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A simple and sensitive $Ce(OH)CO_3/H_2O_2$/TMB reaction system for colorimetric determination of H_2O_2 and glucose

Xiang Zhang, Xueqing Bi, Weihua Di*, Weiping Qin*

State Key Laboratory on Integrated Optoelectronics, College of Electronic Science and Engineering, Jilin University, Changchun 130012, People's Republic of China

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ABSTRACT

The detection for H_2O_2 is essential in many areas, including life activity, medical diagnosis, industry and agriculture production and environmental monitoring, etc. This work developed a simple and sensitive two-step reaction system $Ce(OH)CO_3/H_2O_2$/TMB for H_2O_2 determination. Upon sequential addition of H_2O_2 and TMB to $Ce(OH)CO_3$ powders, a typical color reaction occurred quickly, producing a characteristic blue color in a slightly acidic aqueous solution. The underlying reaction mechanism was proposed based on the color reaction catalyzed by mimetic enzyme. The dependence of the color depth on H_2O_2 concentration enabled the colorimetric determination of H_2O_2. This reaction system responds linearly and quickly in a wide H_2O_2 concentration range of 0–80 μM, and achieves a detection limit of 0.3 μM H_2O_2 and a relative standard deviation lower than 5.1%. This H_2O_2 sensing system was modified to allow for the detection of glucose since H_2O_2 is one of the main products in the oxidation reaction of glucose catalyzed by oxidase enzymes. In addition to a wide linear response, a low detection limit and a high reproducibility, our present reaction system for glucose determination showed a highly specific response to glucose due to the specificity of glucose oxidase to glucose.

1. Introduction

Hydrogen peroxide (H_2O_2), one important representative of the group of reactive oxygen species (ROS), is now known to be acted as one of the signaling molecules in a wide range of signaling transduction processes, such as in normal cell functions or disease progressions [1,2]. H_2O_2 is also involved in chemical, pharma-ceutical, and environmental processes. Therefore, H_2O_2 determination has been required in many areas, including medical diagnosis, industry and agriculture production, environmental monitoring, etc. [3].

On the other hand, H_2O_2 is also the byproduct of many enzymatic reactions by a large number of oxidases, thus enabling quantitative assays of the activity of the enzyme as well as various enzyme substrates such as protein and carbohydrate in living organisms via a H_2O_2-mediated process [4–6].

To date, various analytical techniques have been developed for the detection of H_2O_2, such as high performance liquid chromatography (HPLC) detection [7], optical sensing [8–12], colorimetric method [13–17], electrochemical analysis [18,19], etc. Owing to the advantages of simplicity, cheapness as well as the fact there is no requirement for any sophisticated instrumentation, colorimetric methods show great potential for portable and inexpensive daily life applications [20].

Recently, enzyme mimetics have attracted great attention because they possess several advantages over natural enzyme such as low cost and high stability against denaturation and protease digestion. Meanwhile, the development of enzyme mimics has also opened a new way for colorimetric assay of analyte. In 2007, Fe_3O_4 magnetic nanoparticles were firstly discovered to exhibit an intrinsic peroxidase-like catalytic activity [21], which was used to achieve the colorimetric determination for H_2O_2 and glucose via a color reaction of substrate [13]. Subsequently, various types of nanomaterials including metal oxide, carbon, and noble metal have been developed to possess unique enzyme-mimicking catalytic activities [22–26].

In this work, a simple and sensitive two-step reaction system, $Ce(OH)CO_3/H_2O_2$/TMB, was developed for H_2O_2 detection. $Ce(OH)CO_3$ powders were prepared by a simple hydrothermal reaction. The addition of H_2O_2 solution to $Ce(OH)CO_3$ powders, followed by the addition of TMB at a slightly acidic pH condition, caused a typical color reaction, producing a characteristic blue color in aqueous solution. The color depth of the solution depended on the H_2O_2 concentration, thus enabling the colorimetric determination of H_2O_2. This reaction system was modified to be also suitable

* Corresponding authors at: 2699 Qianjin Street, Changchun 130012, People's Republic of China.
E-mail addresses: whdi@jlu.edu.cn (W. Di), wpqin@jlu.edu.cn (W. Qin).

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for glucose detection via a H_2O_2-mediated process since H_2O_2 is one of the products of the glucose oxidation reaction under the catalysis of glucose oxidase.

2. Experimental

2.1. Chemicals

Analytical grade $Ce(NO_3)_3{\cdot}6H_2O$, $CO(NH_2)_2$ (urea), hydrogen peroxide (H_2O_2), citric acid and sodium citrate were obtained from Beijing Chemicals Reagents, China. Glucose, glucose oxidase (GOx), fructose, maltose, lactose, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich Co. (Shanghai, China). MiliQ water was used throughout. All other chemical reagents were of analytical reagent grade. The citrate buffer was prepared by mixing an approximate ratio of citric acid and sodium citrate solutions.

2.2. Preparation of $Ce(OH)CO_3{\cdot}H_2O$

$Ce(OH)CO_3$ colloidal particles were prepared via a urea-based hydrothermal process according to the previous literatures [27]. In a typical reaction, 1 mmol of $Ce(NO_3)_3{\cdot}6H_2O$ and 20 mmol urea were dissolved in 50 mL of deionized water. The above solution was first homogenized under magnetic stirring at room temperature for 30 min, and then transferred into a 100 mL Teflon-lined autoclave and heated to 160 °C for 2 h. After the autoclave was cooled down to room temperature naturally, the obtained precipitates were collected by centrifugation and washed with deionized water and ethanol several times, and then dried in the air at 80 °C for 12 h.

2.3. H_2O_2 detection

In a typical process of H_2O_2 detection using $Ce(OH)CO_3/H_2O_2$/TMB system, 40 μL of H_2O_2 solution was added into the reaction tube containing 0.5 mg of $Ce(OH)CO_3$ powder, and reacted at room temperature for 4 min. Subsequently, 2 mL of citrate buffer solution (pH = 3.6) and 0.5 mg of TMB was sequentially added to the above mixed solution and incubated at room temperature for 5 min. The supernatant was immediately moved to 4 mL of quartz cuvette for UV–vis absorption measurement. To establish the relationship between the absorbance and H_2O_2 concentration, H_2O_2 concentration was changed from 10 to 80 μM, but other reaction conditions keep the same according to the above experiment procedure. It is known that the solution pH is a key parameter to influence the color reaction [21]. It is found in our experiments that the optimal pH was approximately 3.6 (Fig. S1, supplementary information). Therefore, the color reaction in this work was carried out at pH 3.6 citrate buffer solution.

2.4. Glucose detection

The glucose solution with the concentrations ranging from 10 to 160 μM was prepared and 3.2 mg of GOx was dissolved in 20 mL HEPES buffer solution (pH = 6.5). In a typical process of glucose detection, 40 μL of glucose solution and 100 μL of GOx solution were sequentially added into a 10 mL tube and incubated at 37 °C for 5 min. Then, 0.5 mg of $Ce(OH)CO_3$ powder was added to the above solution and reacted for 4 min. Subsequently, 2 mL of citrate buffer solution (pH = 3.6) and 0.5 mg of TMB were sequentially added to the above mixed solution and incubated for 5 min. The supernatant was immediately moved to 4 mL of quartz cuvette for UV–vis absorption measurement.

2.5. Characterizations

The X-ray powder diffraction (XRD) data were collected on an X'Pert MPD Philips diffractometer (CuKα X-radiation at 40 kV and 50 mA) in the 2θ range from 10° to 70° with a scanning step of 0.02°. The transmission electron microscopy observations were carried out using a JEOL 2200FS microscope. Samples for TEM investigations were prepared by first dispersing the particles in ethanol under assistance of ultrasonification and then dropping 1 drop of the suspension on a copper TEM grid coated with a holey carbon film. Fourier transform infrared (FT-IR) spectra (Mattson 5000) of the samples were measured in the range of 4000–500 cm^{-1} in transmission mode. The pellets were prepared by adding 0.8 mg of the sample powder to 80 mg of KBr. The powders were mixed homogeneously and compressed at a pressure of 10 KPa to form transparent pellets. X-ray photoelectron spectroscopy (XPS) analysis was performed using a PHI Quantera SXM (ULVAC-PHI) device operating at a pressure of 10^{-8} Torr. The photoelectron emission spectra were recorded using a monochromatic Al Kα source (100 W). The angle between the x-ray direction and the emitted electron direction was 45°. The UV–vis absorbance measurements were carried out with a Schimadzu UV-2550 scanning spectrophotometer.

3. Results and discussion

3.1. Preparation and characterization of $Ce(OH)CO_3$

$Ce(OH)CO_3$ was prepared via a urea-based hydrothermal precipitation reaction (Experimental section), in which urea serves as a precipitation agent of metal cations due to self decomposition into the OH— and CO_3^{2-} at elevated temperatures [27–29]. The urea-based reaction is a simple and general route for the preparation of lanthanide hydroxylcarbonate [27]. Fig. 1(a) shows the XRD pattern of the as-prepared product. All diffraction peaks were well indexed to $Ce(OH)CO_3$ phase and no impurity peaks were identified, indicating that the product is single-phased $Ce(OH)CO_3$.

The FT-IR measurement (Fig. 1(b)) provided further evidence for the successful preparation of $Ce(OH)CO_3$. In the FT-IR spectrum, a strong and broad absorption bands peaking at 3400 cm^{-1} and a shoulder located at 1645 cm^{-1} are the characteristic absorption of H_2O molecules and hydroxyl groups (OH^-) [30]. The presence of carbonate anions in the molecular structure is confirmed by the appearance of absorption doublets in the region 1350–1600 cm^{-1} (ν_3 of CO_3^{2-}, peaking at ~1417 and 1504 cm^{-1}) and also by the occurrence of multiple absorptions ranging from 500 to 1000 cm^{-1} (ν_2 and ν_4 of CO_3^{2-}) [27,31].

3.2. Reaction mechanism

In our preliminary experiment, it was interesting to find that, the addition of H_2O_2 solution to $Ce(OH)CO_3$ powders, followed by the addition of TMB at a slightly acidic pH condition, caused a typical color reaction, producing a characteristic blue color in aqueous solution. In comparison, such color reaction was not observed for the mixed solution of TMB and H_2O_2 in the absence of $Ce(OH)CO_3$ or for the mixed solution of TMB and $Ce(OH)CO_3$ in the absence of H_2O_2. This phenomenon excited our curiosity to explore the underlying reaction mechanism in the $Ce(OH)CO_3/H_2O_2$/TMB reaction system.

To this end, several control experiments were performed as follows. (i) 0.5 mg of TMB alone was added to (2 mL + 40 μL) citrate buffer solution. After 5 min, no color transformed. (ii) 40 μL of H_2O_2 (60 μM) was added to 2 mL citrate buffer solution, and subsequently 0.5 mg of TMB was added. After incubation for 5 min,