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Construction of a Concanavalin A electrochemical sensor base on a novel sandwich capture mode

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ABSTRACT

Herein, a concanavalin A (ConA) electrochemical sensor was constructed via a sandwich mode. Firstly chitosan functionalized graphene oxide (CS/GO) composite was introduced on the of glassy carbon electrode (GCE) as the substrate. D–mannose (D-man) was immobilized on the surface of CS/GO *via* Schiff base reaction. Due to the specific affinity between mannose and ConA, the prepared D–man/CS/GO sensing interface could specifically capture ConA which could further react with mannose residues of HRP and form a sandwich configuration. Based on the catalytic effect of horseradish peroxidase (HRP) to H_2O_2, a ConA electrochemical sensor with enzyme catalytic amplification was constructed by taking hydroquinone as electrical mediator. The morphology of CS/GO was investigated by transmission electron microscope and Fourier transform infrared spectroscopy. Electrochemical techniques including electrochemical impedance spectroscopy and differential pulse voltammogram (DPV) were used for reveal the characteristics of the sensing platform. Benefit from the high surface area of GO and enzyme catalytic amplification of HRP, the peak current from DPV is linearly dependent on the ConA concentration ranging from 5×10^{-9} to 5×10^{-7} mol/L with a detection limit of 1.24×10^{-9} mol/L. The results demonstrated that the proposed sensor might exploit a new direction for determination of ConA.

1. Introduction

As a carbohydrate binding protein, concanavalin A (ConA) is a member of the legume lectin family and binds specifically to certain structures found in various molecules and cell plasmon, mainly internal and nonreducing terminal α – D – mannosyl and α – D – glucosyl groups. ConA is the first lectin available on a commercial basis, and is extensively used in biology and biochemistry to characterize glycoproteins and other sugar-containing entities on the surface of various cells. It is also used to purify glycosylated macromolecules in lectin affinity chromatography, as well as to study immune regulation by various immune cells. Like most lectins, ConA is a homotetramer: each sub-unit (25.5 kDa, 235 amino-acids, heavily glycated) binds with a metallic atom [1]. ConA interacts with the surface mannose residues of many microbes and molecules, such as the bacteria E. coli [2–4], plasmon dictyostelium discoideum [5] and horseradish peroxidase [6] (HRP). ConA was found to be sequestered more by hepatic tumor cells, in preference to surrounding normal hepatocytes [7]. Internalization of ConA occurs preferentially to the mitochondria after binding to cell membrane glycoproteins, which triggers an autophagic cell death. ConA was found to partially inhibit tumor nodule growth independent of its lymphocyte activation. So, ConA possesses enormous potential value in the biomedical science, cell biology and cancer diagnosis. Currently, the measurement of ConA mainly depends on agglutination inhibition assays, enzyme-linked lectin assays(ELLA) [8,9], fluorescent assay [10–13], and surface plasmon resonance(SPR) [14], etc. But these methods present some shortcomings, such as low sensitivity, tedious labour work, requiring extensive instruments and technical expertise. Therefore, it's urgent to develop a sensitive, simple and reliable method for determination of ConA.

For rapid and easy determination of ConA, herein, a novel electrochemical sensor with sandwich configuration was constructed based on specific affinity between carbohydrate and lectin. Due to the presence of carboxylic acid groups on the surface of graphene oxide and abundant amino groups in chitosan, chitosan functionalized graphene oxide composite (CS/GO) was successfully obtained according to previous work [15]. As illustrated in Scheme 1, the CS/GO dispersion was dropped onto the surface of a glassy carbon electrode (GCE). D–mannose (D–man) was immobilized on the

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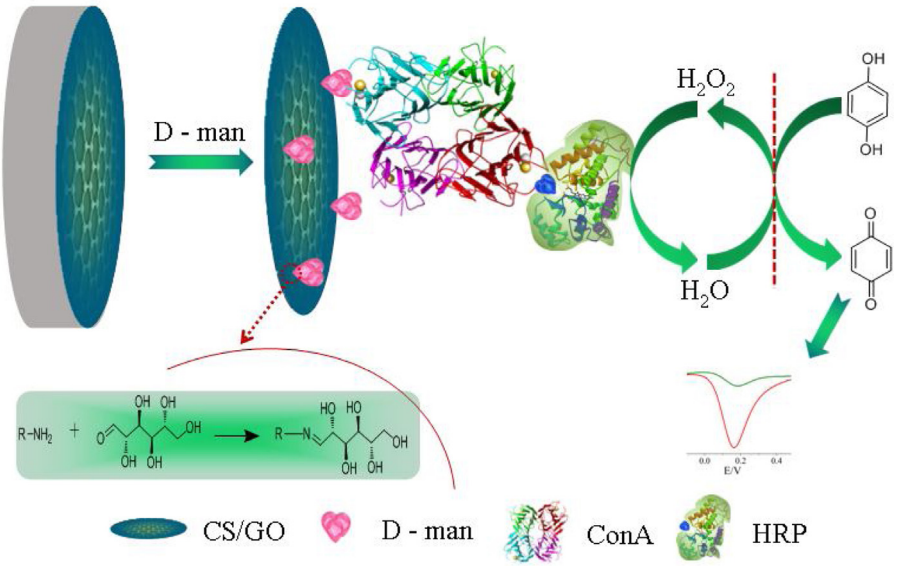

Scheme 1. Construction of a novel sandwich electrochemical sensor for determination of ConA.

modified electrode surface via the Schiff base reaction between aldehyde group on the mannose and the amino groups on the chitosan. In the detection process, ConA was captured by D-man at specific binding sites of its one end, while the binding site of the other end was combined with the HRP containing mannose residues to form a sandwich structure. Finally, taking advantage of the catalytic effect of HRP to H_2O_2, establishment of a ConA electrochemical sensor with enzyme catalytic amplification was achieved when hydroquinone was used as mediator of electron transfer. CS/GO, which firstly acted as a substrate for immobilizing D–mannose, provided multisites for D–man immobilizing due to the larger specific surface area of graphene. On the other hand, the construction of the sensor *via* D-man capturing of HRP provided a new approach for label-free enzymatic determination of ConA sensitively.

2. Experiment section

2.1. Reagents and apparatus

Hydroquinone, HRP and bovine serum protein (BSA) were purchased from Aladdin Reagent (China) Co., Ltd. ConA was provided by Sigma-Aldrich Co. Llc. Chitosan and D–man were obtained from Sinopharm Chemical Reagent Co. Ltd. All other reagents were of analytical grade and used without further purification. All solutions were prepared with ultra-pure water from a high-purity water system (Youpu Super Water Company, Ltd., Chengdu, China). Different concentrations of ConA were prepared with buffer solution (0.02 mol/L PBS, pH = 7.4, containing 0.1 mol/L $MnCl_2$, 0.1 mol/L $MgCl_2$, and 0.1 mol/L $CaCl_2$) according to previous reports [12,14,16].

Electrochemical measurements, such as differential pulse voltammetry (DPV) and cyclic voltammetry (CV), were carried out on a CHI600C work station (Shanghai Chenhua Instrument Co., Ltd., Shanghai, China). Electrochemical impedance spectroscopy (EIS) was performed on an Autolab work station (Metrohm China Co., Ltd.). All the electrochemical measurements were completed in a standard three-electrode system, which consisted of a Ag/AgCl electrode as the reference electrode, a platinum wire electrode as the auxiliary electrode, and a modified GCE (d = 3 mm) as the working electrode. The FT – IR spectrum was performed at a Nicolet Is10 Fourier transform infrared spectroscopy (Thermo Fisher Scientific, USA). The pH of buffer solution was adjusted by a PHS-3C model pH meter (Shanghai Leici Instruments).

2.2. Synthesis of CS/GO

Generally, graphene oxide was prepared by the modified Hummers method [17]. 0.5 g graphite was added to a mixture of 23 mL concentrated H_2SO_4 and 0.5 g $NaNO_3$, followed by stirring for 30 min in the ice bath. Then 3 g $KMnO_4$ was put into the mixture while stirring in a water bath at 35 °C. Next, the mixture was diluted with deionized water (100 mL) and heated up to 90 °C for 1 h under continuous stirring. 3 mL 30% H_2O_2 was dropwise added to the solution, of which the color changed from brown to bright yellow. The product was subsequently washed by 1.0 mol/L HCl and deionized water, and then filtered by using 0.2 μm Nylon film and dried naturally. Finally, the black hydrophobic powder GO was obtained by filtration and dried under vacuum. 10 mg resulted GO was dispersed in 10 mL 0.5% CS solution containing 5% CH_3COOH to form a homogenous mixture of chitosan modified graphene.

2.3. Preparation of ConA electrochemical sensor

The GCE was polished by chamois leather with 1.0, 0.3, and 0.05 μm of aqueous slurry of alumina, and then alternatively washed with water, alcohol, and H_2SO_4 (1 mol/L). Then, 10 μL dispersion of chitosan modified GO was dropped on the polished electrode and dried naturally. Next, the obtained modified electrode was immersed in 1 mg/mL D – mannose solution for 1 h. The prepared D–man/CS/GO/GCE was stored at 4 °C in refrigerator when not in use. In the detection process, the sensor was dipped in ConA solutions of different concentrations for 40 min 20 μL 0.5 mg/mL HRP was subsequently dropped on the electrode surface for 1 h incubation and then washed with deionized water.

2.4. Electrochemical measurement

After being incubated with HRP, the sensor was placed in an electrolytic cell containing 0.02 mol/L pH 7.2 PBS, 6×10^{-4} mol/L hydroquinone and 5% H_2O_2. DPV measurements were performed over a potential range from −0.1 V to +0.6 V at a potential increment of 4 mV and pulse amplitude of 50 mV. The DPV curves for each