Sensors and Actuators B 251 (2017) 503–508

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Carbon dots derived from fungus for sensing hyaluronic acid and hyaluronidase

Kuncheng Yang, Mingxu Liu, Yingyi Wang, Shanshan Wang, Hong Miao, Liqun Yang*, Xiaoming Yang*

College of Pharmaceutical Sciences, State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 26 February 2017
Received in revised form 27 April 2017
Accepted 17 May 2017
Available online 18 May 2017

Keywords:
Carbon dots
Assaying
Hyaluronic acid
Hyaluronidase

ABSTRACT

Herein, one type of facile-green carbon dots (CDs) derived from the mushroom (fungus) has been successfully prepared with a quantum yield (QY) of nearly 15.3%. Interestingly, not only the precursor of this CDs and the whole synthesis procedure were environmental-friendly, but the CDs also exhibited multiple advantages including excellent photostability, non-toxicity and satisfactory stability. Significantly, sensitively assaying hyaluronic acid (HA) and hyaluronidase (HAase) in a maneuverable way have been further proposed on the basis of HA adsorbed on the surface of CDs by static electric and further digested by HAase. To be specific, the CDs exhibited obviously electrostatic adsorption towards HA, resulting in an effective fluorescence quenching through the aggregations appearing. Again, the enzymatic digestion between HA and HAase occurred, thus an immediately fluorescent recovery appeared once HAase was introduced. Thereby, quantitative evaluation of HAase concentration in a linear range from 0.2 U mL^{-1} to 10000 U mL^{-1} was achieved along with the range from 50 pM to 50 μM for HA, and its practicability was subsequently validated by detecting human urine samples, suggesting that the current strategy could broaden the sensing approaches for assaying HA and HAase.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Being a macromolecule of linear polysaccharide along with good water solubility, hyaluronic acid (HA) is composed of repeating D-glucuronic acid and *N*-acetyl-D-glucosamine. As the major component of the extracellular matrix, HA could regulate the cell adhesion, migration and proliferation [1], and several biological characteristics of the tumor were closely related to HA [2]. Accordingly, exploring simple, rapid and sensitive methods for detecting HA are of great importance. Meanwhile, hyaluronidase (HAase) plays the role as an enzyme that degrades HA specifically by cleaving the internal β-*N*-acetyl-D-glucosamine linkages of HA, thus increasing the tissue permeability [3]. As been reported, HAase over expresses in the certain patients with cancers (e.g. bladder, colon, prostate, and beyond), and it has been recognized as a new type of tumor marker accordingly [4,5]. In fact, there have existed various methods for HAase detection mainly including turbidimetric, viscometric, zymography, immunoassay, instrumental and chemical methods such as colorimetric [6], fluorescent detections [7] and chemiluminescence-assisted assay. Among the strategies for HAase sensing, the classical methods (e.g. turbidimetric, viscosimetric and colorimetric methods) usually lack sensitivity and selectivity. Though zymography is simple, but it is not suitable for sensitive quantitative analyses. Immunoassays are known as sensitive and selective, but specialized and expensive reagents are necessary. And instrument-based methods are sensitive and precise, but time-consuming and complicated instruments are usually required. Consequently, developing facile and accurate strategies for sensing HAase are still in demand.

Currently, carbon dots (CDs) are emerging as one type of charming nano materials due to their unique properties including stable photoluminescence, tunable excitation and emission wavelength, lack of optical blinking, small size, low toxicity, favorable biocompatibility and satisfactory fluorescent performance [8–10]. Therefore, this kind of material has drawn considerable attentions in the fields of nanobiotechnology, biological labeling, photocatalysis, sensing, biomedicine and delivering drugs and beyond [11–13]. During the past years, two major approaches have been raised for synthesizing carbon dots. One way was known as the top down, consisting of electrochemical oxidation, acidic oxidation, arc discharge and laser ablation [14–17]. For the other way, hydrothermal,

* Corresponding authors.
E-mail addresses: cysylq@swu.edu.cn (L. Yang), ming4444@swu.edu.cn, xiaoming4444@gmail.com (X. Yang).

http://dx.doi.org/10.1016/j.snb.2017.05.086
0925-4005/© 2017 Elsevier B.V. All rights reserved.

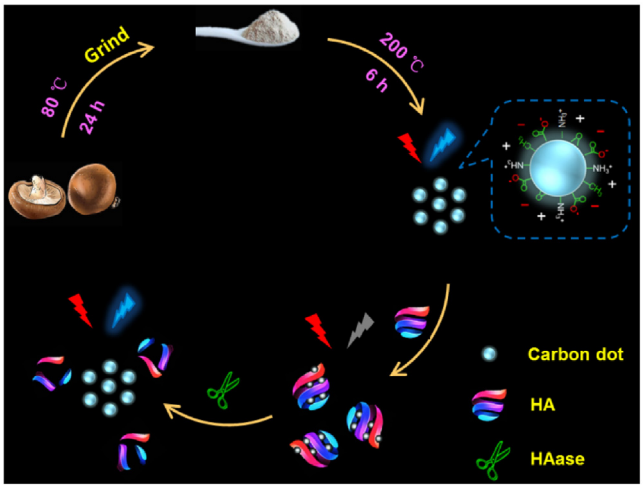

Fig. 1. Schematic illustration of detecting HA and HAase.

microwave, and ultrasonic defined as the bottom up, have been basically applied to prepare carbon dots [18–20]. Compared with conventional organic dyes and semiconductor quantum dots (QDs) that are seriously toxic for human health and our environment, carbon dots exhibited fascinating properties and potentiality not only in theoretic but also in practical aspects [21,22]. Especially, numerous carbon dots have been employed to design fluorescent probes towards analysis purposes [23,24] For instance, various carbon dots have served as biosensors for detecting the clinical proteins.

Hereby, we have succeeded to establish a green synthesis of carbon dots by one-step hydrothermal treatment of fungus within short time (6 h) (Fig. 1). The mechanism for this CDs formation mainly involved polymerization, dehydration and carbonization of the constituents such as saccharides, citric acid, and ascorbic acid in mushroom [25,26]. Besides, the precursor of CDs and the whole synthesis procedure are substantially environment-friendly, so that facilitating their biocompatibility and more extensive applications. Moreover, we developed a label-free fluorimetric assay with high sensitivity for monitoring the levels of both HA and HAase, while the CDs served as the fluorescent sensor and HA as the intermediate. To be specific, the fluorescence of CDs was obviously quenched once HA was introduced, owing to the electrostatic adsorption of HA to the surface of CDs, and thus photoinduced electron transfer (PET) possibly occurring. Subsequently, HA was decomposed by HAase upon adding into HAase, resulting in the recovery of quenched fluorescence.

2. Experimental

2.1. Chemicals

Mushroom was purchased from Yonghui Supermarket (Chongqing, China). Hyaluronic acid (HA) and hyaluronidase (HAase) were obtained from Sigma-Aldrich Co. Ltd. Metal ions (e.g. Co^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, and Ba^{2+}), DNase I, RNase I, EXO I and papain were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Disodium hydrogen phosphate (Na_2HPO_4) sodium dihydrogen phosphate (NaH_2PO_4), glacial acetic acid (HAc), phosphoric acid (H_3PO_4), boric acid (H_3BO_3), sodium hydroxide (NaOH), sodium chloride (NaCl), methanol, ethanol, acetone, acetic acid, acetonitrile, dimethyl sulfoxide, tetramethylene oxide and methyl chloroform were purchased from Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Ultrapure water (18.25 MΩ cm) produced with an Aquapro AWL-0502-P ultrapure water system was employed for all the following experiments.

2.2. Instrumentation

All fluorescence measurements were performed on a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) with an excitation slit set at 5 nm band pass and emission at 5 nm band pass in 1 cm × 1 cm quartz cell. Meanwhile, UV/Vis absorption spectra were recorded by a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). The high resolution transmission electron microscopy (HR-TEM) images were produced using a TECNAI G2 F20 microscope (Portland, America) at 200 KV. The size distribution was obtained via a Zetasizer Nano Dynamic light scattering (DLS) instrument (Malvern, England). Elemental and functional group analyses were obtained by ESCALAB 250 X-ray photoelectron spectrometer (XPS) and Fourier transform infrared (FTIR) spectrometer (Tokyo, Japan), respectively. The quantum yields were obtained by using Absolute PL quantum yield spectrometer C11347 (Hamamatsu, Japan). The powder of CDs was obtained by lyophilization in PiloFD8-4.3V (Charlotte, USA). A Fangzhong pHS-3C digital pH meter (Chengdu, China) was applied to measure the pH values of the aqueous solutions, and a vortex mixer QL-901 (Haimen, China) was used for blending the solutions. The thermostatic water bath (DF-101s) was purchased from Gongyi Instrument Co., Ltd (Gongyi, China).

2.3. Synthesis of CDs

The green CDs derived from fungus here were synthesized for the first time. All glassware and magnetic stir bars used in this experiment were thoroughly cleaned in freshly prepared aqua regia (HCl/HNO3 3:1, v/v), rinsed in distilled water, and further oven-dried prior to use. Briefly, mushrooms were dried at 80 °C for 24 h and ground as powders. Again, 0.6 g of mushroom powder with 6 mL of ultrapure water was added into a 25 mL Teflon-lined stainless-steel autoclave. After heating at 200 °C for 6 h, the autoclave was cooled to the room temperature. This aqueous solution was centrifuged at 10000 rpm for 15 min, and the supernatant was collected. Then, the aqueous solution was filtered with 0.22 μm filter membrane to remove the larger products. Again, the fluorescent carbon dots were collected by dialysis against deionized water through a dialysis membrane (100 MWCO) for 24 h. Finally, the powder of CDs was obtained by lyophilization and further dissolved in ultrapure water at the final concentration of 1 μg mL^{-1}. This CDs were stable for 3 months and stored in the dark at 4 °C for the further applications.

2.4. Fluorescence quenching of CDs by HA

Firstly, 100 μL of CDs (1 mg mL^{-1}) and 50 μL of PBS (20 mM, pH = 7.4) were successively pipetted into a 1.5-mL vial. Subsequently, 50 μL of HA (0.05, 0.5, 2, 5, 20, 70, 100, 500, 2000, 50000 nM) was added separately, and diluted to 800 μL with Milli-Q purified water. The mixtures was incubated at 37 °C for 30 min and further subjected to the fluorescence measurements. To evaluate the interference for assaying HA, HAase, DNase I, papain, Co^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, and Ba^{2+} (5 mg mL^{-1} for each) were introduced to investigate the selectivity of the CDs for HA under the optimal conditions.

2.5. Assaying HAase

At the very beginning, 100 μL of CDs (1 mg mL^{-1}), 50 μL of PBS (20 mM, pH = 7.4) and HA (50 μL, 50 μM) were successively pipetted into a 1.5 mL vial. Next, 50 μL of HAase solutions with varied concentrations (e.g. 0.2, 0.5, 2, 10, 100, 200, 1000, 5000, and 10000 U mL^{-1}) were introduced, and further diluted to 750 μL with Milli-Q purified water along. Finally, all the above mixtures was incubated to react at 37 °C for 30 min and subjected to the

Download English Version:

https://daneshyari.com/en/article/5008897

Download Persian Version:

https://daneshyari.com/article/5008897

Daneshyari.com