Sensors and Actuators B 234 (2016) 255–263

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Amino-functionalized graphene quantum dots based ratiometric fluorescent nanosensor for ultrasensitive and highly selective recognition of horseradish peroxidase

Shan Huang[a,b], Lumin Wang[a], Chusheng Huang[a,b], Wei Su[a], Qi Xiao[a,b,*]

[a] College of Chemistry and Materials Science, Guangxi Teachers Education University, Nanning 530001, PR China
[b] Collaborative Innovation Center of Southwest Ethnic Medicine, Guangxi Normal University, Guilin, 541004, PR China

ARTICLE INFO

Article history:
Received 31 January 2016
Received in revised form 21 April 2016
Accepted 1 May 2016
Available online 3 May 2016

Keywords:
Amino-functionalized graphene quantum dots
Ratiometric fluorescent nanosensor
Horseradish peroxidase
Recognition
Enzymatic activity

ABSTRACT

Herein, a ratiometric fluorescent nanosensor for ultrasensitive and highly selective recognition of horseradish peroxidase (HRP) was reported for the first time, by employing amino-functionalized graphene quantum dots (af-GQDs) as the reference fluorophore and 2,3-diaminophenazine (DAP) as the specific response signal. DAP was the oxidation product of o-phenylenediamine (OPD) that acted as the cosubstrate of HRP. Upon the addition of HRP, OPD could be catalytically oxidized to DAP, then the fluorescence intensity corresponding to DAP at 553 nm increased dramatically with a simultaneous fluorescence quenching of af-GQDs at 440 nm, resulting in a ratiometric fluorescent nanosensor toward HRP. This ratiometric fluorescent nanosensor exhibited a broad linear range and excellent sensitivity toward HRP detection. The fluorescence intensity ratio of DAP to af-GQDs linearly increased with the increasing of HRP concentration in the range of 2 fM–800 fM (0.02 μU mL^{-1}–8 μU mL^{-1}) with a detection limit down to 0.21 fM (2.1 nU mL^{-1}). The present ratiometric fluorescent nanosensor also showed excellent selectivity for HRP over some amino acids, nucleotides, and other heme-containing proteins. The enzymatic activity of HRP was further evaluated by quantitatively calculating the enzymatic velocity and Michaelis–Menten kinetic parameter.

1. Introduction

As an upstart of carbon-based fluorescent nanomaterials, graphene quantum dots (GQDs) have attracted tremendous attentions since their discovery [1–3]. Compared with some semiconductor nanocrystals including toxic/expensive heavy-metals, GQDs are superior in terms of excellent photostability, low cytotoxicity, exceptional biocompatibility, and high water solubility. Therefore, GQDs are expected as the most promising candidates for designing novel fluorescent probes for some chemical and biological assays [4–6]. Recently, ascribed to the significantly boosted quantum yield compared with normal GQDs, amino-functionalized GQDs (af-GQDs) have attracted increasing attention in diverse research fields. Tetsuka and co-workers reported the optically tunable af-GQDs with clear multiple colors and higher luminance quantum yields [7,8]. Chattopadhyay et al. revealed the origin of tunable heterogeneous photoluminescence of af-GQDs from theoretical and experimental analysis [9]. Qu's group fabricated highly photoluminescent af-GQDs for sensing Cu^{2+} ions in aqueous solutions and living cells [10]. Since Cu^{2+} ion could bind and chelate with N and O on the surface of af-GQDs than other transition metal ions, the selectivity of af-GQDs for Cu^{2+} ion was much higher than that of normal GQDs. These results indicate that af-GQDs can be selective for many analytes due to their higher quantum yield and abundant functionalized surface groups.

Based on the reversible fluorescence variation of GQDs during detection process, GQDs have been widely utilized as fluorescent nanosensors for some specific targets through fluorescence "on-off-on" mode [11–13]. However, such single-wavelength-based detecting signal can be easily interfered with various factors, for example, optical path length, drift of light source or detector, and environmental conditions of samples, which otherwise limit the accuracy and sensitivity of quantitative determination. Fortunately, ratiometric fluorescent strategy, which allows the measurement of the changes in the ratio of fluorescence intensities at two well-resolved wavelengths, possesses advantages in terms of improved sensitivity and accuracy [14,15]. Till now,

* Corresponding author at: College of Chemistry and Materials Science, Guangxi Teachers Education University, Nanning 530001, PR China.
E-mail address: qi.xiao@whu.edu.cn (Q. Xiao).

http://dx.doi.org/10.1016/j.snb.2016.05.001
0925-4005/

dual-emission carbon-based nanohybrid materials are designed and utilized as ratiometric fluorescent probes for different substances. Qian's group have developed GQDs-based core-satellite ratiometric fluorescence probe for visual determination of Hg^{2+} ions [16]. Song et al. developed a ratiometric fluorescent nanosensor with carbon dots@europium-based nanoscale coordination polymers for biological warfare agent detection [17]. Shan and coworkers also prepared carbon quantum dots/gold nanoclusters nanohybrid-based ratiometric fluorescent probe for sensitive and selective sensing of Ca^{2+} ions and L-ascorbic acid [18]. Our group also reported a ratiometric fluorescent nanosensor based on fluorescent carbon dots for highly selective recognition of DNA [19]. As a matter of fact, these ratiometric fluorescence measurements can not only efficiently avoid the potential interferences from background fluorescence but also drastically improve the sensitivity of the detection, which undoubtedly exploit the practical application of these carbon-based fluorescent nanomaterials in biochemical and biomedical detections to certain extent.

Horseradish peroxidase (HRP), which possesses highly efficient catalysis ability on the decomposition of peroxide, is one of most widely used enzyme reagent in analytical applications [20,21]. Several analytical strategies have been established for HRP determination, such as UV–vis spectrometry [22], colorimetric assay [23], square wave voltammetry [24], as well as chemiluminescence methods [25,26]. Among these techniques, *o*-phenylenediamine (OPD), hydroquinone, pyrocatechol, *p*-chlorophenol, and tetramethylbenzidine were evaluated as cosubstrates of HRP and could react with HRP to produce different substances with absorption or electrochemical signals. Particularly, OPD was selected as the best cosubstrate for HRP, since the HRP/hydrogen peroxide (HRP/H_2O_2)-catalyzed oxidation of colorless OPD could lead to the formation of various colored products, especially fluorescent 2,3-diaminophenazine (DAP) whose maximum excitation/emission wavelengths of 430 nm/553 nm [22,24].

Recent literatures have shown that carbon-based fluorescent nanomaterials have been successfully utilized to establish fluorescent assays for several enzymes activity evaluation, such as phosphatase and acetylcholinesterase [27–29]. Inspired by these facts, we fabricated an ultrasensitive and highly selective ratiometric fluorescent nanosensor for HRP determination by using af-GQDs (emission wavelength of 440 nm) as the reference fluorophore and DAP as the specific response signal. It has been reported that the π-network of thick planar sheets in sp^2-bonded GQDs can provide a good substrate for anchoring π-conjugated molecules through π–π stacking interaction [30], which is very efficient for fluorescent quenching of af-GQDs by DAP in our system. As shown in Scheme 1, when OPD is oxidized by HRP to form DAP, the fluorescence intensity corresponding to DAP at 553 nm increases significantly with a simultaneous fluorescence quenching of af-GQDs at 440 nm, resulting in a ratiometric fluorescent nanosensor toward HRP. Such hybridized ratiometric nanosensor has been demonstrated ultrasensitive and highly selective for practical bioapplication such as on-site and visual detection of HRP.

2. Experimental

2.1. Reagents

1.0 mg mL^{-1} af-GQDs were purchased from Nanjing XFNANO Materials Tech. Co., Ltd. (http://www.xfnano.com/). HRP (>250 U mg^{-1}), OPD, DAP, amino acids, nucleotides, and other proteins were obtained from Sigma (St. Louis, MO, USA). Hydrogen peroxide (30%, w/w) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents were of analytical-reagent grade and used as received. 10 mM PBS was prepared by mixing stock standard solutions of NaH_2PO_4 and Na_2HPO_4. Ultrapure water with resistivity of 18.2 MΩ cm was produced by passing through RiOs 8 unit followed by Millipore-Q Academic purification set (Millipore, Bedford, MA, USA).

2.2. Apparatus

UV-vis absorption spectra were recorded on Cary 100 UV–vis spectrophotometer (Agilent Technologies, Inc., Australia). Fluorescence spectra were measured with RF-5301 PC luminescence spectrometer (Shimadzu Co., Ltd., Tokyo, Japan). Time-resolved fluorescence decay traces were recorded with Fluorolog-3 system (Horiba Jobin Yvon, France) by using an excitation wavelength of 374 nm. FT-IR spectra were recorded on Nicolet iS10 spectrometer (Thermo, USA). Transmission electron microscopy (TEM) images were taken by JEM 2011FEF high-resolution transmission electron microscope (JEOL Ltd., Tokyo, Japan). All pH measurements were made with a basic pH meter PB-10 (Sartorius Scientific Instruments Co., Ltd., Beijing, China).

2.3. Ratiometric fluorescent sensing of HRP

In a typical run for the determination of HRP, 1.5 mL of 10 mM PBS (pH 6.5), 0.3 mL of 30 mM OPD solution, 0.5 mL of 30 mM H_2O_2 solution, and appropriate aliquot of HRP solution were transferred into a 5 mL eppendorf tube and then the mixture was thoroughly stirred at 25 °C for 20 min in dark. After that, 40 μL of 1.0 mg mL^{-1} af-GQDs solution was added into the mixture and finally diluted to 3 mL with 10 mM PBS (pH 6.5). After 1 min incubation at 25 °C in dark, the fluorescence spectra were measured for quantitative analysis of HRP.

For fluorescence detection, the band-slits of excitation and emission were set as 15.0 nm and 5.0 nm, respectively. The sensitivity was fixed on high and the response time was set as 0.5 s. The fluorescence spectra were recorded from 360 nm to 680 nm under excitation wavelength of 346 nm. The fluorescence intensities of af-GQDs at 440 nm (I_{440}) and DAP at 553 nm (I_{553}) were recorded. The ratiometric fluorescence signal variations at I_{553}/I_{440} were utilized for label-free, ultrasensitive and highly selective determination of HRP.

3. Results and discussion

3.1. Characterizations of af-GQDs

As shown in Fig. 1A, af-GQDs exhibited broad absorption band with a typical absorption peak centered at around 330 nm, which was ascribed to the presence of amino edge functional groups and π–π^* electronic transition in C=C sp^2 carbon domains of carbon-based fluorescent nanomaterials that resulted in the strong fluorescence of af-GQDs [7,9]. Furthermore, the fluorescence spectra indicated that af-GQDs exhibited an obvious, narrow and symmetrical fluorescence spectrum with a strong emission peak centered at 440 nm under 346 nm excitation, with a Stokes shift of 94 nm. As shown in the digital pictures inserted in Fig. 1A, the diluted af-GQDs solution was almost colorless observed by the naked eyes under ambient daylight but emitted very strong blue fluorescence under the radiation of 365 nm UV lamp, demonstrating the higher photoluminescent property of these af-GQDs. The relative quantum yield (QY) of af-GQDs was examined by using quinine sulfate (QY = 54% in 0.1 M H_2SO_4 solution) as the standard substance. The results showed that the relative QY of af-GQDs was calculated to be about 10% under 346 nm excitation (Fig. S1).

As observed from most of carbon-based fluorescent nanomaterials, af-GQDs undoubtedly exhibited the identical excitation-dependent fluorescence property, due to the different surface