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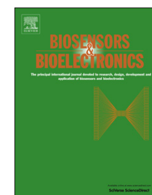

Fluorescent hydrogen peroxide sensor based on cupric oxide nanoparticles and its application for glucose and L-lactate detection

Ai-Ling Hu [a,b], Yin-Huan Liu [c], Hao-Hua Deng [a,b], Guo-Lin Hong [c], Ai-Lin Liu [a,b], Xin-Hua Lin [a,b], Xing-Hua Xia [d], Wei Chen [a,b,*]

[a] Department of Pharmaceutical Analysis, Fujian Medical University, Fuzhou 350004, China
[b] Nano Medical Technology Research Institute, Fujian Medical University, Fuzhou 350004, China
[c] Department of Laboratory Medicine, Second Hospital of Fuzhou, Fuzhou 350007, China
[d] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

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ABSTRACT

A novel fluorescent hydrogen peroxide sensor was developed based on the peroxidase-like activity of cupric oxide nanoparticles. Cupric oxide nanoparticles effectively catalyzed the decomposition of hydrogen peroxide into hydroxyl radicals. Then terephthalic acid was oxidized by hydroxyl radical to form a highly fluorescent product. The linear range of hydrogen peroxide estimated to be 5.0×10^{-6}–2.0×10^{-4} M with a detection limit of 3.4×10^{-7} M. Moreover, this detection system enabled the sensing of analytes which can enzymatically generate hydrogen peroxide. By coupling the oxidation of glucose or L-lactate catalyzed by their corresponding oxidase enzymes with terephthalic acid oxidation catalyzed by cupric oxide nanoparticles, sensitive assays of glucose and L-lactate with detection limits of 1.0×10^{-6} and 4.5×10^{-8} M were realized. The successful applications of this approach in human serum samples have also been demonstrated.

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1. Introduction

Glucose is a source of energy of the living cells and metabolic intermediate in biological systems. It can also provide significant information of many diseases such as hypoglycemia or diabetes. The disease is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. As another important metabolite, L-lactate also plays a key role in clinical analysis. The increase of L-lactate has been widely used as an excellent indicator of hypoxia, poor perfusion of tissue, acute circulatory shock, liver failure, congestive heart failure and diabetic ketoacidosis (Ander et al., 1998; Gajovic et al., 2000). Failure of arterial serum lactate to achieve normal levels has been associated with an increased mortality among medical and trauma patients (McNelis et al., 2001). Therefore, the detection of L-lactate serves as an aid for diagnosing heart disease, exercise physiology, neonatology and neurology studies (Shen et al., 2012; Wyss et al., 2011).

Up to now, various methods such as chemiluminescence (Ballesta-Claver et al., 2008; Lan et al., 2008), fluorometry (Chang et al., 2009; Groegel et al., 2011), electrochemistry (Ghamouss et al., 2006; Hu et al., 2005; Manesh et al., 2010), and spectrophotometry (Sanz et al., 2005; Tumang et al., 2001) have been reported for glucose and L-lactate detection. Among them, horseradish peroxidase (HRP) has been widely used to fabricate sensors for detection of the products from reactions catalyzed by corresponding oxidase. Owing to many disadvantages of natural enzymes like difficult and high-cost purification processes as well as inherent instability, more and more attention has been paid to constructing enzyme mimics with similar functions to natural enzymes in recent years (Wei and Wang 2013; Wiester et al., 2011). A variety of inorganic nanomaterials including ferromagnetic nanoparticles (Gao et al., 2007), carboxyl-modified graphene oxide (Song et al., 2010), gold nanoparticles (Jv et al., 2010; Wang et al., 2012), V_2O_5 nanowires (Andre et al., 2011), AgM bimetallic alloy nanostructures (He et al., 2010), $CoFe_2O_4$ magnetic nanoparticles (Shi et al., 2011b), Au@Pt nanostructures (He et al., 2011; Liu et al., 2012), $BiFeO_3$ (Luo et al., 2010), Co_3O_4 nanoparticles (Mu et al., 2012), helical carbon nanotubes (Cui et al., 2011), and carbon nanodots (Shi et al., 2011a), have been evaluated to possess intrinsic enzyme mimetic activity similar to that found in natural peroxidase. In comparison with HRP, peroxidase nano-mimics demonstrate great catalytic property and high-stability. In our recent studies, we found that cupric oxide

* Corresponding author at: Department of Pharmaceutical Analysis, Fujian Medical University, Fuzhou 350004, China. Tel./fax: +86 591 22862016.
E-mail address: chenandhu@163.com (W. Chen).

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nanoparticles (CuO NPs) are not only highly effective catalysts to peroxidase substrates but also considerably more stable and possess an almost unchanged catalytic activity over a wide range of pH and temperatures (Chen et al., 2011; Hong et al., 2013).

Since fluorescence spectroscopy is one of the most useful analytical tools in bioanalysis, many fluorescent probes, such as europium coordination complexes (Wolfbeis et al., 2003), dichlorofluorescin (Sanchez Ferrer et al., 1990), Amplex Red (Lien et al., 2012), quantum dots (Yuan et al., 2009), gold nanoclusters (Jin et al., 2011), and cationic conjugated polymers (He et al., 2006), have been developed for the detection of H_2O_2. As has already been discovered, terephthalic acid (TA) is a specific fluorescence dosimeter for •OH in a variety of physical and chemical systems (Barreto et al., 1995; Charbouillot et al., 2011; Dutta et al., 2013; Ishibashi et al., 2000). In comparison with other peroxidase substrates like TMB and ABTS, TA has some unique properties such as low cost, pure oxidation products easily obtained, more stability for storage and less vulnerability to degradation. In the present work, therefore, TA is used as a fluorescent peroxidase substrate to establish a TA-CuO NPs system for the determination of H_2O_2. By coupling the oxidation of glucose or L-lactate catalyzed by their corresponding oxidase enzymes with the TA oxidation catalyzed by CuO NPs, fluorometric methods were further developed for quantitative analysis of glucose and L-lactate in human serum.

2. Experimental

2.1. Chemicals and materials

All chemicals and reagents were of analytical grade and used without further purification. Cupric acetate, sodium hydroxide, terephthalic acid, glucose, maltose, D-fructose and 30% (v/v) H_2O_2 were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Glucose oxidase, L-lactate and α-lactose were purchased from Aladdin Reagent Company (Shanghai, China). Lactate oxidase was purchased from Sigma Co. Ltd. Terephthalic acid was neutralized with NaOH and used as 100 mM stock solution in water. The stock solution of glucose was allowed to incubate at room temperature overnight before use. The water used throughout all experiments was purified by a Milli-Q system (Millipore, USA). Clinical serum samples were provided by the Second Hospital of Fuzhou.

The cupric oxide nanoparticles were prepared via a previously reported quick-precipitation method (Chen et al., 2012a). First, 150 mL of 0.02 M copper acetate aqueous solution was mixed with 0.5 mL glacial acetic acid in a round-bottomed flask equipped with a refluxing device. The solution was heated to boiling with vigorous stirring. Then 10 mL of 0.04 g/mL NaOH aqueous solution was rapidly added into the above boiling solution, where a large amount of black precipitate was simultaneously produced. The precipitate was centrifuged, washed three times with absolute ethanol, and dried in air at room temperature. The as-prepared CuO nanoparticles, which are without any surface modification, can well disperse in distilled water and form a transparent brown solution. The appearance of the solution remains unchanged even after 6 months, which performs a perfect stability.

2.2. H_2O_2 sensing

In a typical experiment, (a) 0.8 mL of 18.75 mM TA, 50 μL of the CuO nanoparticles stock solution (0.04 mg/mL), and 500 μL H_2O_2 of different concentrations were added into 3.65 mL of 200 mM phosphate buffer (pH 7.0); (b) the mixed solution was incubated in a 45 °C water bath for 20 min; (c) the resulting reaction solution was measured by using a Cary Eclipse fluorescence spectrometer under the excitation wavelength of 315 nm.

2.3. Glucose detection

62.5 μL of 1 mg/mL GOx and 500 μL glucose of different concentrations were added into 62.5 μL of 200 mM phosphate buffer solution (pH 7.0), and incubated at 37 °C for 20 min. 3.525 mL phosphate buffer solution, 0.8 mL of 18.75 mM TA, and 50 μL of the CuO NPs (0.04 mg/mL) were added to the above glucose reaction solution for another 2 h at 45 °C. The resulting reaction solution was measured by using a Cary Eclipse fluorescence spectrometer under the excitation wavelength of 315 nm.

Glucose detection by oxidase endpoint method was carried out by adding 100 μL of 1 mg/mL GOx, 100 μL HRP, 200 μL TMB, and 500 μL glucose of different concentrations into 3.1 mL of 200 mM phosphate buffer solution (pH 7.0). The mixture was then incubated at 37 °C for 10 min. The resulting reaction solution was measured by using a Shimadzu UV-2450 spectrophotometer.

2.4. L-lactate detection

50 μL of 0.01 mg/mL LOx and 400 μL L-lactate of different concentrations were added into 50 μL of 200 mM phosphate buffer solution (pH 7.0), and incubated at 37 °C for 10 min. 3.65 mL phosphate buffer solution, 0.8 mL of 18.75 mM TA, and 50 μL of the CuO NPs (0.04 mg/mL) were added to the above glucose reaction solution for another 2 h at 45 °C. The resulting reaction solution was measured by using a Cary Eclipse fluorescence spectrometer under the excitation wavelength of 315 nm.

L-lactate detection by oxidase endpoint method was carried out by adding 50 μL of 0.01 mg/mL LOx, 50 μL HRP, 100 μL TMB, and 400 μL lactate of different concentrations into 3.4 mL of 200 mM phosphate buffer solution (pH 7.0). The mixture was then incubated at 37 °C for 10 min. The resulting reaction solution was measured by using a Shimadzu UV-2450 spectrophotometer.

2.5. Serum samples detection

For glucose and L-lactate determination in serum, the samples were first pretreated by ultrafiltration to eliminate the possible interference of proteins. The pretreated samples were diluted by a phosphate buffer solution and determined according to the processes mentioned above for glucose and L-lactate. The results of proposed method were compared with that of the glucose or lactate oxidase endpoint method, which is a widely used clinical method in hospitals for glucose and lactate assay. Standard addition experiments were further conducted by adding three different concentrations of glucose or L-lactate in the real serum samples.

3. Results and discussion

3.1. Sensing protocol

Fig. 1A depicts the principle of the cupric oxide nanoparticles-based fluorescent sensor for hydrogen peroxide. As a peroxidase mimetic, cupric oxide nanoparticles can break up the O–O bond of H_2O_2 into two hydroxyl radicals (Chen et al., 2012b). Then the resulting hydroxyl radicals react with TA, a non-fluorescent molecule, to form highly fluorescent hydroxyterephthalate (TAOH), which shows emission maximum at 422 nm when excited at 315 nm. The fluorescence intensity of TAOH is found to be proportional to the concentration of H_2O_2, which lead to develop a fluorometric sensor for hydrogen peroxide determination.

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