Sensing and Bio-Sensing Research 5 (2015) 112–116

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

Detection of glucose with room-temperature phosphorescent quantum dots without conjugation

Yanming Miao

Shanxi Normal University, Linfen 041004, PR China

ARTICLE INFO

Article history:
Received 26 May 2015
Revised 26 August 2015
Accepted 27 August 2015

Keywords:
Room-temperature phosphorescence
Biological fluids
Glucose

ABSTRACT

A superior method was constructed for rapid and sensitive detection of glucose by utilization of room-temperature phosphorescent (RTP) quantum dots (QDs) without sophisticated conjugation between QDs and glucose oxidase (GOD) nor complex pretreatments, such as oxygen removal, and free from the interference of autofluorescence and scattering light, which can be used to detect glucose in biological fluids. This kind of principle is based on the quenching effect of H_2O_2 on MPA-capped Mn-doped ZnS QDs (MPA: 3-mercaptopropionic acid), and such quenching process is called as Photoinduced Electron Transfer (PIET). The GOD decomposed glucose and released H_2O_2, then H_2O_2 obtained electron from MPA-capped Mn-doped ZnS QDs in order to form H_2O and O_2, and finally quenched RTP of MPA-capped Mn-doped ZnS QDs via PIET, therefore, a new method for glucose detection was built on this basis. However, this biosensor has a detection limit of 0.0029 mM and two linear ranges from 0.005 to 0.1 mM and from 0.1 to 0.4 mM.

1. Introduction

Glucose is the most widely distributed monosaccharide in nature and plays a very important role in the fields of life science [1], biology [2–4], clinical analysis [5], and food industry [6]. As a major source of energy for living cells, the glucose acts as an indispensable metabolic intermediate in various metabolic processes of animals and plants. Furthermore, an abnormal glucose level in human blood or urine is commonly considered as a sign of diabetes or hypoglycemia [7]. Thus, rapid and accurate determination of glucose level in human blood and urine is essential for diagnosis and management of diabetes, which is affecting about 150 million people worldwide [8]. Millions of diabetics need a daily test of blood glucose level, which made the glucose become the most commonly tested analyte. Glucose oxidase (GOD) is widely applied to optical and electrochemical determination of glucose based on the enzyme-catalyzed oxidation mechanism [9–14], but phosphorescence-based detection of blood glucose is not very common [15–18].

Quantum dots (QDs) and GOD were connected through a coupling agent to construct glucose detection sensors [18,19], which were based on the fact that H_2O_2 can quench QDs. Generally speaking, these sensors require complex conjugation between QDs and GOD, which may damage the properties or functions of QDs and GOD in different degrees. Meanwhile, the tedious process and high sample consumption may limit their practical application. In this study, we constructed a rapid and sensitive detection of glucose by using room-temperature phosphorescent (RTP) QDs without conjugation between QDs and GOD. Complex pretreatments are not required in this method, and a great potential for glucose detection was indicated as it was free from the interference of autofluorescence and scattering light.

As so much focus is given to RTP QDs detection recently, it is widely applied to the fields of sensors, especially biomolecular sensors [18,20–31]. Owing to the longer lifetime of phosphorescence than fluorescence, RTP QDs detection shows high reliability and stability without the interference from autofluorescence or scattered light [18,20,22,23]. Moreover, the detection selectivity can be further enhanced since the phosphorescence is not as common as fluorescence [21]. Bright prospect is shown in the development of RTP sensors [18,20–31], any other complicated sample pretreatments are not required for developed biosensors [18,22,28].

In this paper, we reported an RTP glucose detection method without conjugation GOD with QDs based on the principle as follows: GOD decomposed glucose and released H_2O_2, then H_2O_2 obtained electrons from 3-mercaptopropionic acid (MPA)-capped Mn-doped ZnS QDs to form H_2O and O_2, finally, quenched RTP of MPA-capped Mn-doped ZnS QDs via Photoinduced Electron Transfer (PIET) (Fig. 1), thus a new method was established for quantitative detection of glucose accordingly, which can be used to detect glucose in biological fluids.

E-mail address: mym8207@126.com

http://dx.doi.org/10.1016/j.sbsr.2015.08.005
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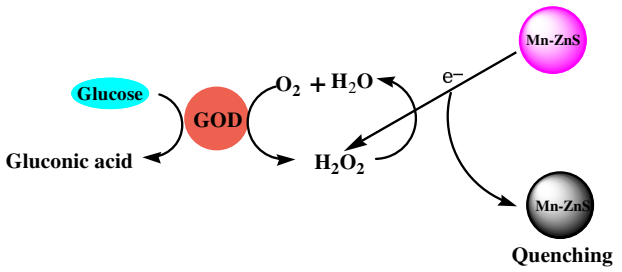

Fig. 1. Schematic illustration of fabrication of Mn-doped ZnS QDs for glucose detection.

2. Experimental

2.1. Materials and chemicals

MPA (J&K Scientific, Beijing, China), $Zn(Ac)_2{\cdot}2H_2O$, $Mn(Ac)_2{\cdot}4H_2O$, and $Na_2S{\cdot}9H_2O$ (Tianjing Kermel Chemical Reagent Co., China) were used to prepare Mn-doped ZnS QDs. Ultrapure water (18.2 MΩ cm) was obtained from a Water Pro water purification system (Labconco Corporation, Kansas City, MO). H_2O_2, glucose and GOD was provided by Tianjing Guangfu Technology Development Co., Tianjing Kermel Chemical Reagent Co., and Sigma respectively.

2.2. Apparatuses

The morphology and microstructure of QDs were inspected by a JEM-2100 transmission electron microscope (TEM, Japan). Phosphorescence and fluorescence were measured by a Cary Eclipse fluorescence spectrophotometer (Varian American Pty Ltd., America), which equipped with a plotter unit and a quartz cell (1 cm × 1 cm) in the phosphorescence mode.

2.3. Synthesis of Mn-doped ZnS QDs

Mn-doped ZnS QDs were synthesized in aqueous solution as per a published method [18,32] with minor modification. The specific steps are as follows: first, add 5 mL of 0.1 M $Zn(Ac)_2$, 2 mL of 0.01 M $Mn(Ac)_2$, and 50 mL of 0.04 M MPA into a three-neck flask and adjust the mixture to pH 11 with 1 M NaOH. Second, keep the mixture under argon conditions and room temperature, and then inject 5 mL of 0.1 M Na_2S into the mixture after ventilation of argon for 30 min. Third, the solution was aged at 50 °C under open air for 2 h after stirring for 20 min. Finally, the purified QDs can be got by precipitation with ethanol, centrifugation, washing with ethanol, and vacuum drying.

2.4. Assay condition and RTP measurement

To study the effect of H_2O_2 on RTP intensity of MPA-capped Mn-doped ZnS QDs, we prepared a 100 mM mother liquor from H_2O_2; then added different volumes of mother liquor into a phosphate-buffered saline solution (PBS, pH 7.4, 10 mM). MPA-capped Mn-doped ZnS QDs were dissolved in water to form a 2.0 mg mL^{-1} solution, which (100 μL) was added to each of the above H_2O_2 solutions, then the RTP intensity can be measured after 5 min. Glucose was made into 5 mM mother liquor in the detection. The assay solutions contained MPA-capped Mn-doped ZnS QDs (100 μL), GOD (500 U L^{-1}) and varying concentrations of glucose (0–0.8 mM), which were prepared in 5 mL of PBS (10 mM), pH 7.4 and placed in water bath at 37 °C for 15 min prior to spectrophotometry. Each experiment was repeated three times.

2.5. Sample pretreatment

The urine and serum samples were collected from healthy volunteers. All samples were subjected to a 100-fold dilution before analysis without any other pretreatments.

2.6. Measurement procedures

PBS (0.2 M, 0.25 mL), GOD (125 μL, 20,000 U L^{-1}), MPA-capped Mn-doped ZnS QDs (2 mg mL^{-1}, 100 μL), and urine or serum (0.05 mL) were sequentially added to 5 mL calibrated test tube, then a recovery study was carried out to samples by spiking with 0.05, 0.2 and 0.4 mM glucose. The mixtures were diluted to 5 mL with ultrapure water, mixed thoroughly, and placed into water bath at 37 °C for 15 min. Then the phosphorescence of the mixtures was measured at an excitation wavelength of 295 nm. Each experiment was repeated three times.

3. Results and discussion

3.1. Characterization of the MPA-capped Mn-doped ZnS QDs

The size of MPA-capped Mn-doped ZnS QDs was tested to be about 3.5 nm (Fig. 2a) by TEM. The maximum excitation peak occurred at 295 nm and the narrow emission band was centered at 595 nm: $h\nu_1$ is the fluorescence occurred from the surface defect of ZnS QDs while $h\nu_2$ is the phosphorescence attributed to the transition of Mn^{2+} from the triplet state ($^4T_1$) to the ground state ($^6A_1$) (Fig. 2b). As reported, Mn-doped ZnS QDs exhibited an orange phosphorescence emission (about 595 nm) [33], which is attributed to the energy transferred from the band gap of ZnS to Mn^{2+} dopant and subsequent $^4T_1$ to $^6A_1$ transition of the Mn^{2+} incorporated into the ZnS host lattice [34].

3.2. The mechanism in detection of glucose using RTP of MPA-capped Mn-doped ZnS QDs

As shown in Fig. 3, the RTP of MPA-capped Mn-doped ZnS QDs was not impacted by glucose within 0–1 mM basically, which indicated that the RTP of MPA-capped Mn-doped ZnS QDs was not quenched by glucose significantly.

H_2O_2 not only contains an effective QDs quencher [15,19,35–38], but also is the product of many enzyme-decomposed substrates in life activities, such as GOD, lysine oxidase and chlorine oxidase. As shown in Fig. 4, H_2O_2 affected the RTP of MPA-capped Mn-doped ZnS QDs. Along with the increased concentration of H_2O_2, the RTP intensity of MPA-capped Mn-doped ZnS QDs was regularly decreased, which indicated that H_2O_2 significantly quenched the RTP of MPA-capped Mn-doped ZnS QDs.

The principle just like explaining the conjugation of GOD and QDs [18,19]. The quenching of RTP of MPA-capped Mn-doped ZnS QDs by H_2O_2 should also be achieved via an electron-transfer mechanism [18,19]: H_2O_2 captured electrons from the conduction band of MPA-capped Mn-doped ZnS QDs, and then inhibited radiative recombination of the photoinduced electrons and holes.

Therefore, the ability of the MPA-capped Mn-doped ZnS QDs for glucose detection can be attributed to the quenching of their RTP by H_2O_2. The O_2 produced from the QDs-induced reduction of H_2O_2 can participate in the oxidation of glucose again because of its availability to glucose and GOD in solutions (Fig. 1). This cyclic use of O_2 is favorable for the biosensor system since the potential quenching of O_2 on the phosphorescence of MPA-capped Mn-doped ZnS QDs can be avoided [18,39].