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Chinese Chemical Letters 23 (2012) 221–224

CHINESE CHEMICAL LETTERS

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Ascorbate oxidase electrochemical biosensor based on the biocompatible poly(3, 4-ethylenedioxythiophene) matrices for agricultural application in crops

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Received 19 July 2011
Available online 21 December 2011

Abstract

The vitamin C (VC) in crops was successfully determined using ascorbate oxidase (AO) electrochemical biosensor based on the biocompatible poly(3, 4-ethylenedioxythiophene) (PEDOT) matrices, which was easily prepared by one-step electrodeposition technique in ionic liquid microemulsions. The fabricated biosensor displayed excellent bioelectrocatalytic performance to the oxidation of VC, wide linear range, low detection limit, fast response time, good operational and storage stability, the good results of the determination of VC in vegetable crops indicated that the fabricated biosensor will be a good candidate for the physiological and biochemical studies of crops in near future.
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Keywords: Biosensor; Agricultural application; Ionic liquid microemulsions; Ascorbate oxidase; Poly(3, 4-ethyl-enedioxythiophene); Vitamin C

The importance of vitamin C (VC) in various physiological and biochemical studies of crops has extensively been reviewed elsewhere [1,2]. VC is an irreplaceable cofactor in several pathways including the xanthophyll cycle, synthesis of hormones and hydroxyproline-rich proteins as well as important cell wall components [3]. VC also plays a number of important roles in physiological and biochemical fields of crops [4,5] such as flowering phase induction and adjustment, growth and development. Most importantly, VC also provides essential information on cellular redox state and influences gene expression associated with biotic and abiotic stress responses. Hence, the detection of VC in crops is very essential for physiological and biochemical research in agricultural applications.

Many traditional methods such as titrimetry, fluorometry, spectrophotometry, and chromatography have been widely reported for the determination of VC [6–8]. However, they have many disadvantages: tedious and time-consuming multi-step procedures, interference resulting from colored substances and impurities, and high equipment, reagent, and training costs, *etc*. Enzymatic method is more sensitive, accurate, specific, and faster than other methods. Unfortunately, expensive enzyme is uneconomical for routine tests. However, the ascorbate oxidase biosensors are

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1001-8417/$ – see front matter © 2011 Published by Elsevier B.V. on behalf of Chinese Chemical Society.
doi:10.1016/j.cclet.2011.11.010

considered as an economical, practical, inexpensive, specific, efficient, sensitive and simple method for the detection of VC [9,10].

In our previous work, a nontoxic and biocompatible sugar-based surfactant *N*-dodecyl-*β*-D-maltoside (DM) could enhance the water-solubility of 3, 4-ethylenedioxythiophene (EDOT), lower onset oxidation potential of EDOT, facilitate the biocompatibility and bioactivity of the resulting poly(3, 4-ethylenedioxythiophene) (PEDOT) film containing biologically-active species [11]. 1-Ethyl-3-methylimidazolium ethyl sulfate (EMIES), a halide-free and relatively hydrolysis-stable hydrophilic ionic liquid, could not only improve the electrochemical activity and the performance of inherently conducting polymer matrices [12], but also enhance performance and anti-interference of the biosensors [13–15]. In addition, low polymerization potentials could form high-quality PEDOT film, prevent overoxidation and degradation of PEDOT matrices, as well as avoid the performance deterioration of the biosensor [16]. The low working potential of the biosensor could avoid the interference from reducing agents in real samples [17,18]. The development of ascorbate oxidase (AO) biosensors based on PEDOT-single or multi-walled carbon nanotubes matrices for the detection of VC has also been studied [17,18]. To the best of our knowledge, there is, so far, no report on the agricultural application in crops of the AO electrochemical biosensor.

In this paper, we report the agricultural application of AO electrochemical biosensor based on the biocompatible PEDOT matrices, which was one-step electrosynthesized in ionic liquid microemulsions.

1. Experimental

The matrices were prepared electrochemically in ionic liquid microemulsions containing 20 mmol/L EDOT, 0.1 mol/L EMIES, and 0.1 mol/L DM at a constant potential of 1.1 V *vs.* SCE at 25 °C for 90 s on the bare surface of GCE. 5 μL of 0.3 g/L AO was dip-coated on the surface of the matrices. After drying in air, 5 μL of 5% Nafion was covered on the top of the surface of AO layer to provide a biocompatible environment for biologically active species, prevent peeling off of films, and avoid foreign interferences and possible enzyme leakage [19]. Then the enzyme electrode was allowed to dry in air, and stored in 50 mmol/L phosphate-buffered saline (PBS, pH 6.5) at 4 °C prior to use. The response current of the biosensor for the detection of VC was recorded by chronoamperometry at working potential of 0.2 V *vs.* SCE at 25 °C. For the detection of VC in vegetable crops, 100 g materials were homogenized, and the homogenate was filtered to remove the cellulose residue, then the filtrate obtained was kept at 4 °C in dark when not in use. All electrochemical experiments were performed in the three-electrode cell with a potentiostat–galvanostat.

2. Results and discussion

Fig. 1 shows bioelectrocatalytic activity of the fabricated biosensor on the oxidation of VC. It could be seen that the bioeletrocatalytic oxidation of VC began to appear from approximately −0.051 V and reached the maximum value at 0.21 V, which was different from cyclic voltammetry of the biosensor in PBS without VC. This fact also suggested that the biosensor could catalyze the oxidation of VC, indicating that the biosensor possessed a good bioelectrocatalytic performance for the oxidation of VC.

Fig. 2 presents the relationship between the response current and the concentrations of VC. Obviously, response current increases with the increasing concentrations of VC (Fig. 2), indicating that the fabricated biosensor possessed an excellent bioelectrocatalytic performance for the oxidation of VC. The relationship between the response current and the concentrations of VC (Fig. 2 inset) showed good linearity from 8.0×10^{-7} to 1.9×10^{-2} mol/L, pronounced sensitivity of 56.63 mA L mol^{-1} cm^{-2} and low detection limit of 0.41 μmol/L (S/N = 3), implying that the fabricated biosensor could successfully detect the unknown content of VC in crops. Moreover, the response time was less than 20 s (Fig. 2). In addition, the operational stability of the construction protocol was investigated by measuring the response current of 13 electrodes from the same batch in 0.5 mmol/L VC, a relative standard deviation (RSD) of 0.57% was obtained. One enzyme electrode for fifty replicate determinations gave a RSD of 0.48% in 0.5 mmol/L VC solution. No loss of the bioactivity in current response was observed for 8 days and decreased approximately 10.58% after being stored for 32 days, showing a fairly good operational and storage stability of enzyme electrode. In comparison with our previous studies [17,18], the fabricated biosensor had wider linear range, lower detection limit, faster response time, better operational and storage stability.Table 1 shows the specificity of the fabricated biosensor. Many different substances existing in vegetable crops such as carbohydrates, amino acids, organic acids and alcohols

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