Biosensors and Bioelectronics 24 (2009) 1792–1795

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Inner epidermis of onion bulb scale: As natural support for immobilization of glucose oxidase and its application in dissolved oxygen based biosensor

Jitendra Kumar, S.F. D'Souza*

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre Trombay, Mumbai 400085, Maharashtra, India

ARTICLE INFO

Article history:
Received 16 June 2008
Received in revised form 28 July 2008
Accepted 11 August 2008
Available online 26 August 2008

Keywords:
Onion bulb scales
SEM
Glucose oxidase
Dissolved oxygen
Glucose biosensor

ABSTRACT

Inner epidermal membrane of the onion bulb scales was studied as a natural polymer support for immobilization of the glucose oxidase (GOD) enzyme for biosensor application. Onion epidermal membrane was used for immobilization of glucose oxidase and was associated with dissolved oxygen (DO) probe for biosensor reading. Glucose was detected on the basis of depletion of oxygen, when immobilized GOD oxidizes glucose into gluconolactone. A wide detection range between 22.5 and 450 mg/dl was estimated from calibration plot. A single membrane was reused for 127 reactions with retention of ~90% of its initial enzyme activity. Membrane was stable for 45 days (~90% activity) when stored in buffer at 4 °C. Surface structure studies of the immobilized membranes were carried under SEM. To our knowledge, this is the first report on employing inner epidermal membrane of onion bulb scales as the solid support for immobilization of enzyme.

1. Introduction

Immobilization of enzymes on a variety of different matrices has always attracted researchers to improve their functionality and reusability. Immobilized enzymes are used as a biocomponent for development of bioprocess and biosensors. For biosensor, a large number of techniques and support have been used for the immobilization of biocatalyst (Turner et al., 1987; D'Souza, 2001a,b; Kumar et al., 2006; Tembe et al., 2006; Rauf et al., 2006; Kumar and D'Souza, 2008). The choice of support and techniques should be such that it maintains the enzyme activity and has reusability as well as storage stability. A variety of synthetic as well as natural polymers have been exploited for immobilization of enzymes and their use in biosensor preparation. Natural polymers like eggshell membrane and bamboo inner shell membrane have been proved to be useful support for enzyme immobilization for biosensor application (Wu et al., 2004; Yang et al., 2006).

Since natural polymers in living organisms are composed of biomolecules like carbohydrates, lipid and protein, inner epidermal membrane of the onion bulb scales can provide biocompatible microenvironment for the enzyme immobilization. The cell wall is an elaborate extracellular matrix consisting of a microfibrillar cellulose phase and a matrix phase that contains a variety of polymers such as polygalacturonic acid (PGA), hemicelluloses, proteins, and phenolics, including lignin (Carpita and Gibeaut, 1993; Brett and Waldron, 1996). The inner epidermis of the onion bulb scales consists of elongated tubular cells, blunt or tapering ends alongwith numerous guard cells (Scott et al., 1958; Bruce and Hepworth, 2004). Structural features of cell wall of inner epidermis cells are such that it can provide a biocompatible environment and support for enzyme immobilization. This natural membrane is mechanically stronger than the reported other natural membrane because it consists of microfibrillar cellulosic biological components and can be a biocompatible immobilizing support for optimum enzyme activity. Significance of the present work is to employ this new natural polymer as an enzyme immobilization support for biosensor development.

Glucose oxidase (GOD) was selected for immobilization since it is a well studied and applicable in the field of biosensor (Shan et al., 2007; Newman and Turner, 2005). In order to improve the enzyme properties like reusability, operational stability and storage self life, GOD was immobilized on different matrices (Wu et al., 2004; Yang et al., 2006; Rauf et al., 2006; Kumar and D'Souza, 2008) and associated with transducers for biosensor application.

Most of glucose biosensors are based on the glucose oxidation catalysed by glucose oxidase:

$$\text{D-glucose} + O_2 \xrightarrow{\text{GOD}} H_2O_2 + \text{D-gluconolactone}$$

The amperometric response of consumed O_2 was monitored for glucose sensing as depletion of oxygen using oxygen-sensitive electrode (Clark and Lyons, 1962).

* Corresponding author. Tel.: +91 22 25593632; fax: +91 22 25505151.
E-mail addresses: jkumar@barc.gov.in (J. Kumar), sfdsouza@barc.gov.in (S.F. D'Souza).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.08.022

In present work, GOD was immobilized on inner epidermal membrane of onion bulb scales and their operational reusability as well as stability was investigated. To our knowledge, this is the first report on employing inner epidermal membrane of onion bulb scales as the solid support for enzyme immobilization for use in biosensor application. Our studies indicated that this natural polymer can serve as a biocompatible and inexpensive stable support for immobilization of enzyme for further application in biosensor. Our proposed enzyme immobilization matrices and method are simple and shown a better sensitivity and wide detection ranges. Applicability is also demonstrated with real blood sample analysis.

2. Materials and methods

2.1. Materials

Glucose oxidase (E.C.1.1.3.4), type II from *Aspergillus niger* lyophilized powder (100 units/mg protein) and glutaraldehyde (25%, w/w) solution were purchased from Sisco Research Laboratories, Mumbai. Glucose and other chemicals from Qualigens Fine Chemicals, Mumbai, India. Clark dissolved oxygen electrode probe was purchased from M/S Century Instruments, Chandigarh, India. Onions were purchased from local market.

2.2. Immobilization of GOD on onion membrane

Onions (*Allium cepa* L.) were purchased and stored at room temperature until used. The onions were cut into half, bulb scales were separated and inner epidermis from outer fleshy scale was stripped. Circular pieces of 1.5 cm diameter were cut and stored at 4 °C in the refrigerator. An aliquot of 100 μl GOD (250 units/ml prepared in 50 mM sodium phosphate buffer, pH 7.0) was added on each membrane. After 30 min 10 μl of 2% glutaraldehyde solution was dropped onto the surface of the membrane and spread uniformly. The cross-linking was allowed to take place for 30 min. Some membranes were prepared without glutaraldehyde as control.

2.3. Operating condition and calibration of the DO probe

Commercial Clark-type dissolved oxygen (DO) electrode in conjunction with GOD immobilized onion membrane was used for biosensor reading as mentioned in earlier report (Kumar and D'Souza, 2008) (Supplementary Fig. 1). The Clark electrode measures oxygen tension amperometrically. DO electrode produces a current, at a constant potential (−0.6 V vs. Ag/AgCl) which is directly proportional to the partial pressure of oxygen diffusing to the reactive surface of the electrode (Clark and Lyons, 1962). The electrode was filled with 1 M potassium chloride solution. Blank readings of the probe with air and 50 mM sodium phosphate buffer (pH 6.0) were taken without the membrane being attached to the electrode. Membrane was tightly attached to the electrode using O-ring with the support of cheesecloth. The DO probe was allowed to obtain a base line corresponding to the buffer volume 20 ml in the reaction vessel. 500 μl of standard glucose solutions (concentration 4.5, 22.5, 45, 90, 225, 450 and 900 mg/dl) were introduced into reaction vessel and oxidation reaction occurred at electrode surface was observed. Oxygen was consumed from sample during oxidation of glucose and depletion of oxygen was measured using DO probe. All experiments were performed at room temperature.

2.4. SEM study of the membrane

A scanning electron microscope (SEM, Model 435 VP, Leo Electron Microscopy Ltd, Cambridge, UK) was employed to observe the surface structure of the inner epidermal membrane of the onion bulb scales. For scanning electron microscopy study, pieces of the membranes were mounted on stubs and were then coated with gold using a sputter coater. The SEM micrographs of the onion membranes (before and after immobilization) were studied at magnifications 500 and 5000×.

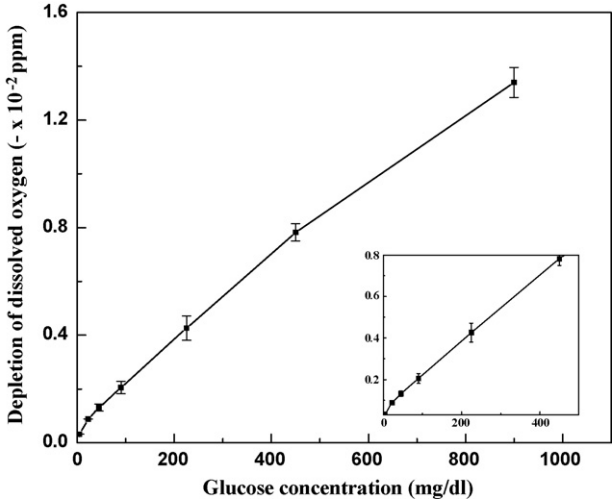

Fig. 1. Calibration of DO based biosensor in association with GOD immobilized onion membrane: glucose concentration 4.5, 22.5, 45, 90, 225, 450 and 900 mg/dl. Inset: linearity range 22.5–450 mg/dl; $Y = 0.0517 + 0.0016X$ ($R^2 = 0.999$; S.D. = 0.318).

3. Results and discussions

3.1. SEM study of the membrane

Structural morphology of immobilized membrane was also studied under SEM. Result showed (Supplementary Fig. 3a) that membrane consisted of elongated tubular cells with blunt or tapering ends as reported in literatures (Carpita and Gibeaut, 1993; Brett and Waldron, 1996). The main cell framework consists of cell wall, is an elaborate extracellular matrix consisting of a microfibrillar cellulose phase and a matrix phase that contains a variety of polymers like pectin and hemicelluloses (Scott et al., 1958; Bruce and Hepworth, 2004) biomolecules, therefore it was considered for immobilization of enzyme for optimum enzyme activity. After GOD immobilization on surface of onion membrane, many changes like depressions and uneven distribution of microfibrous structure were observed on the surface morphology (Supplementary Fig. 3b). Changes in surface morphology might be because of cross-linking of enzyme with membrane using glutaraldehyde. SEM micrographs indicated that enzyme was successfully immobilized on the surface of onion epidermal membrane.

3.2. Immobilization of GOD on onion membrane and response time

GOD of different concentrations (10, 50, 100, 250 and 500 units/ml) was immobilized on onion membranes and optimum activity was obtained when 250 units/ml concentration was used. There after there was no further significant increase in activity. As a result, 250 units/ml concentration of GOD was used for the subsequent experiments.

Glutaraldehyde concentration was optimized for optimum activity of enzyme. Optimum enzyme activity was obtained when 2% glutaraldehyde was used for cross-linking of enzyme on onion epidermal membrane. It was found that high concentration (>5%, v/v) of glutaraldehyde inactivated the enzyme and low