic chemicals and organic solvents were of reagent grade or better and were purchased from Aldrich Chemical Co. (St. Louis, MO). The pre-analyzed blood plasma samples from patients were obtained fresh from the Veteran General Hospital—Taichung (VGHTC). The use of these samples in no way contradicts the Helsinki Declaration. De-ionized distilled water was obtained from a Milli-Q system (Milford, MA).

2.2. Methods

2.2.1. Capillary modification

For a sensitive flow-injection analysis (FIA) enzyme reactor, we required a high-enzyme loading comparable to the dead volume of the bed. For such an enzyme assay, the immobilization support must be rigid and have a mild, very stable, covalent immobilization chemistry. Our group has previously demonstrated the successful immobilization of biomolecules such as antibody without loss of activity and decreased stability on the inner surface of capillary column precoated with a glycerylpropyl layer to minimize the adsorption of the analyte. In the current study the microcapillary enzyme reactor was modified based on previously described procedures [31–36]. Detailed modification procedures were as follows:

Step 1: The 85 cm fused-silica capillary (0.53 mm i.d.) was treated with 1 M NaOH overnight.
Step 2: 1 M HCl and distilled water were used to rinse the capillary, which was subsequently filled with 3-glycidoxypropyltrimethoxysilane (GPTMS) and heated at 90 °C for 2 h.
Step 3: The capillary was rinsed and treated with 10 mM sulfuric acid at 90 °C for 10 min to convert the residual epoxy groups to diols.
Step 4: After washing with distilled water, diols were cleaved and oxidized to aldehydes with sodium metaperiodate containing potassium carbonate at room temperature for 2 h.
Step 5: 190 μL of GOx (200 mg/mL) and sodium cyanborohydride (5 mg/mL) in 0.1 M phosphate buffer (pH 7.3) were passed slowly into the capillary and incubated overnight to reduce the Schiff base.
Step 6: The capillary was rinsed with 0.2 M triethanolamine buffer (pH 8.2), 1 M NaCl, 0.1 M glycine/HCl buffer (pH 2.5), and Tris buffered saline (TBS), pH 7.0, sequentially. Finally, the capillary enzyme reactor, filled with TBS (pH 7.0), was then stored at 4 °C until use. In this way glucose oxidase was covalently attached on the inner wall of capillary column.

2.3. Flow-injection analysis system

The flow-injection analysis system (schematic diagram shown at Fig. 1) consists of a Hewlett Packard 1050 HPLC pump (Agilent, Foster City, CA) at the inlet of the capillary glu-