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Monodispersed silica nanoparticles as carrier for co-immobilization of bi-enzyme and its application for glucose biosensing

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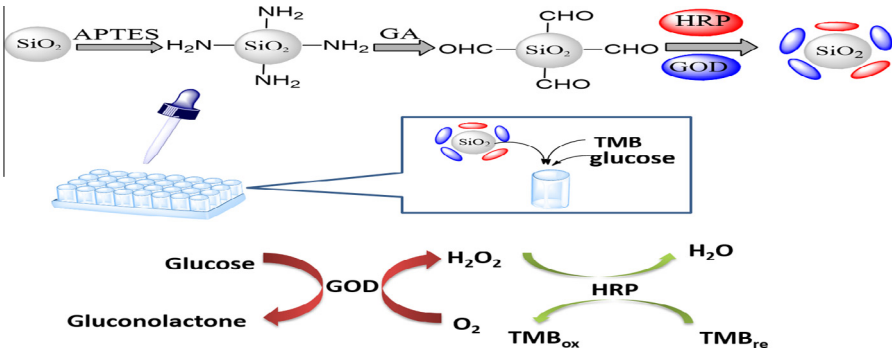
HIGHLIGHTS

- The bi-enzyme was easily and tightly immobilized on silica NPs.
- SiO_2-GOD/HRP was used to detect glucose by a visible method.
- The method is sensitive and reliable.
- It is possible to be applied in various fields by using SiO_2-GOD/HRP to make pills.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel glucose sensing strategy by using bi-enzyme coated monodispered silica nanoparticles (SiO_2) was proposed. The monodispered SiO_2 was synthesized according to our previously reported seed-growth methods. Glucose oxidase (GOD) and horseradish peroxidase (HRP) were simultaneously covalent immobilized on the surface of SiO_2 nanoparticles through the cross-linker of glutaraldehyde. The immobilized bi-enzyme remained their bioactivities well for the substrate reaction. Thus, the resultant SiO_2-GOD/HRP nanocomposites could be used as catalyst for enzymatic substrate reactions in the presence of 3,3′,5,5′-tetramethylbenzidine (TMB) as chromogenic reagent and glucose as substrate. The factors of affecting the catalytic activities of enzymes were optimized. Under optimal conditions, the absorbance at 450 nm in UV–visible spectra increased with the glucose concentration, which could be used for glucose detection with a linear range from 0.5 μM to 250 μM and a detection limit of 0.22 μM at a signal-to-noise ratio of 3σ. Considering the potential of making pills using this SiO_2-GOD/HRP, the present strategy has good prospect in the clinic science and other fields in future.

Introduction

With the excellent properties of high surface area, high chemical purity, good stability, well dispersion and ease of modification, nanosized silica has become one of the widely used nanomaterials [1]. It has been vastly applied in many aspects including paper, food painting, cosmetics, and so on. Furthermore, it has an important effect on the biochemical and medical fields. For example, SiO_2 as a perfect bio-carrier has been vastly occupied in biomedical engineering such as drug delivery, DNA transfection, cancer diagnosis, DNA mismatching, biomolecule detection and enzyme immobilization [2–6]. And the application of enzyme immobilization is outstanding in biomedical engineering.

Compared with the free enzymes, the enzymes immobilized on nanoparticles showed many advantages. The abilities of resisting some unusual conditions such as high temperature or extreme pH in the environment are improved. Basically, methods of enzymes immobilization can be divided into three categories: binding to a support, entrapment and cross-linking. Each of these ways has several characters, separately. The method of binding to a support

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can be included physical (such as hydrophobic and Vander Waals interactions), ionic, or covalent. Although the process is simple, it is usually sensitive to the synthesis conditions. Entrapment is via inclusion of an enzyme in a polymer network, typically organic/inorganic polymer matrices or a membrane device such as a hollow fiber. In general, additional covalent to attachment is often required to fix enzyme. Cross-linking, employing a bi-functional reagent, is a general method to fix enzyme [7]. It vastly improves the capacity to fix enzymes, but also cause some descents in enzyme activity.

The glucose detection has been deeply researched because of the increasing related diseases [8,9]. And the scientists have made many efforts to construct the bi-enzyme biosensors of glucose oxidase (GOD) and horseradish peroxidase (HRP) to detect glucose. When only GOD existed, glucose was converted into gluconic acid and O_2 changed to H_2O_2. So there are many experiments by detecting H_2O_2 to measure glucose in early work [10,11]. But they are usually unapparent and complex. When GOD and HRP are co-immobilized on the same carrier, the process would be altered. The product of H_2O_2 could be catalyzed to H_2O and O_2. If there were some chromogenic reductants, they can be oxidized by H_2O_2 and the color of the solution changed. In this way, the glucose detection would be visible and simplified. The common chromogenic reductant was 3,3′,5,5′-Tetramethylbenzidine (TMB) in practice experiments.

In this work, a simple and convenient approach to immobilize glucose oxidase (GOD) and horseradish peroxidase (HRP) on the surface of monodispersed SiO_2 to detect glucose in UV–visible spectra was proposed. The catalytic reactions of co-immobilized enzymes were investigated by using glucose and 3,3′,5,5′-tetramethylbenzidine (TMB) as substrates (Fig. 1). Through this method, the concentration of glucose could be monitored quickly and accurately in the serum samples, especially when there were plentiful specimens at the same time. Furthermore, considering the potential of making pills in industry, it would provide a lower-cost and more portable way to detect glucose in clinic and other fields.

Experimental

Chemicals and apparatus

Tetraethyl orathosilicate (TEOS), glucose oxidase (GOD, EC 1.1.3.4, from Aspergillus niger, 181.3 μ/mg) and (3-aminopropyl)-triethoxysilane (APTES) were purchased from Sigma–Aldrich Chemical Co., 3,3′,5,5′-Tetramethylbenzidine (TMB), horseradish peroxidase (HRP, EC 1.11.1.7, RZ ⩾ 3.0, 250 μ/mg), L-histidine, D-(+)-mannose, b-cyclodextrin, D-(−)-fructose, ascorbic acid and D-galactose were obtained from Nanjing Sunshine Biotechnology Co., Ltd. (Jiangsu, China). All other chemicals were of analytical grade and used without further purification. Phosphate buffer solution (PBS) was prepared by mixing NaH_2PO_4 and Na_2HPO_4. Twice-distilled water was used throughout the study.

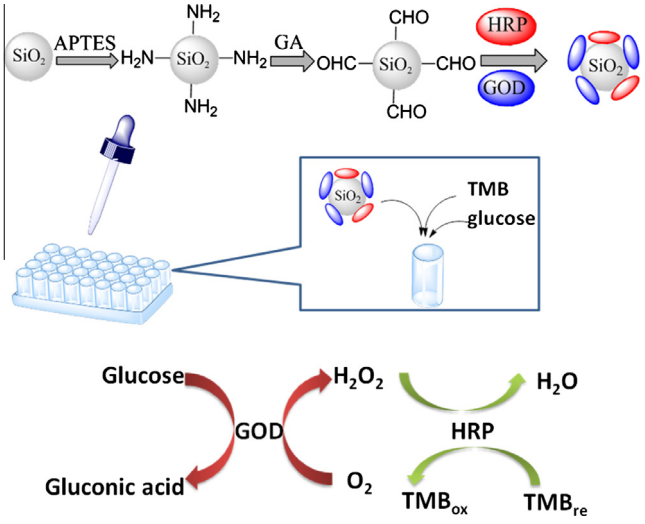

Fig. 1. Schematic illustration in colorimetric detection of glucose with SiO_2-GOD/HRP and TMB.

The morphology and size of mono-dispersed silica nanoparticles were analyzed with a transmission electron microscope (TEM, S-2400N, HITACHI, Japan). Before the measurements, 10 mL of silica suspension in water was dropped onto copper grids and dried in vacuum overnight. The optical density absorbance readings (450 nm) were taken by using the iMark Microplate Absorbance Reader (Bio-Rad, USA).

Synthesis of amino-functionalized silica particles (SiO_2–NH_2)

Monodispersed SiO_2 nanoparticles were synthesized according to our previously reported seed-growth methods [12–14]. The diameters of the as-synthesized silica nanoparticles were 130 ± 5.0 nm, determined by TEM microscopy. The synthesis of SiO_2–NH_2 was according to the literature with slightly modifications [15]. As-synthesized silica nanoparticles (0.04 g), anhydrous ethanol (6 mL) and 3-aminopropyltriethoxysilane (0.2 mL) were placed in a 10 mL glass tube. The mixture was stirred for 1 h at room temperature. Then, the solution was washed with dehydrous ethanol three times. Finally, the resultant product (SiO_2–NH_2) was dried under vacuum at room temperature for 12 h.

Co-immobilization of GOD and HRP on silica particles (SiO_2-GOD/HRP)

Typically, 4 mg amino-functionalized silica particles were ultrasonically dispersed in 500 μL glutaraldehyde (GA, 1%) followed by stirring at room temperature for 20 min. Then, the mixture was quickly centrifuged and cleaned up with PBS (0.01 M pH 7.4) several times. Next, the above mixture of SiO_2–GA was re-dispersed in 300 μL PBS containing a certain amount of GOD and HRP. The solution was then gently stirred overnight at room temperature. After that, the final product of GOD/HRP co-loading SiO_2 nanoparticles (SiO_2-GOD/HRP) was collected by centrifugation, washing and re-dispersing in a certain volume of 0.1 M pH 7.4 PBS.

Analysis procedure

0.5 μL 0.1 M TMB and 2 μL SiO_2-GOD/HRP suspension were added to 100 μL 0.1 M pH 6.0 PBS buffer. After a certain amount of glucose was added, the mixture was incubated at room temperature for 10 min. Then 50 μL 2 M H_2SO_4 was added to stop the enzymatic reaction as terminator. The absorbance at 450 nm of the resultant solution was measured. For glucose determination in human serum, the fresh serum samples were diluted 100 times using 0.1 M pH 6.0 PBS buffer. In control experiments, 2 mM L-histidine, D-(+)-mannose, b-cyclodextrin, D-(−)-fructose, ascorbic acid, D-galactose and PBS buffer were used instead of glucose for the experiments.

Results and discussion

Characterization of SiO_2-GOD/HRP nanocomposites

SiO_2 nanoparticles were employed as carriers for co-immobilization of glucose oxidase and horseradish peroxidase. Thus, silica nanoparticles with good monodispersion and similar surface morphology were vital for consistent loading of the same amount