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Dual-emission carbon nanodots as a ratiometric nanosensor for the detection of glucose and glucose oxidase

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ABSTRACT

In this paper, utilizing dual-emission carbon nanodots (DECNDs), both single signal and ratiometric detection could be developed for glucose and glucose oxidase (GOx) with high sensitivity and selectivity. Glucose could be efficiently catalyzed by GOx to generate gluconic acid and hydrogen peroxide (H_2O_2); in the presence of Fe^{2+}, based on Fenton reaction (Fe^{2+} and H_2O_2), hydroxyl radicals ($^{\bullet}OH$) could be easily achieved and caused the fluorescence quenching of DECNDs. Under optimum conditions, this fluorescent nanosensor showed excellent sensitivity with the limit of detection (LOD) of glucose 0.03 μM and LOD of GOx 1.5×10^{-5} U/mL. The proposed method was successfully applied to the determination of glucose in real samples with satisfactory recoveries.

1. Introduction

Glucose distributed widely in nature as an important monosaccharide. By virtue of glucose acting as a source of energy of the living cells and a metabolic intermediate product in the formation of other complex molecules, the level of glucose in blood was used to diagnose diabetes or hypoglycemia [1]. Therefore, glucose detection was of practical importance in the food and fermentation analysis, environmental monitoring, medical diagnosis [2–4] and so on.

Benefiting from the excellent optical properties of nanomaterials, such as good chemical stability, high quantum yield, and biocompatibility, various nano-probes were constructed for detection of glucose through multiple approaches. (1) Colorimetric detection: Apoferritin paired gold clusters could efficiently catalyze oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by hydrogen peroxide (H_2O_2) to produce a blue color reaction, while glucose could be highly catalyzed by glucose oxidase (GOx) to generate gluconic acid and H_2O_2, so an indirect detection of glucose could be developed with the linear range from 2.0 to 10.0 mM [5]. Although this method was simple, convenient and low cost, the sensitivity was not satisfactory. (2) Electrochemical detection: at first, CdTe quantum dots (CdTe QDs) and Au nanoparticles (Au NPs) were integrated on the glassy carbon electrode, and in this reaction, Au NPs acted as an efficient GOx-mimickess to catalytically oxidize glucose to produce H_2O_2 based on the consumption of dissolved oxygen, resulting in a quenching effect on the electrochemiluminescence [6]. (3) Fluorescence detection: a 3D porous carbon foam, consisting of Fe and N-incorporated carbon nanotubes, could catalyze H_2O_2 to generate hydroxyl radicals ($^{\bullet}OH$), which could oxidized the non-fluorescence of terephthalic acid to the fluorescent hydroxyterephthalate [7]. The fluorescence approach exhibited simplicity, rapid response and high specificity, but these fluorescent nanoprobes for glucose were mainly based on single-signal modulation (turn-off or turn-on). However, it was well known that many interference factors would influence the single-signal detection, for instance, probe concentration, illumination intensity, optical path length, or environmental effect in complex samples. Hence, ratiometric fluorescence detection attracted special interest due to anti-interference ability and reliability [8–13] by calculating the ratio of fluorescence intensities at two different wavelengths.

On the other hand, as an emerging luminescent material, fluorescent carbon nanodots (CNDs) were a new platform for designing multifunctional sensors. In comparison with semiconductor quantum dots and fluorescent dye molecules, CNDs showed such advantages as low toxicity, low cost, and environment friendly. For example, water-soluble CNDs were prepared by pyrolysis of ethylenediamine-tetraacetic acid (EDTA) salts, and these CNDs with no further chemical modification could detect Hg^{2+} and

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biothiols; [14] through hydrothermal process, aloe as a carbon source could also be used to synthesize CNDs, which exhibited high sensitivity and selectivity toward tartrazine [15]. More importantly, the development of CNDs eliminated the need to use quantum dots, organic dyes and organic solvents, and this was more environment-friendly.

In previous report, we developed a green and simple synthesis of dual-emission carbon nanodots (DECNDs), which contained two types of emitters, ascorbic acid (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) [16]. Herein, glucose was catalyzed by GOx to produce H_2O_2, and through Fenton reaction between ferrous iron (Fe^{2+}) and H_2O_2, the reactive •OH could effectively quench the fluorescence of these two emitters. By virtue of the unique dual-emission property, both of single emission and ratiometric dual emissions could be used to develop standard calibrations of glucose and GOx. This fluorescent nanosensor showed highly sensitivity with the limit of detection (LOD) of glucose 0.03 μM and LOD of GOx 1.5×10^{-5} U/mL, which were better than the reported literature [5–7,17]. Moreover, it also exhibited high selectivity owing to the high specificity of the enzymatic oxidation, and this developed DECNDs ratiometric sensor was successfully applied to the determination of glucose in glucose injection and human plasma with good accuracy and precision.

2. Experimental

2.1. Materials

Ascorbic acid (AA), ethylene glycol, glucose, glucose oxidase (GOx), 3,3′,5,5′-tetramethylbenzidine (TMB), dimethylsulfoxide (DMSO), lactic acid, KCl, sodium citrate, fructose, lactose, four butyl phosphonium hydroxide (TBPH), NaClO, glutathione, KO_2, pepsin, trypsin, human serum albumin (HSA), transferrin, bovine serum albumin (BSA), HCl, acetonitrile, CH_3COOH, CH_3COONa, $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$, Na_2HPO_4, NaH_2PO_4 and H_2O_2 were purchased from Aladdin (Shanghai, China). Glucose injections (0.25 M) were purchased from local pharmacy. HCl-CH_3COONa buffers (1 M, pH = 3.09) were prepared by mixing 1 M HCl and 1 M CH_3COONa according to suitable proportion. Phosphate Buffer (PB) buffers (0.2 M, pH = 7) were prepared by mixing 0.2 M Na_2HPO_4 and 0.3 M NaH_2PO_4 according to suitable proportion. All the solutions were prepared using ultrapure water produced with a Millipore-Q water system.

2.2. Apparatus

The pH values of solutions were measured using a pH meter (Mettler Toledo FE20, Switzerland). The fluorescence spectra were recorded with a Hitachi F-7000 fluorescence spectrophotometer. The ultraviolet–visible (UV–vis) absorption measurements were carried out using a Cary 300 Bio UV–vis spectrophotometer.

2.3. Synthesis of DECNDs

In our previous work, the synthetic method of DECNDs was reported [16]. In a typical procedure, 0.8 g AA was dissolved in ethylene glycol-water binary reaction media. The total volume of the mixture was 20 mL and the volume fraction of ethylene glycol was 50%. Subsequently, under the vigorous stirring, the mixture was heated at 160 °C in a constant temperature drying oven for 70 min. After cooling to room temperature, the mixture was centrifuged for 10 min at 12,000 rpm. Finally, a clear yellow aqueous dispersion containing DECNDs was gained, and these CNDs were stored at ambient environment.

2.4. Fluorescence assay of H_2O_2

A typical H_2O_2 detection procedure was conducted as follows. Briefly, the as-prepared DECNDs solutions were diluted 100 times before detection (10 μL/mL). On the basis of the different fluorescence intensities, the probe concentration was different for these two emitters. For blue emitters, 30 μL DECNDs (10 μL/mL) were added to different volumes of H_2O_2 (1 mM), respectively, and these mixtures were diluted to 700 μL with water. Subsequently, the pH values of these solutions were adjusted to 3.0 with 200 μL of HCl-CH_3COONa buffers (1 M, pH = 3.09). Then, 100 μL Fe^{2+} solutions (1 mM) were added to the above mixtures, which were stirred vigorously and incubated for 5 min at ambient temperature. Eventually, the fluorescence spectra of the mixtures were recorded by a spectrofluorophotometer. The fluorescence intensity decreased linearly with the increase of the concentration of H_2O_2, and the quenching rate was expressed as $(F_0\text{-}F)/F_0$, in which F_0 and F represented the fluorescence intensities in the absence and presence of H_2O_2, respectively.

For yellow emitters, 150 μL DECNDs (10 μL/mL) were added to different volumes of H_2O_2 (1 mM), respectively, and these mixtures were diluted to 750 μL with water. Subsequently, the pH values of these solutions were adjusted to 3.0 with 200 μL of HCl-CH_3COONa buffers (1 M, pH = 3.09). Then, 50 μL Fe^{2+} solutions (1 mM) were added to the above mixtures, which were stirred vigorously. The fluorescence spectra were recorded after reaction for 5 min at room temperature. Subsequently, a working curve was established by plotting the emission ratios (F_{410}/F_{530}) vs. H_2O_2 concentrations.

2.5. Fluorescence detection of glucose

The procedure for glucose detection was described as follows. Firstly, 20 μL GOx (50 U/mL) and different volumes of glucose (1 mM) were added into 100 μL phosphate buffer (PB) solution (pH 7.0). The mixtures obtained were kept in room temperature for 60 min before adjusting the pH of solution to 3.0 with 200 μL HCl (0.1 M). Then, the as-prepared DECNDs solutions were diluted 100 times before detection (10 μL/mL). For blue emitters, 30 μL DECNDs (10 μL/mL) were added to the obtained mixtures, respectively, and these solutions were diluted to 900 μL with water. Finally, 100 μL Fe^{2+} solutions (1 mM) were added, respectively, to the above solutions, which were stirred vigorously and incubated for 5 min at ambient temperature. The emission spectra were recorded by a spectrofluorophotometer. The fluorescence intensity decreases linearly with the increase of the concentration of glucose, and the quenching rate was expressed as $(F_0\text{-}F)/F_0$, in which F_0 and F represented the fluorescence intensities in the absence and presence of glucose, respectively.

For yellow emitters, 150 μL DECNDs (10 μL/mL) were added to the obtained mixtures and these solutions were diluted to 950 μL with water. Then, 50 μL Fe^{2+} solutions (1 mM) were added to the above mixtures, which were stirred vigorously. The emission spectra were recorded after reaction for 5 min at room temperature. Finally, a linear equation could be developed to describe the relationship between the emission ratios (F_{410}/F_{530}) and glucose concentrations.

2.6. Fluorescence detection of GOx

The different volumes of GOx and 30 μL glucose (1 mM) were added into 100 μL PB solution (pH 7.0). These mixtures obtained were kept for 60 min before adjusting the pH to 3.0 with 200 μL HCl (0.1 M). The following experiments were same as above mentioned in part 2.5 “Fluorescence detection of glucose”.