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Sensors and Bioselective Reagents

Fluorescent blood glucose monitor by hemin-functionalized graphene quantum dots based sensing system

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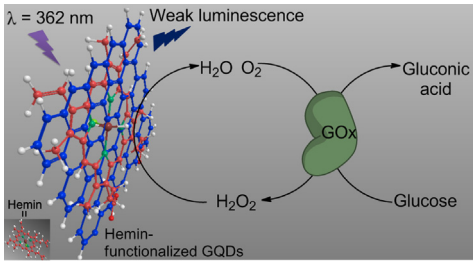
HIGHLIGHTS

- Hemin is assembled onto the surfaces of graphene quantum dots (GQDs).
- With the aid of hemin, H_2O_2 could quench the FL signal of GQDs obviously.
- Based on this effect, a fluorescent platform is proposed for the sensing of glucose.
- The proposed method provides a new pathway to explore practical application of GQDs.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present work, a highly sensitive and specific fluorescent biosensor for blood glucose monitoring is developed based on hemin-functionalized graphene quantum dots (GQDs) and glucose oxidase (GOx) system. The GQDs which are simply prepared by pyrolyzing citric acid exhibit strong fluorescence and good water-solubility. Due to the noncovalent assembly between hemin and GQDs, the addition of hemin can make hydrogen peroxide (H_2O_2) to destroy the passivated surface of GQDs, leading to significant fluorescence quenching of GQDs. Based on this effect, a novel fluorescent platform is proposed for the sensing of glucose. Under the optimized conditions, the linear range of glucose is from 9 to 300 μM, and the limit of detection is 0.1 μM. As unique properties of GQDs, the proposed biosensor is green, simple, cost-efficient, and it is successfully applied to the determination of glucose in human serum. In addition, the proposed method provides a new pathway to further design the biosensors based on the assembly of GQDs with hemin for detection of biomolecules.

1. Introduction

Semiconductor quantum dots (QDs), as photoluminescent nanomaterials, have been intensively studied in sensing and cell imaging due to their excellent performance and size-tunable optical properties [1,2]. Even with these advantages, heavy metals which are the essential elements in available high-performance semiconductor QDs have suffered from intrinsic limitations such as potential toxicity and environmental hazards [3,4]. Therefore, the search for benign alternative has become increasingly important and urgent. Recently, graphene quantum dots (GQDs) as an exciting class of carbon nanomaterial, have emerged as potential new platform in fluorescent (FL) biosensing and imaging [5,6]. GQDs, which are graphene sheets smaller than 100 nm, exhibit bright fluorescence due to quantum confinement and edge effects [7]. In addition, GQDs have the excellent properties of high biocompatibility, low-toxicity, chemical inertness and good water-solubility [8,9]. Therefore GQDs are currently the eco-friendly alternatives to toxic metal-based QDs. However, most studies are focused on the preparation and property of GQDs,

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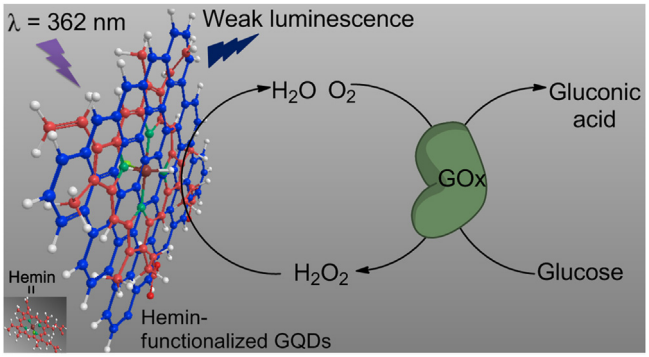

Scheme 1. Schematic illustration of the FL biosensor by hemin-functionalized GQDs.

and their analytical applications still remain at an early stage [10].

The functional modification and assembly of GQDs are essential for GQDs related applications [11]. In principle, GQDs are graphene-like smaller polycyclic aromatic hydrocarbon molecules and contain many carboxylic acid moieties at their surface, thus they can be functionalized by covalent and noncovalent interactions with various organic, inorganic, or biological species [12]. When organic molecules are covalently bound to the surface of GQDs, their extended aromatic character would be perturbed and result in the impairment of optical properties [13]. Noncovalent functionalization by π-interactions is an attractive method, because it offers the possibility of attaching functional groups without disturbing the electronic network. Hence coupling GQDs and biomolecules through π-interactions to develop high-performance sensors for biomolecular recognition is highly expected.

As a well-known natural metalloporphyrin, hemin which has intrinsic peroxidase-mimicking activity can be assembled onto the surfaces of grapheme through π–π stacking and electrostatic interactions [14–17]. Label-free colorimetric detection of single-nucleotide polymorphism has been reported based on hemin-graphene hybrid nanosheets [18]. Wu et al. constructed a label-free electrochemiluminescence sensor for telomerase detection based on porphyrin-functionalized graphene [19]. Tu et al. designed direct electrochemistry and amperometric biosensing based on picket-fence porphyrin and reduced graphene oxide through π–π interactions [20]. Song et al. reported a selective, quantitative and fast colorimetric detection of cancer cells using a folic acid conjugated graphene-hemin composite [21]. Inspired by the above reports, we designed a label-free ultrasensitive FL biosensor to detect hydrogen peroxide (H_2O_2) and glucose based on hemin-functionalized GQDs. As shown in Scheme 1, the FL sensing platform was constructed by the noncovalent assembly of hemin on GQDs through electrostatic and π–π stacking interactions. With the aid of hemin, H_2O_2 could destroy the passivated surface of GQDs and quench the FL signal obviously. As the FL quenching of hemin-functionalized GQDs was H_2O_2-concentration-dependent, a label-free, green and simple FL biosensor was established for the determination of H_2O_2 and glucose.

2. Experiment section

2.1. Materials and instruments.

Hemin and citric acid were purchased from Aladdin chemistry Co. Ltd (Shanghai, China). Hemin stock solution (5 mM) was prepared in dimethyl sulfoxide (DMSO) and stored in the dark at −20 °C. 30% H_2O_2, glucose were purchased from Shanghai Chemical Reagent Co., Ltd (Shanghai, China). Glucose oxidase was obtained from Shanghai Sagon Biotech Laboratory, Ltd (Shanghai, China). Other reagents were brought from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All the other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiments.

Transmission electron micrographs (TEM) were obtained with a Tecnai G220S-TWIN transmission electron microscope (FEI).The fluorescence spectra were performed using a Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with a quartz cell (1 cm × 1 cm). UV–vis absorption spectra were obtained using a UV-3010 recording spectrophotometer (Hitachi, Japan). FTIR spectra were measured on a FTIR-8400S spectrometer in the 480–4000 cm^{-1} region using a powered sample on a KBr plate (Japan, Shimadzu).

2.2. Synthesis of GQDs.

The GQDs were synthesized according to the literature [22]. Briefly, 2 g of citric acid was put into a 5 mL beaker and heated to 200 °C for about 20 min, until the citric acid changed to an orange liquid. Under vigorous stirring, the obtained orange liquid was added by dropwise into 100 mL of 10 mg mL^{-1} NaOH solution. The obtained GQDs solution was adjusted to pH 7 with 10 mg mL^{-1} HCl solution. The product was subsequently purified by dialyzing through the molecular porous membrane tubing (Retained molecular weight: 1000 Da).

2.3. Procedures for FL detection of H_2O_2 and glucose

FL detection of H_2O_2: In a series of 10.0 mL volumetric flasks, 1 mL 0.5 g L^{-1} GQDs, 1.0 mL of 25 μM hemin solutions, and various amounts of H_2O_2 were added. Then the mixture was diluted to the mark with 20 mM PBS and incubated at room temperature for 10 min. The fluorescence intensity of the mixture was measured with 1 cm × 1 cm quartz cells of the F-4500 spectrofluorometer. The excitation was set at 362 nm. The slit of the excitation and emission wavelengths were set at 5 nm and the voltage was set at 400 V.

Glucose detection was tested as follows: (a) 100 μL of 20 mg mL^{-1} GOx and 8 mL of different concentrations of glucose in 20 mM PBS (pH 7.0) were incubated at 37 °C for 30 min. (b) 1.0 mL of 0.5 mg mL^{-1} GQDs and 1.0 mL of 25 μM hemin were added into the above glucose reaction solution, and incubated at room temperature for 10 min. The mixed solution was used to perform the spectroscopy measurement.

3. Result and discussion

3.1. Self-assembly of GQDs and hemin

The as-prepared GQDs aqueous solution showed bright blue emission under excitation of 362 nm UV light. The quantum yield of GQDs was calculated to be about 14% using quinine sulfate as the standard. Hemin is introduced via simple adsorption on the surface of GQDs to form hemin-GQDs hybrid nanocomposites. GQDs in aqueous dispersion are negatively charged because of their residual carboxyl groups and thus can be viewed as a 0D anionic conjugated polymer [16]. Therefore, as a positively charged 18 π-electron porphyrin, hemin molecules can be assembled onto the surfaces of GQDs to form complexes through electrostatic and π–π stacking interactions. The interplay between GQDs and hemin molecules during the process of assembly was studied by the use of UV–visible spectroscopy. As shown in Fig. 1A, GQDs exhibited an apparent UV–visible absorption band centered at 362 nm, which corresponded well to the excitation spectra. The spectrum of hemin solution contained a strong peak at 386 nm attributed to the Soret band. Upon hemin absorbed on the surface of GQDs, an absorption