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A mediated turnip tissue paper-based amperometric hydrogen peroxide biosensor

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ABSTRACT

A novel inexpensive turnip tissue paper-based mediated amperometric hydrogen peroxide (H_2O_2) biosensor was developed based on screen-printed carbon electrodes (SPCEs). The use of cellulose paper proved to be an "ideal" and simple biocompatible immobilization matrix for raw turnip peroxidase as it was successfully embedded within the fiber matrix of paper *via* physical adsorption. The mediator potassium hexacyanoferrate (II) was also embedded onto the paper matrix together with the raw enzyme. The biosensor allowed a minute amount (0.5 μL) of sample solution for analysis. The linear calibration range of the biosensor was between 0.02 and 0.50 mM (R^2 = 0.999) with a detection limit of 4.1 μM H_2O_2, calculated based on IUPAC convention. Its analytical performance was demonstrated in real sample analysis and the results obtained corroborated well with the classical titration method. Without any complex packaging modification, the biosensor retained *ca.* 70% of its activity after a storage period of 25 days at 4 °C.

1. Introduction

Hydrogen peroxide (H_2O_2) is an important by-product in various enzymatic catalyzed biochemical reactions [1]. It is commonly used in environmental, pharmaceutical, clinical, food analyses and drug manufacturing [2–4]. However, H_2O_2 is extremely harmful to the environment and public health. Therefore, H_2O_2 determination based on a simple, precise and economical method is very desirable. Recently, the development of biosensors with low-cost robust novel biological materials as biocatalysts has received considerable interest and this has been applied for various plant tissue-based biosensors for H_2O_2 monitoring [5,6]. Recent research has shown that raw unpurified peroxidases found in young leaves, root tips, fruits and seeds of plants have been used in the fabrication of inexpensive plant tissue-based H_2O_2 biosensor [7]. Plant tissue-based biosensors are advantageous over their enzymatic counterparts as tedious and time-consuming enzyme extraction as well as purification steps can be avoided [8,9] and thus, they are relatively cheaper.

Different analytical techniques have been employed for the determination of H_2O_2, such as titrimetry, voltammetry, spectrophotometry, fluorimetry, chemiluminescence and electrochemistry [10–13]. However, electrochemical method is one of the advantageous techniques because of its robustness, possible miniaturization, low-cost and ease of operation. Direct electrochemical determination of H_2O_2 based on oxidation occurs at a relatively high potential of *ca.* 0.70 V *versus* SCE, thus resulting in interference during analysis of real samples and electrochemical analysis in the presence of peroxidase as a biocatalyst can overcome the above problem.

In order to improve the performance of the plant tissue-based H_2O_2 biosensors, a suitable immobilization method for the raw unpurified enzyme must be developed. We selected paper as an immobilization matrix as it is inexpensive, disposable and biodegradable [14–16] and it has been used in our previous works for the immobilization of pure glucose oxidase enzyme [17,18]. Naturally made of cellulose fibers, paper allows the wicking of aqueous fluids into its hydrophilic fiber matrix and this wicking makes passive transport of fluids without active pumping to be very practical [19]. Here, we report on a simple enzyme immobilization procedure whereby raw peroxidase extracted from freshly

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pounded turnip tissue was pipetted directly onto cellulose paper thus enabling physical adsorption of enzyme within the porous paper matrix.

Previously, we reported on the development of a Swede turnip (*Brassica napobrassica*) tissue-modified carbon paste biosensor for the determination of H_2O_2 in the presence of ferrocene as a mediator [20]. In this study, due to the desirable properties of both paper as a simple and inexpensive green immobilization matrix along with plant tissue as an advantageous low-cost biocatalytic component, a mediated amperometric H_2O_2 biosensor was fabricated by immobilizing raw turnip peroxidase and potassium hexacyanoferrate (II) mediator within the porous structure of paper discs placed on top of SPCEs. The peroxidase immobilized *via* physical adsorption onto paper was obtained directly from the original turnip tissue without any complex extraction procedures. This biosensor allowed for the analysis of H_2O_2 to be performed using only 0.5 μL of analyte. Furthermore, working with micro-volumes result in cost savings due to a reduction in reagent consumption and prevents incessant exposure to bulk solution. Various parameters for the optimization of biosensor development such as applied potential, pH, mediator concentration and tissue composition were investigated.

2. Experimental

2.1. Reagents and instrumentation

All reagents used were of analytical grade. The Swede turnip was purchased from a local supermarket. Hydrogen peroxide (30%, w/w extra pure) was purchased from Scharlau (Scharlab S.L., Sentmenat, Spain). Potassium hexacyanoferrate (II) ($K_4[Fe(CN)_6]$) was obtained from Sigma–Aldrich (St Louis, MO, USA).

For validation studies, real samples such as antiseptic H_2O_2 and AOSept® Plus contact lens cleaning solution were purchased from a local pharmacy and optician respectively. All solutions were prepared with 18 MΩ cm ultrapure water obtained from Millipore Alpha-Q water system (Bedford, MA, USA). Hexacyanoferrate (II) (50 mM) mediator solution was freshly prepared daily in 0.1 M phosphate buffer solution (PBS, pH 7.0). Electrochemical characterizations and measurements were performed using a four-channel system (eDAQ QuadStat, e-Corder 8 and Echem software, eDAQ Europe, Poland).

SPCEs (DRP-110) and the boxed connector for SPEs (DRP-DSC) were purchased from DropSens (Asturias, Spain). The working electrode (*ca.* 4 mm diameter) was made up of carbon while Ag/AgCl and a carbon ring made up the reference and counter electrodes respectively. Paper discs were cut from Grade 1 filter paper [Whatman Asia Pacific Pte Ltd (Singapore)]. Data points were plotted using Microsoft Excel (USA) and ORIGIN (Northampton, MA, USA).

2.2. Preparation of turnip peroxidase paper discs and electrochemical characterization

Grade 1 filter paper was cut into round discs with *ca.* 8 mm diameter using a paper punch. Accurately weighed raw turnip tissue was finely ground thoroughly using a mortar and then fixed volume of 0.1 M PBS (pH 7.0) was added into the homogenous tissue paste. The buffer and turnip tissue were thoroughly mixed together and the supernatant of this mixture was then used for subsequent experiments. Typically, 5 μL of turnip tissue extract (3%, w/v tissue in 0.1 M PBS (pH 7.0) was carefully added to each paper disc and allowed to dry at room temperature (25 °C). Upon drying, 5 μL of mediator solution (50 mM hexacyanoferrate (II) in 0.1 M PBS, pH 7.0) was added to each disc and allowed to dry at room temperature. These paper discs laden with both raw turnip peroxidase and mediator solution were used for H_2O_2 analyses and stored at 4 °C for stability study of the biosensor.

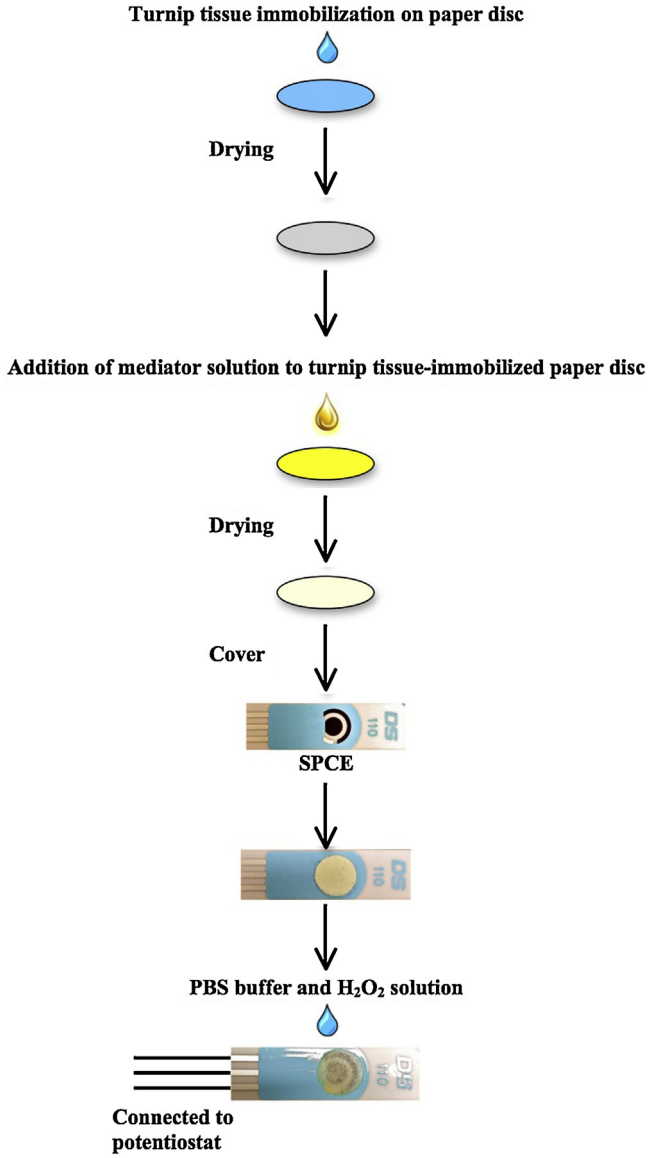

Fig. 1. Schematic illustration of turnip paper disc preparation and integration with the SPCE. The paper disc with immobilized turnip tissue and mediator were placed on top of the SPCE surface for complete coverage of working, counter and reference electrodes.

The SPCE was slotted into the boxed connector that was linked to the potentiostat. Typically, as illustrated in Fig. 1, the paper disc with immobilized raw turnip peroxidase and mediator solution was placed on top of the SPCE to completely cover the working, counter and reference electrodes before each measurement. Typically, 12 μL of 0.1 M pH 7.0 PBS buffer solution was added onto the paper disc and good contact was formed with the SPCE with this volume of buffer. All amperometric experiments were carried out at a potential of −0.10 V. Unless otherwise indicated, all measurements were performed in triplicates on paper discs prepared using 5 μL from a 3% (w/v) turnip tissue extract, 5 μL of 50 mM hexacyanoferrate (II) solution and H_2O_2 detection was performed using 0.5 μL of 2 mM H_2O_2 solution. Furthermore, all different compositions of turnip tissue extract and H_2O_2 solutions were prepared using 0.1 M pH 7.0 PBS, unless stated otherwise. The H_2O_2 concentrations in real samples were determined by a two-point standard addition method and a classical titration method [21].