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Detection of glucose based on the peroxidase-like activity of reduced state carbon dots

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ABSTRACT

It was found that reduced state carbon dots (r-CDs) possessed intrinsic peroxidase-like activity, and could catalytically oxidize 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 to produce a color reaction. The effects of temperature, pH, incubating time and the concentration of H_2O_2 and TMB on catalytic activity of r-CDs were investigated. Finally we calculated the kinetic constant was ca. 0.00729 min^{-1} and applied r-CDs to glucose sensing by coupling glucose oxidase. As low as 2 μM H_2O_2 could be detected with a linear range from 0.010 to 0.40 mM *via* this method. This study offered a simple, sensitive, and high selectivity method for glucose determination even in serum.

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1. Introduction

As a new member of the carbon nanomaterials family, carbon dots (CDs) have garnered intensive interest due to excellent fluorescence performances [1], such as excitation-dependent emission [2] and high photostability against photobleaching and blinking [3]. In addition, CDs are good biocompatible, small in size and low in molecular weight [4]. These unique properties make the CDs great potential substitutes for semiconductor quantum dots and organic dyes in biolabeling and bioimaging [5].

Recently, tremendous efforts have been made to develop nanomaterial peroxidase mimics since Fe_3O_4 magnetic nanoparticles-based peroxidase was reported [6]. A variety of nanomaterials, including AuNPs [7,8], BSA-stabilized Au clusters [9], Pt nanoparticles [10], Ag nanoparticles [11], carbon-based nanomaterials [12,13], and CuO nanoparticles [14,15] have been also demonstrated to possess intrinsic peroxidase-like activity. These peroxidase-like nanomaterials have shown the obvious advantages of good stability, simple preparation and low cost compared to natural enzyme [16]. However, to one's disappointment, some of these peroxidase mimics have low catalytic activity [7,16]. Thus, it is highly necessary to improve the peroxidase-like activity of nanomaterials.

The peroxidase-like activity of CDs has attracted extensive attentions since the first report in 2011 [12]. Herein, we demonstrated the intrinsic catalytic activity of r-CDs in the TMB-H_2O_2 system to produce a blue color. Therefore r-CDs were used successfully for hydrogen peroxide and glucose detection based on the TMB-H_2O_2 reaction. The effects of temperature, pH, incubating time and the concentration of H_2O_2 and TMB on catalytic activity of r-CDs were investigated. Finally we calculated the kinetic constant ca. 0.00729 min^{-1}. This study offered a simple, sensitive, and high selectivity method for glucose determination even in serum.

2. Experimental sections

2.1. Chemicals and reagents

Glucose, fructose, lactose, maltose and sucrose were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Glucose oxidase (GOx, EC 1.1.3.4. 47, 200 Umg^{-1}) was purchased from Sigma-Aldrich (St. Louis, MO) and stored in a refrigerator at −20 °C. 3,3′,5,5′-tetramethylbenzidine (TMB) was purchased from Sangon Biotech Co. Ltd. (Shanghai, China). H_2O_2 and sodium borohydride were obtained from Aladdin reagent Co. Ltd. (Shanghai, China). Serum samples were obtained from Southwest University Hospital (Beibei, Chongqing). Dialysis bags (with cutoff molecular weight of 3000 Da) were purchased from Shanghai Green Bird Science & Technology Development Co. (Shanghai, China). Ultrafiltration tubes (with cutoff molecular weight of 30 kDa and 5 kDa) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were of analytical reagent grade and used without

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further purification, and all solutions were prepared using ultra-pure water.

2.2. Preparation of r-CDs

Reduced state carbon dots were prepared following our previous report [17]. Firstly, the lampblack (0.5 g) was added in 150 mL of nitric acid solution (5 M), and the mixture was heated under reflux at 140 °C for 12 h. The dark-brown CD solution was neutralized with sodium carbonate, and then, the solution was dialyzed against ultra-pure water for two days. For the reduction of the CDs, excess $NaBH_4$ (0.5 g) was mixed with 150 mL of the CDs (about 100 μg/mL) under gentle stirring for 2 days at room temperature. Excess reductant was removed by heating at 80 °C and dialysis for two days.

2.3. Characterization of r-CDs

The morphologies of r-CDs were examined by a transmission electron microscopy (TEM) (Tec – nai G^2 F20 S – TWIN, FEI, USA). The TEM sample was prepared by dropping r-CDs solutions onto a 400-mesh carbon coated copper grids (SPI, USA) and dried in vacuum at 40 °C. Absorption spectra and fluorescence spectra was recorded by a UV-2450 spectrophotometer (Shimadzu, Japan) and an F-4500 fluorescence spectrophotometer (Hitachi, Japan), respectively.

2.4. Kinetic analysis and relative activity comparison

The reaction kinetics measurements were performed by recording the absorption value at 652 nm. Experiments were conducted using 100 μg/mL r-CDs in 0.2 M acetate buffer (pH 3.0) containing 5 mM TMB and 50 mM H_2O_2. The mixture was further incubated at 60 °C for 30 min. The absorbance at 652 nm were recorded after 0, 1, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50, 60 min incubation, respectively, to determine the reaction process and kinetic constant.

2.5. Determination of H_2O_2 and glucose

A typical response curve for H_2O_2 was realized as follows: 200 μL of 50 mM TMB, 200 μL of 1 mg/mL r-CDs and 200 μL of H_2O_2 with different concentrations were added into 0.2 M acetate buffer (pH 3.0) successively. Then the mixed solution was diluted to 2 mL and incubated in 60 °C water bath for 30 min. Finally, the color reactions were observed and the absorption values were recorded by the adsorption spectroscopy measurement.

The response curve for glucose was realized as follows: 200 μL of 10 mg/mL glucose oxidase and 200 μL of glucose with various concentrations in 0.2 M phosphate buffer (pH 7.0) were incubated at 37 °C in water bath for 30 min. Then, 200 μL of 50 mM TMB, 200 μL of 1 mg/mL r-CDs, and 200 μL of 0.2 M acetate buffer (pH 3.0) were added into the above solution, and the final mixture was further incubated at 60 °C for 30 min. Finally, the absorbance at 652 nm was recorded by a UV-2450 spectrophotometer.

2.6. Determination of serum glucose

Fresh blood samples were centrifugated at 3000 rpm for 10 min, and the supernatant was collected. Immediately after ultra-filtrated at 3000 rpm for 30 min, glucose oxidase and PBS (pH = 7.0) were added and incubated at 37 °C for 30 min to produce H_2O_2. The other detection procedure was the same as that of determination of H_2O_2.

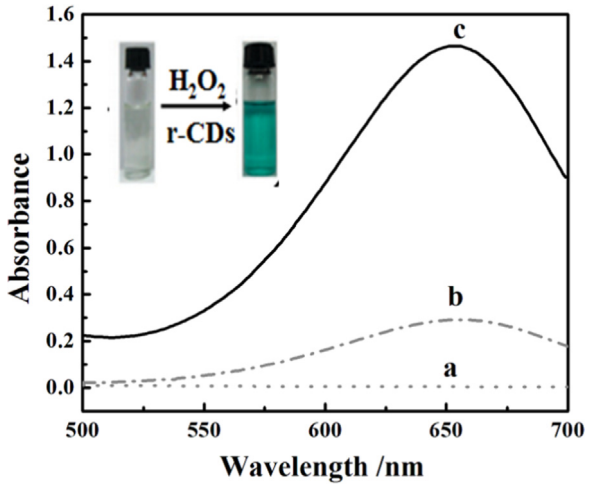

Fig. 1. Observed changes in the absorbance spectra at 652 nm for different reaction systems: (a) r-CDs+TMB, (b) TMB+H_2O_2 and (c) TMB+r-CDs+H_2O_2 in a pH 3.0 NaAc buffer (0.2 mol/L) at 35 °C.

3. The results and discussion

3.1. Morphology and spectral characterization of r-CDs

TEM was carried out to characterize the morphology and particle size of r-CDs. As shown in Fig. S1(a), r-CDs were spherical morphology with homogeneous particle size, the average diameter was ca. 3.4 ± 0.8 nm (n = 100). The absorbance and fluorescence spectra of r-CDs and CDs were shown in Fig. S1(b) and (c), supplementary materials. The optimal excitation and emission wavelength of r-CDs were 280 and 450 nm, respectively (Fig. S1(c), supplementary materials). The features of the obtained spectra are consistent with previous report [17], which indicated the successful preparation of r-CDs.

3.2. Peroxidase-like activity of r-CDs

The peroxidase-like activity has been reported for CDs [12] but there is no information whether r-CDs possesses it. In this paper, we investigated the peroxidase-like activity of r-CDs via TMB-H_2O_2 chromogenic system. As shown in Fig. 1, r-CDs can catalyze the oxidation of TMB by H_2O_2 to produce the typical blue color reaction. The absorbance peak of the reaction mixture locates at 652 nm, which attributes to the oxidation products of TMB [18]. Additional control experiments using TMB in the absence of r-CDs or H_2O_2 showed no oxidation reaction, indicating that both the components are required for the reactions and r-CDs possess peroxidase mimics activity.

3.3. Factors affect the catalytic activity of r-CDs

The catalytic activity of r-CDs is pH and temperature-dependent. The effects of these two parameters on the catalytic relative activity of TMB oxidation are shown in Fig. 2(a) and (b). The peroxidase-like activity of r-CDs was monitored by varying the pH from 1 to 10, and the maximum catalytic activity of r-CDs was obtained at pH 3.0. The peroxidase-like activity of r-CDs was monitored by varying the temperature from 20 to 90 °C, and the maximum catalytic activity of r-CDs was obtained at 60 °C. In addition, we also investigated the influence of the concentration of H_2O_2, TMB (Fig. 2(c) and (d)) and r-CDs (Fig. S2, supplementary materials) on the catalytic activity. The optimal concentration of H_2O_2, TMB and r-CDs for this system were 50 mM, 5.0 mM and 0.10 mg/mL, respectively.

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