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Highly sensitive D-alanine electrochemical biosensor based on functionalized multi-walled carbon nanotubes and D-amino acid oxidase

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ABSTRACT

With an excellent electron-transfer ability of 3,4,9,10-perylene tetracarboxylic acid functionalized multi-walled carbon nanotubes (PTCA-MWCNTs), and successful maintenance of D-amino acid oxidase (DAAO) activity by the protection of bovine serum albumin (BSA) and glycerol, a signal amplification biosensor for chiral recognition of D-alanine (D-Ala) has been designed. PTCA worked as redox probe due to its self-derived redox activity. The proposed biosensor was characterized by scanning electron microscopy (SEM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). After the biosensor interacting with D-alanine and L-alanine respectively, a larger current response was obtained from D-alanine. The linear range of the biosensor under the optimum working conditions was investigated by current-time response in successive addition of D-Ala from 1.0×10^{-8} to 1.0×10^{-3} M with a lower detection limit of 3.3×10^{-9} M (S/N = 3). Possible explanations for substrate specificity of the biosensor were discussed. This method showed high sensitivity and selectivity for chiral recognition of D-alanine. It also exhibited good stability, repeatability and reproducibility. The proposed biosensor was applied for real sample measurement.

1. Introductions

Chiral discrimination of amino acids has received increasing attention due to its critical impacts on biological, chemical, and pharmaceutical sciences [1,2]. It's well known that there is a large amount of L-amino acids in human body and D-isomers of amino acids are common constituents of bacterial cell walls [3]. Recent studies reported that D-alanine as one of D-amino acids has been found in the central nervous system and peripheral tissues of mammals [4,5]. Besides, D-alanine has also been used for the synthesis of sweeteners and chiral drugs. L-alanine is an important raw material for synthesis of Vitamin B6, which belongs to a part of the vitamin B group. And the direct absorption of L-alanine by cells can help people rapidly recover from the exhausting status [6]. To recognize alanine enantiomers and determine their amounts is important for researchers to better understand their physiological functions. In recent years, different methods have been adopted to achieve recognition of chiral amino acids, including highly efficient but costly means like capillary electrophoresis, high-performance liquid chromatography (HPLC) and gas chromatography (GC). Among the various techniques, electrochemical biosensor based on enzyme-nanocomposite attracts our attention due to its simple preparation, short measuring time, and good sensitivity [7,8].

D-amino acid oxidase (DAAO) is a flavine adenine dinucleotide (FAD)-containing flavoenzyme which can specifically catalyze a variety of D-amino acids with a strict stereospecificity to produce hydrogen peroxide and ammonium. The stereospecific oxidative deamination of D-amino acids follow the equation: D-amino acids + O_2 + $H_2O \xrightarrow{DAAO}$ α-keto acid + NH_3 + H_2O_2 [9,10]. The current response was due to the generation of H_2O_2 [11–13]. In this study, DAAO has been chosen to fabricate biosensor that can achieve high sensitivity and selectivity for the D-alanine detection. In order to maintain its activity and stability, bovine serum albumin (BSA), which can form DAAO-BSA homogeneous solution in presence of glycerol, was used as cross-linking co-reagent to protect it from excessive reaction with EDC/NHS [14,15].

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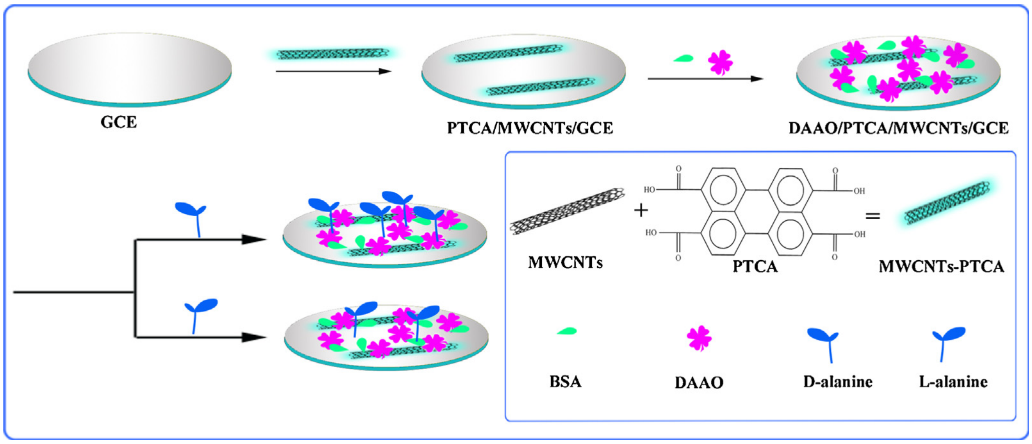

Scheme 1. Fabrication process of the proposed biosensor.

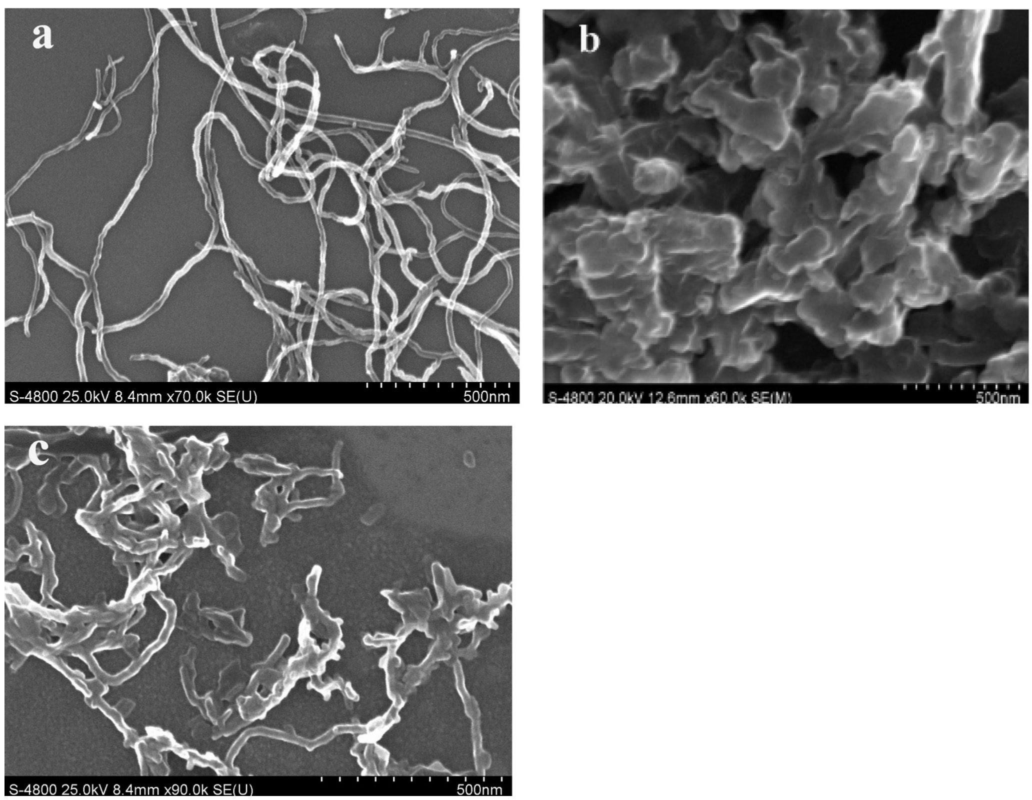

Fig. 1. SEM images of (a) MWCNTs, (b) PTCA, and (c) PTCA-MWCNTs.

To obtain large current response, novel nanomaterial with good conductivity was crucial. Among numerous candidates, multi-walled carbon nanotubes (MWCNTs), which possesses high surface active area, excellent electrical conductivity, outstanding stability [16–18] and electrocatalytic activity [19,20], gets considerable attention since it's been discovered. However, pristine MWCNTs exhibits poor dispersibility and easy agglomeration in water, which limits its further application for biosensors [21,22]. Traditional strategy to get high dispersion of MWCNTs in water is the introduction of carboxyl groups (-COOH) onto the MWCNTs surface by HNO_3 or HNO_3/H_2SO_4 mixture [23], which may cause structural damage and loss of the electronic conductivity of MWCNTs [24]. To avoid this problem, 3,4,9,10-perylene tetracarboxylic acid (PTCA), an typical π-stacking organic perylene dye which contains an aromatic pyrenyl group and four carboxyl groups and possesses characteristics of redox-activity [25], desirable electronic conductivity, excellent stability, and film-forming property [26,27], has been used to functionalize MWCNTs via π-π stacking. The obtained PTCA functionalized MWCNTs (PTCA-MWCNTs) showed high dispersibility and excellent electron-transfer ability. Additionally, the effective areas and binding sites of PTCA-MWCNTs for immobilization of DAAO has been increased [28]. Moreover, the non-permeable character of the PTCA might be helpful to maintain the activity of DAAO. Therefore, a biosensor based on PTCA-MWCNTs and DAAO to recognize D-alanine was designed. The specifically catalytic properties of DAAO combined with an excellent electron-transfer ability of PTCA-MWCNTs nanocomposite successfully achieved signal amplification, which was crucial to further decrease detection limit and increase sensitivity. Compared with other electrochemical methods, a much lower detection