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One-step self-assembled epoxide-containing nanodots as an enzyme-immobilized platform for biosensing

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ABSTRACT

3-Glycidoxypropyl trimethoxysilane-containing self-assembled SiOx nanodots (GPS-SANDs) bearing epoxide functional groups were successfully fabricated via a facile one-step heat treatment process. The GPS-SANDs possessed spherical-like particles with an average diameter of 3.1 ± 0.3 nm. This material exhibited a broad band, and a multicolor. Glucose oxidase (GOx) enzyme was further immobilized on GPS-SANDs via aminolysis of the epoxide groups to detect glucose using photoluminescence (PL) spectroscopy. The PL intensity changed inversely with the glucose concentration. The glucose detection limit of GOx-immobilized GPS-SANDs was determined to be 100 μM. Therefore, GPS-SANDs provide a promising GOx-immobilized platform for the design of optical glucose biosensors with high sensitivity. In addition, this platform may allow for the further development of enzyme biosensing systems through conjugations between GPS-SANDs and a target enzyme.

1. Introduction

Fluorescent nanoparticles, such as semiconductor quantum dots (QDs), exhibit a strong photoluminescence (high quantum yields), multiple fluorescence and excellent photostability [1–3]. Based on their excellent optical properties, QDs have attracted much attention in various areas, such as bioimaging, catalysis, biosensing [4,5], and especially glucose sensing [6,7]. Various types of quantum dots (e.g., CdSe/ZnS QDs, CdTe QDs, Silicon QDs and Carbon QDs) have been used to monitor glucose based on their fluorescence quenching by H_2O_2 in assembled QDs-glucose oxidase (GOx) complexes [6,8–12]. Nevertheless, some QDs require modification by a secondary chemical process because they are toxic, environmentally unsafe and chemically unstable as well as being susceptible to ultraviolet light excitation [11,13,14]. Importantly, the development of novel fluorescence-enhanced nanomaterials with excellent selectivity and sensitivity to serve as efficient fluorescent probes is desirable.

Recently, several groups have focused on the design of QD-based on optical sensing systems for glucose determination. CdSe/ZnS QDs have been used for the specific and quantitative detection of glucose based on the quenching the photoluminescence (PL) emission of the QDs by H_2O_2 and acidic changes by GOx-catalyzed oxidation of glucose [10]. Carbon QDs have been prepared from polyethylene glycol for use as a PL probe for glucose detection based on PL enhancement with H_2O_2 produced from GOx-catalyzed oxidation of glucose [11]. Ionic liquids may catalyze H_2O_2 decomposition into radicals, which could quench the fluorescence of CdSe QDs [8]. A medium pH and heavy metal ions (i.e., Cu^{2+}, Pb^{2+} and Cd^{2+}) may affect the GOx activity and PL intensity [15]. The assembly consisting of Concanavalin (ConA)-conjugated CdTe QDs and thiolated β-cyclodextrin-modified Au nanoparticles was constructed for use as a nanobiosensor for glucose detection from human serum with good selectivity [16]. CdTe QDs-Au NPs on mesoporous silica microspheres have been fabricated for glucose detection. Au NPs in a mesoporous silica shell were able to catalytically oxidize glucose as a mimic of glucose oxidase (GOx). The resulting hydrogen peroxide can quench the photoluminescence (PL) signal of the QDs in a microsphere core [17].

A promising strategy for biosensor design is the immobilization of an enzyme onto a platform or mediator [18]. Nanostructures can play an important role in the immobilization of biomolecules due to their high effective surface area, enhancement of mass transport, suitable microenvironment and good conductivity [19]. Enzymes can be immobilized through crosslinking, adsorption, covalent binding, crystallization, entrapment and encapsulation [20]. GOx is a homodimer flavoprotein containing two active sites per molecule.

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GOx can be chemically functionalized with other functional groups, such as covalent bonding with carboxylic acid groups using nucleophiles (e.g., amine or hydroxyl groups), covalent attachment of the epoxide groups via ring opening reactions with amines (aminolysis) and non-covalent functionalization via van der Waals or π–π interactions [21]. However, the enzymatic glucose sensors in which GOx is immobilized on various materials suffer from insufficient stability and loss of enzyme activity during the immobilization process [22].

In our previous study, one-step self-assembled SiO_x nanodots (SANDs) were prepared via hydrolysis and a condensation reaction using alkoxysilane [23] and 3-aminopropyltriethoxysilane [24] as molecular precursors. These nanodots exhibited band adsorption, multicolor fluorescence and very low toxicity. This combination of properties was exploited in an intravital imaging experiment using zebrafish [23] and cell cultures [24]. In this study, 3-glycidoxypropyl trimethoxysilane (GPS), which contains reactive epoxide groups that are widely used to conjugate with thiol-, amine- or hydroxyl-containing ligands in bioconjugate applications, was used as precursor to prepare GPS-containing SANDs (GPS-SANDs) through a one-step heat treatment process for amine conjugation and enzyme immobilization. GPS-SANDs exhibited multicolor photoluminescence and was further applied for the immobilization of GOx via aminolysis under ambient conditions to prevent disruption of the enzyme activity. The fluorescence of GOx-immobilized GPS-SANDs (GOx-GPS-SANDs) was quenched by H_2O_2, which was produced from the GOx-catalyzed oxidation of glucose. The fabricated GPS-SANDs demonstrated the possibility of preparing a novel platform for an enzyme biosensor system, and further application using bioconjugation is expected to produce a platform for use as an optical biosensor.

2. Material and methods

2.1. GPS-SANDs synthesis

GPS-SANDs were synthesized using a one-step heat treatment process. GPS (3 mL, Sigma-Aldrich) was placed into a thoroughly clean glass vial and heated at a constant temperature of 350 °C with stirring at 1000 rpm for 90 min under an ambient air atmosphere. Then, the sample was cooled to room temperature with continuous stirring to afford GPS-SANDs.

2.2. Characterization

The PL spectra were acquired using a Hitachi F-7000 fluorescence spectrometer. The excitation wavelengths were 340–540 nm at 20 nm intervals in the excitation domain. The emission spectra were recorded from 200 to 800 nm. Atomic force microscopy (AFM, MFP-3D, Asylum Research) was performed to analyze the particle size of GPS-SANDs. A GPS-SANDs/toluene solution (10 μL, Ratio of GPS-SANDs to toluene was 1:200 V/V) was dropped and casted on a silicon wafer substrate. An atomic force microscope was operated in tapping mode under ambient conditions. A silicon cantilever (Olympus, AC240TS) with a nominal spring constant of 2 N/m was used to obtain all of the images at a scan rate of 1.0 Hz and an image resolution of 512 × 512 pixels. The transmission electron microscopy (TEM) images of GPS-SANDs were acquired using a JEOL JEM-2100 operated at 200 kV under a vacuum of 2×10^{-5} Pa. The TEM sample was prepared by depositing a GPS-SANDs solution (0.5 μL) onto a TEM carbon grid with 200 mesh copper (No. 01801, Ted Pella Inc.) and allowed to dry. X-ray photoelectron spectroscopy (XPS, JEOL JPS 9010 MX) was performed with a monochromatic Mg Kα X-ray radiation source to determine the elemental compositions of the GPS-SANDs sample, and the sample was prepared by placing a GPS-SANDs (5 μL) solution onto clean gold substrates at room temperature. Surface-enhanced Raman scattering (SERS) was performed to analyze the functional groups on the samples. The samples were deposited on a gold substrate and then dried under ambient temperature. The SERS experiment was performed using a Raman microscope (WiTec alpha 300R) with a 532 nm incident laser and 40 mW of power. A holographic grating (600 g/mm) and a 1024 × 127 pixel back-illuminated CCD detector with a 5 s total accumulation time were used.

2.3. Alkylamine-functionalized GPS-SANDs

GPS-SANDs (300 μL) were mixed with alkylamine (6 μg) which included propylamine (C3N, TCI America) dodecylamine (C12N, TCI America) and octadecylamine (C18N, TCI America). Then, all of the mixtures were sonicated at room temperature for 30 min. The PL of the obtained samples was investigated.

2.4. Immobilization of GOx on GPS-SANDs

GOx (2 mg, Sigma) was dissolved in a phosphate buffer solution (10 μL, Sigma). GPS-SANDs (590 μL) was added to the GOx solution and further sonicated for 30 min to afford GOx-GPS-SANDs. The GOx-GPS-SANDs was further analyzed using PL and SERS to confirm the crosslinking between GOx and GPS-SANDs.

2.5. Glucose assay

The GOx-GPS-SANDs was mixed with 1 μL of glucose at various different concentrations ranging from 8 to 800 μM. The mixture was sonicated for 20 min at room temperature. Then, the oxidation reaction of glucose with GOx-GPS-SANDs was investigated by PL at an excitation wavelength of 400 nm. For comparison, free GOx was mixed with 1 μL of glucose at various concentrations ranging from 8 to 800 μM and the mixture was allowed to react for 20 min. Then free GOx-glucose solution was used to directly mix GPS-SANDs, and then characterized by PL at an excitation wavelength of 400 nm.

3. Results and discussion

3.1. Characterization

The particle size distribution of the GPS-SANDs particles was investigated using AFM topography image (Fig. 1a). Fig. 1b shows the corresponding particle size histograms. The GPS-SANDs particles were well dispersed on the silicon surface, indicating minimal aggregation in solution, and exhibited spherical-like particles with a narrow size distribution and an average diameter of 3.1 ± 0.3 nm based on statistical analysis of 250 particles. This particle size distribution was in agreement with the particle size (i.e., 3.1 ± 1.4 nm) obtained from a high-resolution TEM image (Fig. 1c). Because nanoparticles can exhibit bright and multicolor photoluminescence [25], they are capable of being applied as an important tool for biomedical applications, e.g., biolabeling, biosensing and bioimaging [26,27]. PL spectroscopy was used to characterize the optical properties of GPS-SANDs. Fig. 1d shows the PL spectra of a GPS-SANDs solution that was investigated under excitation wavelengths (λ_{ex}) that varied from 340 to 540 nm with a 20 nm increment. The GPS-SANDs solution exhibited a broad band and multicolor fluorescence emission ranging from 480 (blue) to 580 nm (yellow). Moreover, the intensity of the band depends on the excitation wavelength. The PL peak exhibited a maximum fluorescence emission (λ_{em}) of 505 nm at λ_{ex} of 420 nm.

The result from the XPS spectra of GPS-SANDs are shown in Fig. S1. GPS-SANDs primarily contained Si 2p, O 1s and C 1s. The Si 2p peak that was located at 104.8 eV was derived from