APPLICATION OF IMMOBILIZED ENZYME AND CELLS IN BIOSENSORS FOR THE DETECTION OF GLUCOSE AND METHYL PARATHION

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Abstract

Biosensors are analytical devices composed of a recognition element of biological origin and a physico-chemical transducer. Immobilization plays a key role in developing stable biocomponent for integration with transducers, which lead to the development of biosensor. This article describes the study carried out on immobilization of glucose oxidase (GOD) enzyme and microbial cells on suitable matrices for the development of stable biocomponent and its integration with the transducer for the detection of glucose and methyl parathion.

Introduction

A biosensor is a compact analytical device, incorporating a biological or biologically derived sensing element, either closely connected to, or integrated within a transducer system. The principle of detection is the specific binding of the analyte of interest to the complementary biorecognition element immobilized on a suitable support matrix. The specific interaction results in a change in one or more physico-chemical properties (viz. pH change, electron transfer, mass changes, heat transfer, uptake or release of gases or specific ions) which can be detected and measured by the transducer. The aim is to produce an electronic signal, which is proportional to the concentration of a specific analyte or group of analytes, to which the biosensing element binds. Biosensors can be classified according to biorecognition system. The biological elements used in biosensor technology are enzymes, antibody/antigens and nucleic acids/complementary sequences. In addition, microorganisms, animal or plant whole cells and tissue slices, can also be incorporated in the biosensing system. Depending on the method of signal transduction, biosensors can also be divided into different groups: electrochemical (amperometric, potentiometric or conductometric), optical, thermometric and piezoelectric. Biosensors offer many advantages over conventional analytical techniques. The selectivity of the biological sensing element offers an opportunity for the development of highly specific devices for real-time analysis in complex mixtures, without the need for extensive sample pre-treatment or large sample volumes. The function of a biosensor will depend on the biochemical specificity of the biologically active material. Biosensors also promise highly sensitive, rapid, reproducible and simple-to-operate analytical tools.

The basic requirement of a biosensor is that the biological material should bring the physico-chemical changes in close proximity of a transducer. In this direction, immobilization technology has played a major role. Immobilization not only helps in forming the required close proximity between the biomaterial and the transducer, but also helps in stabilizing it for reuse. The biological material has been immobilized directly on the transducer or in most cases, in membranes, which can subsequently be mounted on the transducer. Biomaterials can be immobilized either through adsorption, entrapment, covalent binding, cross-linking or a combination of all these techniques. The choice of technique and support should be such that, it should retain the enzyme activity and have reusability as well.
as storage stability. A variety of synthetic as well as natural materials have been exploited in our laboratory, for immobilization of biocatalysts and their use in biosensor development.

This article summarizes all our studies related to immobilization of glucose oxidase (GOD) and microbial cells having OPH enzyme on different supports using suitable techniques for the development of biosensors for methyl parathion and glucose monitoring.

**Immobilization of GOD enzyme for glucose biosensors**

Enzymes are well-known as biological sensing materials in the development of biosensors due to their specificity and play a key role in clinical diagnosis. Detection of glucose has been the most studied analyte in clinical diagnosis for diabetic patients. Most of the glucose biosensors are based on glucose oxidation, catalyzed by GOD enzyme. In our study GOD enzyme was immobilized on suitable support for better stability and reusability and associated with suitable transducers for biosensor monitoring of glucose. Immobilized GOD converts glucose into gluconolactone with the consumption of oxygen. Amperometric response was monitored, by measuring the depletion of oxygen from sample using oxygen-sensitive dissolved oxygen (DO) electrode.

In one of our study a method was optimized to prepare a synthetic polyvinyl alcohol (PVA) membrane from a homogenous mixture of 12% low DOP PVA, 8% high DOP PVA and 2% benzoic acid in 50 mM sodium phosphate buffer containing 20% acetone. 2 mL GOD (2000 units/mL) was added to the mixture and mixed homogenously. The mixture was overlaid as a thin membranous layer, air-dried, peeled off carefully and placed under UV light for cross-linking and integrated with the DO probe for biosensor application (Fig. 1). Detection range of this biosensor was estimated to be between 0.9 and 225 mg/dL glucose. GOD-PVA membrane was reused for 32 reactions and was stable for 30 days without significant loss of activity.

In another study GOD was immobilized on inner epidermal membrane of the onion bulb scales as a natural support and applied for biosensor application for detection of glucose in association with DO probe. Natural polymers in living organisms are composed of biomolecules like carbohydrates, lipids and proteins and therefore they can provide a biocompatible microenvironment for enzyme immobilization. This natural membrane is also mechanically stronger because it consists of microfibrillar cellulosic biological components and served as a biocompatible immobilizing support, for optimum enzyme activity.

Onions (*Allium cepa* L.) 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 50mM sodium phosphate buffer) was added on each membrane. After 30 min, 10 µL of 2% glutaraldehyde solution was added to the surface of the membrane and spread uniformly. After GOD immobilization on surface of onion membrane, many changes like depressions and uneven distribution of microfibrous structure were observed on the surface morphology (Fig. 2). GOD immobilized onion membrane has shown a high reusability.
Methyl parathion pesticide is extensively used in the field of agriculture despite its high toxicity and contributes major share in terms of restricted uses in India. Among the various biosensors for methyl parathion determination, systems based on acetylcholinesterase (AChE) and organophosphorus hydrolase (OPH) enzymes contribute major share. AChE biosensor is based on enzyme inhibition mechanism; hence it requires longer incubation time and also has poor specificity because of interference from carbamate pesticide and metals. OPH catalyzes hydrolysis of methyl parathion pesticide into detectable product \( p \)-nitrophenol (PNP) and generates two protons as a result of the cleavage of the P-O bond. Products that are chromophoric and/or electroactive can be detected by colorimetric and electrochemical methods, and were exploited to develop biosensors for detection of methyl parathion pesticide. The analyte can be determined as the rate of product formation is directly proportional to the concentration of the analyte. As the OPH is a periplasmic enzyme, whole cells can be immobilized directly on the matrix and integrated with transducers for biosensor development.

In our laboratory different types of microbial biocomponents (from disposable to reusable) were developed by immobilizing microbial cells on different supports and associated with different transducers (optical and electrochemical) for analysis of single to multiple samples at a time.

In our first study for detection of methyl parathion, an optical microbial biosensor was developed in which disposable biocomponent was prepared by immobilizing whole cells of *Flavobacterium sp.* on glass fibre filter paper disc and associated with an optical fibre transducer. Detection range of the biosensor was 4 – 80 \( \mu \)M methyl parathion which required only 75 \( \mu \)L of sample and its biocomponent was disposable in nature and can be used for field sample analysis.

In the second study, *opd* gene, which codes for OPH enzyme, was cloned to make recombinant *E.coli* with high periplasmic expression of enzyme. Recombinant *E.coli* cells were immobilized on screen-printed carbon electrode (SPCE) for preparing the biocomponent. Surface morphologies of the working area of both blank and whole cells immobilized SPCE were studied by SEM. As observed in SEM micrograph (Fig. 3), bacterial cells (size 0.5–2 \( \mu \)m) were present in immobilized SPCE and were absent in the micrograph of blank SPCE. An

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**Fig. 2: SEM micrograph of onion membrane after immobilization of glucose oxidase**

**Immobilization of microbial cells for methyl parathion biosensors**

**Fig. 3: SEM micrograph of E.coli immobilized SPCE**
Fig. 4: Schematic diagram of experimental set up of electrochemical biosensor

electrochemical microbial biosensor was developed based on cyclic voltammetry for the detection of methyl parathion using cells immobilized SPCE (Fig. 4). In this study, detection range of the biosensor was 2 - 80 µM methyl parathion but the biocomponent was reusable upto 32 reactions and biosensor requires low volume (20 µL) of sample hence it can be used when very low sample amount is available.

In our third study, an optical biosensor was developed for detecting large number of samples in a short period of time (5 min). For this, a bacterium was isolated from soil and identified as Sphingomonas sp. JK1 which hydrolyzes methyl parathion. Microbial cells were then immobilized onto the surface of the wells of microplate (96 wells) and used as a reusable (upto 75 reactions) biocomponent. Surface morphologies of the immobilized area of both blank and whole cells-immobilized microplate were imaged by SEM (Fig. 5). SEM micrographs were acquired on interior surface of the wells of microplates. A bunch of bacterial cells (sizes 0.4 - 0.8 µm) were observed in the micrograph of cells-immobilized surface of well and were absent in the micrograph of unimmobilized surface of well. Microplate based technology was utilized to develop an effective biosensor tool for detection of multiple numbers of samples because of the time independent measurement of the whole microplate plate irrespective of the number of samples present on the plate (96 wells) that enable to handle multiple number of samples simultaneously on a single platform (Fig. 6). In another experiment, Sphingomonas sp. JK1 was also immobilized on natural support, inner epidermis of onion bulb scale and a bunch of bacterial cells (sizes 0.4–0.8 µm) were observed in the micrograph of cells immobilized inner
epidermis of onion bulb scale and was absent in the micrograph of unimmobilized surface (Fig. 7). Cells immobilized biocomponent was directly placed in the wells of microplate and associated with the optical transducer for monitoring of methyl parathion pesticide.

**Conclusion**

GOD was successfully immobilized on PVA membrane as a synthetic support and inner epidermal membrane of the onion bulb scales as a natural support for application in DO based glucose biosensors. Microbial cells having OPH was immobilized on different types of supports for developing biocomponents (from disposable to reusable) and associated with different transducers for biosensor detection of methyl parathion pesticide.

**References**


