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Fluorescent turn-off/on bioassay for hemoglobin based on dual-emission carbon nanodots-graphene oxide system with multi-detection strategies

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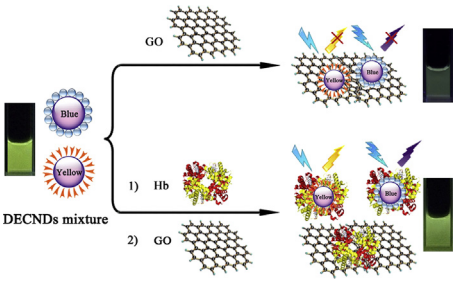
HIGHLIGHTS

- Fluorescence of dual-emission carbon nanodots could be quenched by graphene oxide.
- Fluorescence of DECNDs-GO system would recover in the presence of hemoglobin.
- Multi-detection strategies could be employed to sense hemoglobin.
- This Hb sensor showed superior sensitivity and selectivity.
- The proposed approach was applied for detection of Hb in practical blood samples.

GRAPHICAL ABSTRACT



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ABSTRACT

As two members of the carbon materials family, carbon nanodots (CNDs) and graphene oxide (GO) possess many excellent optical properties resulting in a wide range of applications. In this work, the fluorescence of resultant dual-emission carbon nanodots (DECNDs) could be quenched by GO. In the presence of hemoglobin (Hb), the fluorescence would recover resulting from two interactions: one was the direct stacking effect of Hb on GO; the other one was that Hb could cover the surfaces of DECNDs; both of them would prevent the fluorescence quenching of DECNDs by GO. In the light of this mechanism, a novel fluorescent turn-off/on method has been developed for the detection of Hb based on DECNDs-GO system. By virtue of the dual emissions of these CNDs, it is noteworthy that both a single emission and ratiometric of dual emissions can be used to establish linear relationships of Hb: 0.05 −300 nM (λem = 386 nm), 5−500 nM (λem = 530 nm), and 50−500 nM (I_{530}/I_{410}), with the corresponding limit of detection (LOD) as low as 20 pM, 2 nM and 20 nM, respectively. This present system is highly selective toward Hb over other proteins and this reliable method has been successfully applied for the detection of Hb in whole blood samples.

1. Introduction

Hemoglobin (Hb), a tetrameric metalloprotein, consists of a protein part (globin) and four iron-containing parts (heme). The

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existence of Hb in red blood cells is responsible for carrying oxygen from the lungs to various parts of the body through blood and carrying the main portion of carbon dioxide from these parts to the lungs [1,2]. The amount of Hb in blood is associated with many clinical diseases such as leukemia, anemia, heart diseases, etc. [3,4], while its normal level displays the well-functioning of the organism. Up to now, numerous analytical methods, including fluorometric [1,2,4,5], electrochemical [6,7], colorimetric [8] and chemiluminescence methods [9] have been reported for sensitive determination of Hb. However, some of these methods require expensive materials such as DNA aptamers, complicated probe preparation, or use of fluorescent dyes that suffer from photobleaching for sensitive detection of Hb [4]. Thus, there is a strong need for developing simple, reasonably accurate, and sensitive Hb measurement in human blood.

As new star members of the carbon family, graphene oxide (GO) and carbon nanodots (CNDs) have attracted tremendous attention, in which GO has been known as a superquencher of organic dyes, and CNDs are considered as a favorable candidate for the conventional heavy-metal-based quantum dots (QDs) because of their convenience in surface functionalization, low cytoxicity, good biocompatibility, and photostability [10–12]. In addition, some reports have studied the quenching effect of GO on CNDs [13–15] resulting from the energy transfer (ET) processes from photoexcited CNDs to the aromatic GO lattice [14] or the ultrafast electron transfer from CNDs to GO [15]. Utilizing the interaction between GO and CNDs, Qu et al. established protein sensors for recognition for ten proteins [16], however, in which the sensitivity of Hb was not satisfactory and the mechanism of detection was not discussed fully by the authors.

In our previous work, dual-emission carbon nanodots (DECNDs) were synthesized successfully in ethylene glycol-water binary systems, using ascorbic acid (AA) as carbon source. The dual emissions were ascribed to two types of CNDs due to the different modified ligands on their surfaces, such as AA capped on the blue emitters (λex = 315 nm, λem = 386 nm) and alcohol molecules capped on the yellow emitters (λex = 365 nm, λem = 530 nm) [17]. In this assay, we found that both fluorescence of these two emitters could be quenched effectively by GO. With addition of Hb, the dual emissions restored rapidly based on two reasons: (1) formation of GO-Hb composite via $\pi-\pi$ stacking interaction [18], and (2) Hb capping on the surfaces of DECNDs. Both of them would prevent the fluorescence quenching of DECNDs by GO. Therefore, a simple, highly sensitive and selective fluorescent Hb sensor based on DECNDs-GO was developed without complicated, costly and time-consuming operations.

On the other hand, suffered from “false positive” of one emission-based probe due to multiple factors, such as the concentration of sensors, similar interferences, or environmental effects in complex samples, ratiometric fluorescent sensors were developed with high stability, anti-interference, and good reproducibility. In this paper, benefiting from the unique dual-emission property, both a single emission and ratiometric dual-emissions could be used as criteria for Hb detection, thus multi-detection strategies were available. For instance, for the blue emitters (λem = 386 nm), the linear range of Hb was 0.05–300 nM with limit of detection (LOD) 20 pM; for the yellow emitters (λem = 530 nm), the linear fashion was 5–500 nM with LOD 2 nM; taking advantage of intensity-ratiometric measurement of dual emissions (I_{530}/I_{410}), the linear scope was from 50 to 500 nM with LOD 20 nM. Besides, the present DECNDs-GO system exhibited high selectivity toward Hb over other proteins, such as myoglobin, bovine serum albumin (BSA), β-casein, transferrin, and cytochrome c. By virtue of the superior sensitivity and selectivity, this approach has been successfully applied for the detection of Hb in whole blood samples. To our best knowledge, this is the first time for the application of quenching interaction between DECNDs and GO. And this assay would inspire future investigations in developing dual-emission sensors and lead to new discoveries.

2. Experimental

2.1. Chemicals and materials

Ascorbic acid (AA), ethylene glycol, NaCl, hemoglobin (Hb), myoglobin, bovine serum albumin (BSA), β-casein, transferrin, cytochrome c, and glucose were purchased from Aladdin (Shanghai, China). Natural graphite powder (325 mesh) was purchased from Qingdao Huatai lubricant sealing S&T Co. Ltd (Qingdao, China). Double distilled water was used for the preparation of all the solutions. All chemicals and solvents were obtained from the commercial sources and used without any further purification.

2.2. Instrumentation and characterization

The fluorescence and resonant Rayleigh scattering spectra were collected with a Hitachi F-7000 fluorescence spectrophotometer. The ultraviolet–visible (UV–vis) absorption spectra were obtained on a Cary 300 Bio UV–vis spectrophotometer. The transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) measurements were obtained on a Tecnai G2 F20 electron microscope. The Fourier transform infrared (FT-IR) spectra of the samples were analyzed by a WQF-520A FTIR spectrophotometer using KBr pellets. The X-ray photoelectron spectroscopy (XPS) measurements were performed on a Thermo Scientific/k-Alpha photoelectron spectrometer.

2.3. Preparation of DECNDs and GO

In this paper, the DECNDs were synthesized via one-pot hydrothermal method using AA as carbon source in ethylene glycol-water binary systems according to our previous report [17]. Typically, 0.8 g AA was dissolved in ethylene glycol-water reaction media. The total volume of the mixture was 20 mL and the volume fraction of ethylene glycol was 50%. Subsequently, under vigorous stirring to form a homogeneous solution, the mixture was heated to 160 °C in a constant temperature drying oven for 70 min. During this process, the color of the mixture changed from colorless to dark yellow, indicating the formation of DECNDs. After cooling to room temperature, the mixture was centrifuged at 12000 rpm for 10 min. Finally, a clear yellow aqueous dispersion containing DECNDs was obtained, and the DECNDs were stored at ambient environment. Using this method, the concentration of the synthesized DECNDs was ~36 mg mL^{-1} and the mass yield of DECNDs reached ~90%. Although the blue emitters and yellow emitters could be separated through chloroform extraction in our previous report [17], the DECNDs used to detect Hb were mixed emitters in this paper because chloroform was not suitable for bioassay.

Graphene oxide (GO) was synthesized by oxidation of graphite powder (3 g) according to the Hummers method [19]. The final concentration of GO was 0.33 mg mL^{-1}.

2.4. Fluorescence detection of Hb

At first, a suitable amount of Hb (5 μM) was added to the DECNDs solution (0.72 mg mL^{-1}) with vigorous stirring. And then the GO solution (0.33 mg mL^{-1}) was added to the mixture. After mixing to form a homogeneous solution, the signal output value was calculated according to the relative fluorescence intensity $(FF_0)/F_0$, in which F_0 and F represented the fluorescence intensities