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Preparation of bright fluorescent polydopamine-glutathione nanoparticles and their application for sensing of hydrogen peroxide and glucose

Li Tang, Shi Mo, Shi Gang Liu, Na Li, Yu Ling, Nian Bing Li*, Hong Qun Luo*

Key Laboratory of Eco-environments in Three Gorges Reservoir Region (Ministry of Education), School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

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ABSTRACT

A novel water-soluble fluorescent polydopamine derivative, polydopamine-glutathione nanoparticles (PDA-G (-S-)NPs), was synthesized by the Michael addition reaction between dopamine (DA) and reduced glutathione (GSH). Compared to a direct polymerization of dopamine, the as-prepared PDA-G (-S-)NPs have stronger fluorescence emission intensity. Also, the synthesis does not need any hazardous organic solvents and the process is simple. Additionally, the roles of GSH and hydrogen peroxide (H_2O_2) in enhancing the fluorescence intensity are discussed in detail. The thioether in PDA-G (-S-)NPs is easily oxidized by hydrogen peroxide to sulfoxide and sulfone groups, accompanied by a decrease in fluorescence intensity. Therefore, the PDA-G (-S-)NPs can be applied to construct a fluorescent sensor for the sensitive detection of hydrogen peroxide. Based on the transformation of glucose into gluconic acid and H_2O_2 in the presence of glucose oxidase, the PDA-G (-S-)NPs system was further utilized to sensing glucose. The linear ranges and detection limits of hydrogen peroxide and glucose are (0.5–6 μM, 2–130 μM) and (0.15 μM, 0.6 μM), respectively.

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1. Introduction

In recent years, the thiol-X reaction has gained extensive attention in polymer modification and organic synthesis [1–5] due to its various advantages, such as mild reaction conditions, fewer by-products, higher functional group compatibility, and higher conversion [6–9]. Since Allen et al. initially proposed the thiol-Michael addition reaction in the 1960s [10], it rapidly became indispensable to organic synthesis and essential issues on fundamental analysis and practical implementation in polymer chemistry [11,12].

Dopamine [13–15] is inclined to be oxidized and polymerized to form polydopamine (PDA) under aerobic and alkaline conditions [16–18]. With excellent biocompatibility and many other prominent properties, polydopamine nanoparticles are widely investigated in the field of optics, electricity, or magnetism [19–22]. The particles were always generated by the oxidation and polymerization of dopamine. However, it is an intrinsic fluorescent polymer with weak fluorescence intensity. Therefore, enhancing the fluorescence intensity of PDA has become a meaningful research topic. In 2015, Tseng et al. utilized hydroxyl radicals in H_2O_2 to degrade large particles of PDA into small polydopamine dots (PDs), leading to the great enhancement of the fluorescence intensity [23]. In addition, Zuo et al. prepared a novel water-soluble polydopamine-polyethyleneimine (PDA-PEI) copolymer by the Michael addition reaction between PEI and PDA. As a result, the hydrogen bonds formed between PEI and PDA twisted the plane structure of PDA, which decreased the intro- or intermolecular coupling and enhanced the fluorescence intensity of PDA-PEI [24].

Inspired by the above-mentioned studies, we synthesized polydopamine derivatives by a thiol-Michael addition reaction between thiols and dopamine. This work can identify the sulfhydryl compounds by a colorimetric method and establish a fluorescent sensor for the detection of H_2O_2 and glucose based on the interaction between thioether of PDA-G (-S-)NPs and H_2O_2. The preparation procedures of fluorescent nanoparticles were described as follows: Firstly, dopaquinone (the oxidation product of dopamine (DA)) reacted with GSH to form a dopamine derivative by the Michael addition reaction under alkaline conditions, and then its cyclization and polymerization took place with the addition of H_2O_2, resulting in the formation of polydopamine-glutathione nanoparticles (PDA-G (-S-)NPs). Interestingly, H_2O_2 can oxidize the structure of

* Corresponding authors.
E-mail addresses: linb@swu.edu.cn (N.B. Li), luohq@swu.edu.cn (H.Q. Luo).

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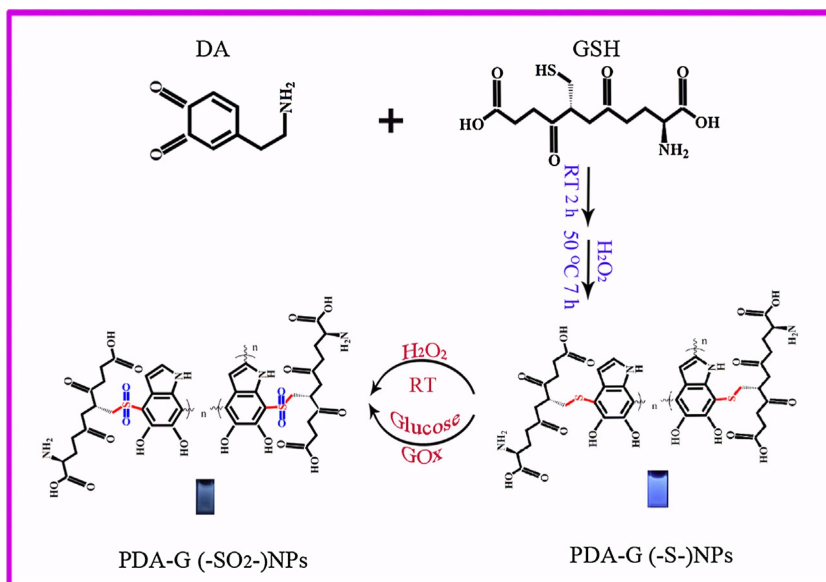

Scheme 1. Schematic illustration of the sensing mechanism of PDA-G (-S-)NPs for H_2O_2 and glucose.

thioether in the product to sulfoxide and sulfone groups, with a decrease of the fluorescence signal. Therefore, using these fluorescent PDA-G (-S-)NPs, we can establish a sensor to detect H_2O_2. The level of glucose in the blood is usually closely linked with various diseases including diabetes and hypoglycemia [25–28]. Therefore, the determination of glucose content plays an important role in the diagnosis of diabetes and clinical trials. Because the glucose oxidase (GOx)-catalyzed oxidation of glucose by molecular oxygen yields gluconic acid and H_2O_2, the constructed fluorescent sensor can also be used to detect glucose. The preparation and sensing mechanism of PDA-G (-S-)NPs are shown in Scheme 1.

2. Experimental section

2.1. Reagents and materials

3-Hydroxytyramine hydrochloride (dopamine) was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). Tris(hydroxymethyl) aminomethane (Tris), glutathione (GSH, reduced), cysteine (Cys), N-acetyl-cysteine (N-Cys), phenylalanine (Phe), glutamic (Glu), arginine (Arg), lysine (Lyb), tyrosine (Tyr), tryptophan (Try), histidine (His), alanine (Ala), and threonine (Thr) were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2, 30 wt%) was ordered from Chengdu Kong Chemical Reagents Factory, China. GOx was received from Songon Biotech Co., Ltd. (Shanghai, China). Glucose, fructose, sucrose, lactose, mannose, and maltose were purchased from Sigma-Aldrich (USA). NaH_2PO_4-Na_2HPO_4 (200 mM, pH 6.0-7.0) and Tris-HCl (50 mM, pH 7.0–10.0) buffers were needed. Ultrapure water with a resistivity 18.2 MΩ cm was used in all the experiments. Human serum samples were provided by the local hospital and diluted to one hundred fold with ultrapure water after centrifugation at 1000 rpm for 5 min.

2.2. Instruments

Excitation and emission spectra of the systems were measured on a Hitachi F-2700 spectrofluorometer (Tokyo, Japan). Slit widths of all spectra were fixed at 10 nm with a PMT voltage of 400 V. A UV–vis 2450 spectrophotometer (Shimadzu, Japan) was utilized to record UV–vis absorption spectra. A Bruker AVANCE III (600 MHz) (Bruker, Germany) was used to measure nuclear magnetic resonance (NMR) spectra by dissolving the freeze-dried product in D_2O. Fourier transform infrared (FT-IR) spectra were measured on a Bruker IFS (Germany) 113 v spectrometer. A JEOL-2100 (Tokyo, Japan) system was used to record transmission electron microscopy (TEM) images at 200 kV. Zeta potential and hydrodynamic diameter measurements were performed on a Zetasizer Nano-ZS90 (Malvern Instruments Ltd.).

2.3. Synthesis of the fluorescent polydopamine-glutathione nanoparticles

PDA-G (-S-)NPs were synthesized from dopamine and GSH via the Michael addition reaction. Typically, 4 mg of dopamine was firstly dissolved in 2 mL of Tris–HCl buffer solution (pH 8.5) with magnetic stirring for 2 min, and then 4.8 mg of GSH was added. The mixture continuously reacted for 2 h with the color changing from brown to pale yellow. All the above experiments were carried out at room temperature. Subsequently, 200 μL of H_2O_2 (30 wt%) was injected into the above solution dropwise and incubated via hydrothermal treatment at 50 °C for 7 h. For purification, the as-prepared PDA-G (-S-)NPs solution was dialyzed against ultrapure water through a dialysis membrane (molecular weight cutoff 1000 Da) for 24 h. The purified product was freeze-dried under vacuum and stored in the refrigerator (−20 °C) for long-term preservation and dissolved with ultrapure water if needed (PDA-G (-S-)NPs: 2.5 mg mL^{-1}).

2.4. The effect of pH, H_2O_2, and glucose on PDA-G (-S-)NPs

The effect of pH on the fluorescence intensity of PDA-G (-S-)NPs was investigated. 100 μL of PDA-G (-S-)NPs (2.5 mg mL^{-1}) was mixed completely with 400 μL of Tris-HCl (50 mM) buffer solution with pH ranging from 6.0 to 10.0. Then the fluorescence emission spectra were recorded at a maximum excitation of 380 nm. All measurements were carried out at room temperature.

The effect of H_2O_2 on the fluorescence intensity of PDA-G (-S-)NPs was studied. Firstly, 100 μL of PDA-G (-S-)NPs (2.5 mg mL^{-1}) was introduced to 380 μL of Tris-HCl buffer (50 mM, pH 9.0) with stirring sufficiently. Then, 20 μL of H_2O_2 with different concentrations (0–25 μM) were added and the mixtures were incubated at room temperature for 10 min. The subsequent operations and fluorescence measurements were the same as above.

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