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Simple method for preparing glucose biosensor based on in-situ polypyrrole cross-linked chitosan/glucose oxidase/gold bionanocomposite film

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ABSTRACT

A film of chitosan–polypyrrole–gold nanoparticles was fabricated by in-situ chemical synthesis method and its application in glucose biosensor was investigated. The obtained biosensor exhibited a high and reproducible sensitivity of 0.58 μA/mM, response time ~4 s, linear dynamic range from 1 to 20 mM, correlation coefficient of $R^2 = 0.9981$, and limit of detection (LOD), based on S/N ratio (S/N = 3) of 0.068 mM. A value of 1.83 mM for the apparent Michaelis–Menten constant was obtained. The resulting bio-nanocomposite provided a suitable environment for the enzyme to retain its bioactivity at considerably extreme conditions, and the decorated gold nanoparticles in the bio-nanocomposite offer good affinity to enzyme.

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1. Introduction

The detection of glucose level in physiological liquids such as blood and urine has an importance to make a diagnosis and treatment of diabetes mellitus. Amperometric glucose biosensors based on the glucose oxidase enzyme are more frequently employed biosensors due to its specificity [1–3]. The active site of the glucose oxidase contains two tightly bound flavin adenine dinucleotide (FAD) cofactors and catalyses the oxidation of β-D-glucose to gluconic acid, by utilizing molecular oxygen as an electron acceptor with simultaneous production hydrogen peroxide. The quantification of glucose can be achieved via electrochemical detection of the enzymatically liberated H_2O_2 [4–6].

$$\text{Glucose} + \text{GOx}\ (\text{FAD}) \rightarrow \text{GOx}\ (\text{FAD}_2) + \text{glucono lactone}$$
$$\text{GOx}\ (\text{FAD}_2) + \text{O}_2 \rightarrow \text{GOx}\ (\text{FAD}_2) + \text{H}_2\text{O}_2$$

In recent years, remarkable process in the synthesis and characterization of conducting polymers has offered a great possibility for novel applications in various fields due to considerable flexibilities in modifying their chemical structures [7,8]. By chemical modeling and synthesis, it is possible to modulate their electrical and mechanical properties [9]. Polypyrrole (PPy) is found to be of particular interest among various conducting polymers [10,11]. Conducting polymers are interesting materials for sensor and biosensor constructions due to their effective mediation of electron transfer in redox or enzymatic reactions and can be used as a suitable matrix for immobilization of biomolecules. The immobilization of desired biomolecules on the conducting polymers surface was achieved by various methods. To control over the shape and dimensions of conducting polymers by varying synthesis or processing conditions is likely to result in desired physical and electrochemical properties for biosensing application [12,13].

Chitosan is a natural polysaccharide generally obtained by the deacetylation of the natural chitin [14]. Due to its excellent biocompatibility, nontoxicity, cheapness, easy-handling and high mechanical strength, chitosan (Ch) has been widely used as a modifying reagent to prepare modified electrode [15]. It is preferable to maintain the high biological activity of the immobilized biomolecules and enhance the sensitivity of the sensor. To improve the properties of chitosan, the combination of chitosan with other materials such as ethylene diamine, thiourea, ferrocene, carbon nanotubes and urocanic acid was reported [16–19].

Metal nanoparticles have attracted much interest in the development of biosensors. Among all metal nanoparticles, gold nanoparticles have been widely used to construct biosensors due to their unique properties such as catalytic activities, optical properties, and biocompatibility. Gold nanoparticles dispersed onto different materials such as carbon paste electrode, self-assembled monolayer, conducting and non-conducting polymers to obtain biosensor interface [20,21]. The

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stabilization of gold nanoparticles with chitosan has been extensively studied [22]. For the composite of polymer and gold nanoparticles various monomers and polymers could be chosen. Chitosan can be adsorbed onto the surface of the gold nanoparticles due its positively charged structure and protection of gold nanoparticles [23]. In a previous study, gold nanoparticles electrochemically deposited on chitosan layer and immobilize GOx effectively [24]. Also, gold nanoparticles prepared simple chemical reduction process by using pyrrole monomer to construct biosensor through simple one-step process [25,26]. Herein, the reduction ability of the pyrrole monomer is used to prepare gold nanoparticles.

This work proposed a simple method for immobilization of GOx by using pyrrole branched chitosan as host material for enzyme and reducing agent for the formation of gold nanoparticles. Due to the distinct advantages of the PPy, chitosan and the Au nanoparticles one by one, the Chi-Py/Au cross-linked nanocomposite designed in this work for highly sensitive and stable amperometric biosensor, in which the sensing film could benefit from the good conductivity, and the superior stability and conductivity of Au nanoparticles.

2. Experimental

2.1. Materials and apparatus

Glucose oxidase (GOx) (EC 1.1.3.4), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-(2-cyanoethyl)pyrrole (Py-CN) were obtained from Aldrich Chemical Co. All other chemicals were of analytical grade and used without further purification.

The FTIR-ATR measurements (4000–400 cm^{-1}) were recorded with a Bruker spectrometer. 64 spectra in the range of 4000–400 cm^{-1} were recorded with a resolution of 1 cm^{-1} and averaged. ^{1}H NMR spectra were recorded at room temperature in $CDCl_3$ (containing 0.03% tetramethylsilane as an internal reference $\delta = 0.00$ ppm) on Bruker 400 MHz spectrometer at 300 or 400 MHz.

X-ray powder diffraction (XRD) analysis was performed on a Rigaku SmartLab Diffractometer operated at 40 kV and 35 mA using Cu Kα radiation. Ch-Py, Chi-Py/Au and Chi-Py/Au/GOx layers prepared on glass slides and used for measurements of XRD.

Electrochemical impedance spectroscopy (EIS) measurements were carried out using a CHI Model 6005 electrochemical analyzer in a background solution of 5 mM Fe^{3+}/Fe^{2+} phosphate buffer (pH 7.0) at a normal potential. The alternating voltage was 5 mV and the frequency range was 5.0×10^{-2} to 1.0×10^{6} Hz.

All amperometric measurements were carried out at room temperature in stirred solutions by applying the desired potential and allowing the steady state current to be reached. Once prepared, the GOx electrodes were immersed in 10 ml of 10 mM PBS solution at pH 7.5 and the amperometric response to the addition of a known amount of glucose solution was recorded. Data collected with freshly prepared enzyme electrodes refer to the average of three experiments.

2.2. Synthesis of 1-(2-carboxyethyl)pyrrole (PyPA)

1-(2-Carboxyethyl)pyrrole was obtained by hydrolysis of 1-(2-cyanoethyl)pyrrole (Py-CN) according to literature [27]: a mixture of 25 g of Py-CN and 100 ml of 15% potassium hydroxide solution was stirred at 50 °C for 40 h. Then the mixture was cooled to room temperature and acidified by hydrochloric acid. After extraction with ether, the crude product (colorless crystals) was collected on evaporation of ether. The product was dissolved in ether and purified by recrystallization from the ether solution. The product was identified as 1-(2-carboxyethyl)pyrrole by means of FTIR-ATR, ^{1}H, and ^{13}C NMR spectroscopy (not shown).

2.3. Preparation of pyrrole branched-chitosan and enzyme electrode

The pyrrole-branched-chitosan was prepared by the formation of amide linkages through the EDC-mediated reaction following the similar method of Liu et al. [28] as shown in Fig. 1A. 1 g of chitosan was dissolved in 100 ml acetic acid solution (1% w/v) and diluted with 85 mL of methanol. PyPA was added to the chitosan solution at 0.54 mol/mol glucosamine residue of chitosan followed by a dropwise addition of 15 mL methanol solution of EDC (0.07 g/L) while stirring at room temperature. The 1:1 mole ratio of EDC to PyPA was used in this study. After 24 h, the reaction mixture was poured into 200 mL of methanol/ammonia solution (7/3, v/v) with stirring. The crude product was filtered, washed with distilled water, methanol, and ether, and then dried under vacuum for 24 h at room temperature.

The Chi-Py/Au/GOx bionanocomposite biosensor electrodes were prepared by mixing 2.5 μl of 0.0925 M Chi-Py, 10 mg/ml GOx and 2.5 μl of 0.0125 M $HAuCl_4$ solutions (in 100 mM PB pH 6.5) onto the electrode surface, respectively (Fig. 1B). The mixture on the GCE allows reacting and drying for 30 min. The electrode was then thoroughly rinsed with distilled water before use.

3. Results and discussion

3.1. Spectroscopic characterization of pyrrole branched-chitosan and gold-nanobiocomposite

The FT-IR spectra of chitosan, PyPA and pyrrole branched-chitosan are shown in Fig. 2A. Characteristic peaks of pyrrole at 1495 cm^{-1} and 1090 cm^{-1} in pure PyPA and pyrrole branched-chitosan, these have

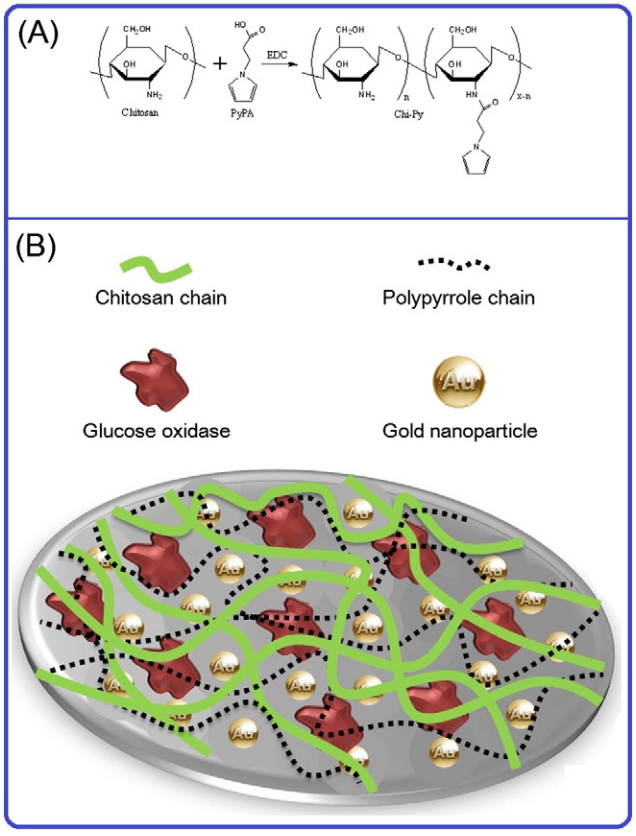

Fig. 1. A) Synthesis of pyrrole branched-chitosan. B) Schematization of the surface of GOx immobilized gold electrode.

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