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Colorimetric detection of glucose based on ficin with peroxidase-like activity

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ABSTRACT

In this work, we developed a colorimetric biosensing system for glucose detection by coupling the peroxidase-like of ficin and the glucose oxidase (GOx). GOx can catalyze the oxidation of glucose to produce H_2O_2, then, ficin catalyzes the oxidation of peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 to produce a blue color reaction. The present sensing system showed a linear response toward glucose detection over range of 2.0–100 μM with a detection limit of 0.5 μM. This system is simple, low cost, highly sensitive and selective for glucose detection, and was also applied to measuring glucose in human serum. Furthermore, in order to expand the application of ficin in biological sensing, we immobilized ficin onto the SiO_2@Fe_3O_4 NPs, which exhibited the merits of recycling as well as allowing the repeated detection of glucose. Thus it may provide great potential applications in biomedicine, biotechnology and environmental chemistry.

1. Introduction

Diabetes mellitus is one of the most common chronic diseases globally, and its related complications lead to increase disability, shorten life expectancy and the enormous health costs for virtually every society [1–3]. It is a group of metabolic disorders in which the individual has a high concentration of glucose in their blood. The past couple of years have witnessed the prevalence of a noteworthy social phenomenon which diabetes mellitus in the world showed a rapid increasing trend [4]. Confronted with this matter, the efforts to develop a variety of methods for accurate detecting of glucose in the blood for the diagnosis of diabetes have aroused strong interest in scientific researchers [5].

As glucose is one of the most important metabolic carbohydrate energy sources, there is of great significance in the determination of blood glucose concentrations to ensure the effective treatment of prediabetes and diabetes [6,7]. To date, there are lots of analytical techniques have been reported for the detection of glucose, including fluorescent [8,9], electrochemical [10,11], bioelectrochemical [12] and colorimetric [13,14] based sensing approaches. Among these methods, colorimetric analysis approach would be a more desirable method due to the characteristics of low cost, simplicity and practicality [15]. Meanwhile, this method does not require sophisticated instrumentation and can transform the detection events into color changes, which can be read by naked eye [16,17]. For instance, Coltro et al. reported a microfluidic paper-based analytical device (μPAD) that can effectively enhance the analytical performance for colorimetric measuring glucose [18,19]. With the development of nanotechnology, an enzyme-like nanomaterials based biosensor has emerged as an important colorimetric tool for the detection of biomolecules. A series of nanomaterials such as Fe_3O_4 [20], V_2O_5 nanowires [21], CeO_2 nanoparticles [22], Au nanoparticles [23], graphene oxide [24] and carbon nanodots [25], etc., have been reported to exhibit intrinsic peroxidase-like activity and been successfully used for glucose detection. Nevertheless, the preparation of nanomaterials requires a great deal of manpower and most of them are prone to aggregation in preparation process, which might affect their catalytic activity because of the decrease of specific surface area [26]. Therefore, exploring new biological catalysts and applying them in glucose detection still remains challenging.

Ficin (EC 3.4.22.3) is a kind of plant protease and mainly exists in the latex of fig tree, which is recognized as a sulfhydryl enzyme and contains cysteine residue at the active site essential for its protease activity [27]. Because of its good stability, high protein hydrolysis ability and a good degradation effect on many kinds of proteins, it is widely used in various fields, such as food processing, industrial production and medical and health fields [28,29]. Recently, our group has reported that ficin exhibited significant peroxidase-like activity [30]. As a kind of biological catalysts, ficin has properties of high catalytic efficiency under favorable biological reaction conditions. In the colorimetric assay, ficin can catalyze the reaction of a series of peroxidase substrate including 3,3′,5,5′-tetramethylbenzidine (TMB), 2,2′-azinobis-3-ethylbenzthiazoline-6-sulphonate) (ABTS) and *o*-phenylenediamine (OPD) in the presence of H_2O_2 to produce blue, green and orange colors, respectively [31]. On the basis, a novel colorimetric method for glucose detection by combining of the catalytic reaction of glucose with glucose oxidase and chromogenic reaction catalyzed by ficin was established in this work (Scheme 1).

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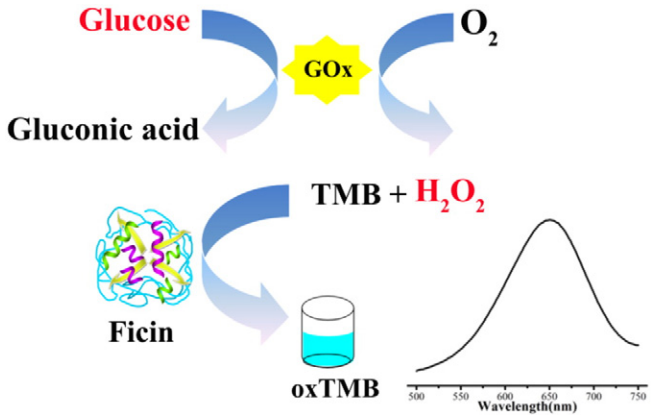

Scheme 1. Schematic illustration of the colorimetric method for glucose detection by using glucose oxidase (GOx) and ficin.

Furthermore, by immobilizing ficin onto the surfaces of SiO_2@Fe_3O_4 nanoparticles, our sensing platform permits easy sample separation, recycling as well as allowing the repeated detection of glucose.

2. Experimental Section

2.1. Materials

Glucose, fructose, sucrose, lactose, maltose, and hydrogen peroxide were purchased from Sinopharm Chemical Reagent Co., Ltd. Glucose oxidase (GOx, 200 U mg^{-1}) and premium grade ficin (≥0.1 U mg^{-1}) were purchased from Sigma-Aldrich and stored in a refrigerator at about −20 °C. 3,3′,5,5′-tetramethylbenzidine (TMB) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Amino-modified SiO_2@Fe_3O_4 was purchased from BaseLine Chrom Tech Research Center (Tianjin, China). Glutaraldehyde (50% aqueous solution) was obtained from Aladdin (Shanghai, China). All other chemicals were of analytical reagent grade and used without further purification. Ultrapure water purified with a Milli-Q system (18.2 MΩ, Millipore) was used in all runs.

2.2. Measurements

The absorption spectra were recorded by a UV–2450 spectrophotometer (Shimadzu, Japan). Transmission electron microscopy (TEM) was performed on a JEM-2100 instrument (JEOL Ltd., Japan). Fourier transform infrared (FT-IR) spectra were recorded on Shimadzu Varian 4300 spectrophotometer. The thermal gravimetric analysis (TGA) were carried out using a thermal gravimetric analysis instrument (Shimadzu TGA-50H) with a heating rate of 10 °C min^{-1} under N_2 atmosphere. A pH Meter PB-10 (sartorius, shanghai, China) was used to adjust the pH values of aqueous solutions.

2.3. Preparation of Ficin-conjugated SiO_2@Fe_3O_4 NPs

First, 1.0 mL of 5 mg mL^{-1} aminated SiO_2@Fe_3O_4 NPs was centrifuged and washed with PBS (pH 7.4) and water. Subsequently, the particles were immersed in a 500 μL of 5% glutaraldehyde solution (in 50 mM PBS, pH 7.4) for 2 h at 37 °C, followed by washing with water. In the next step, 500 μL of 0.010 g mL^{-1} ficin was mixed with the glutaraldehyde-functionalized SiO_2@ Fe_3O_4 NPs. The mixture solution was incubated for 2 h at room temperature on a shaker. Magnet was utilized to separate the ficin modified SiO_2@Fe_3O_4 (SiO_2@Fe_3O_4-ficin), followed by successively washing three times with washing buffer (10 mM PBS, pH 7.4, containing 0.05% Tween-20) to remove unbound ficin. Finally, the SiO_2@Fe_3O_4-ficin was put into 1 mL of blocking buffer (10 mM PBS, pH 7.4, containing 0.5% BSA) for another 24 h under stirring to occupy the nonspecific binding sites. After centrifugation and washing with washing buffer three times, the SiO_2@Fe_3O_4-ficin was redispersed in l.0 mL of H_2O, and stored at 4 °C for later experiments.

2.4. Colorimetric Detection of Glucose Using Ficin and GOx

Glucose detection was performed as follows: a) 0.10 mL of 0.80 mg mL^{-1} GOx, 0.20 mL of glucose with different concentrations and 0.10 mL of 0.20 M Na_2HPO_4 buffer (pH 7.0) were incubated at 37 °C for 30 min; b) 0.20 mL of 6.0 mM TMB, 0.20 mL of the ficin stock solution (10 μg mL^{-1}) and 1.2 mL of 0.20 M Na_2HPO_4 buffer (pH 4.0) were added to the above 0.40 mL reaction solution; and c) the mixed solution was incubated at 30 °C for 3 h, and at last, absorbance at 652 nm was recorded.

2.5. Glucose Determination in Serum Samples

Serum samples were obtained from three healthy volunteers. For glucose determination, the collected samples were first treated by ultrafiltration with 1 kDa at 3000 rpm for 30 min. And then, the filtrates were diluted 10 times using ultrapure water. The subsequent operations for glucose detection in serum samples were the same as described above except the replacement of glucose with serum samples.

3. Results and Discussion

3.1. Feasibility of Glucose Detection-based Colorimetric System

Hydrogen peroxide (H_2O_2) participates in a wide range of enzymatic reactions, playing an important role in the fields of chemistry and biology, therefore, considerable research interest has been paid toward its detection [32]. Recently, our group has reported that ficin was a novel peroxidase-like catalyst, which can be successfully used for H_2O_2 detection [30]. On the basis of the intrinsic peroxidase catalytic activity, H_2O_2 can interact with ficin to generate the •OH radicals [30], which is a kind of strong oxidizing agent for the oxidation of TMB to produce a blue product (oxTMB). Therefore, the absorbance of oxidation product of TMB at 652 nm was employed to indirectly measure H_2O_2. Fig. S1 showed a typical H_2O_2-concentration response absorption profile. The linear range for H_2O_2 was from 2 to 70 μM ($R^2 = 0.992$) and the limit of detection (LOD) was as low as 0.34 μM.

In addition, H_2O_2 can produce by the catalytic oxidation of glucose in the presence of GOx. Herein, we establish a TMB-ficin-GOx system to achieve quantitative detection of glucose. To investigate whether colorimetric detection of glucose based on ficin with peroxidase-like activity could be realized by GOx-triggered H_2O_2 generation, several control tests were carried out under the different conditions. From Fig. 1, it

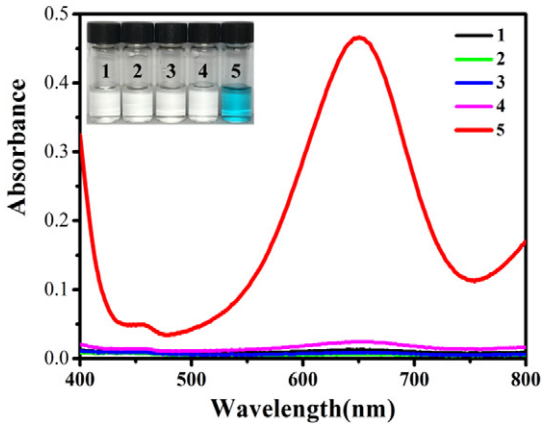

Fig. 1. UV–vis spectra of (1) TMB, (2) TMB + ficin, (3) TMB + ficin + GOx, (4) TMB + Glucose + GOx and (5) TMB + ficin + Glucose + GOx. The inset gives the photograph of the color changes of different samples under corresponding conditions. (Reaction conditions: 1.0 μg mL^{-1} ficin, 0.40 mg mL^{-1} GOx, 0.60 mM TMB and 20 μM glucose.)