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Evaluation of fluorogenic substrates for Ni/Co LDHs peroxidase mimic and application for determination of inhibitory effects of antioxidant

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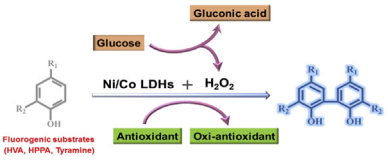
HIGHLIGHTS

- Three fluorogenic substrates were suitable for basic condition.
- Substrates were evaluated by experiment condition, reaction kinetic and detection assay.
- Inhibitory effects of antioxidant was studied based on Ni/Co LDHs-HVA-H_2O_2 system.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanomaterial-based peroxidase-mimetics are an emerging research field that promises to produce alternatives to horseradish peroxidase for a variety of applications. Generally, some peroxidase-mimetics substrates are used in acidic condition (pH $\leq$ 7). Then, it is necessary to screen some peroxidase-mimetics substrates suitable for basic condition because that some peroxidase-mimetics leached ions in acidic solution. In this paper, using Ni/Co layered double hydroxides (LDHs) as a nano-peroxidase mimic model, we evaluated three fluorogenic substrates suitable for basic condition though experimental conditions, reaction kinetic and glucose detection assay. And the detection of glucose method based on homovanillic acid (HVA) as fluorescent substrate gave wide linear range (0.02–20 μM) and low detection limit (0.01 μM). We also developed a novel platform that could study the inhibitory effects of ascorbic acid and glutathione based on the system of Ni/Co LDHs-HVA-H_2O_2.

1. Introduction

In the past few decades, researchers have developed large number of artificial enzymes to mimic the structures and functions of natural enzymes. Recently, nanozymes, nanomaterials with enzyme-like characteristics, are emerging as novel artificial enzymes, and attracting researchers' enormous interest. Compared with traditional horseradish peroxidase (HRP), varieties of nanozymes with peroxidase activity such as metal nanomaterials [1–3], metal oxide nanomaterials [4–6], carbon nanomaterials [7,8] have

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been developed because of their advantages including low cost, ease of production, high stability, long-term storage and so on [9,10]. Benefiting from these advantages, these peroxidase-mimetics have been extensively investigated for diverse applications in the detection of H_2O_2 [11–14], glucose [15–19], nucleic acid [20–22], metal ions [23–25]and so on. In these peroxidase mimic-based sensors, colorimetric detection is one of the most common detection strategies.

In general, colorimetric peroxidase substrates, such as 3,3′,5,5′-tetramethylbenzidine (TMB), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and *o*-phenylenediamine (OPD), were oxidized by H_2O_2 under the catalyze of peroxidase mimics to produce blue, green, and orange oxidation product, respectively [26–29]. And the color changes can be read by the naked eye or UV-visible spectrophotometers. Colorimetric detection possess some advantages, which included that it does not require expensive or sophisticated instrumentation, and is easy to be operated. However, colorimetric method process the disadvantage of the lower sensitivity and the color fading of colorimetric peroxidase substrate (such as ABTS) [30,31]. These make colorimetric peroxidase substrates are not the perfect substrates of peroxidase mimics. In particular, the reaction of TMB, ABTS and OPD with H_2O_2 by the catalyze of peroxidase-mimetics mostly occurred in acidic solutions [32]. While, some peroxidase-mimetics, such as Fe_3O_4 magnetic nanoparticles [12], $CuFe_2O_4$ magnetic nanoparticles [33], and CuO nanoclusters [11] leached metal ions in acidic solution, which required these peroxidase-mimetics catalyze the reactions of these substrates with H_2O_2 in alkaline environment. However, in basic solution, these substrates could not react with H_2O_2 even in the presence of peroxidase-mimetics. Therefore, it is necessary to screen substrates suitable for alkaline environment.

In this paper, as fluorogenic peroxidase substrates applying in alkaline condition, three phenolic compounds including homovanillic acid (HVA), 3-(4-hydroxyphenyl) propionic acid (HPPA) and tyramine were evaluated though experimental conditions, reaction kinetic and glucose detection assay. In this evaluation process, we selected Ni/Co layered double hydroxides (LDHs) leaching ions in acidic solution as a model nano-peroxidase mimic due to ease of preparation, low-cost, and water-solubility. As another potential application of nano-peroxidase mimic, Ni/Co LDHs was used for detection of antioxidant and investigation the antioxidant capacity. Antioxidants are reducing agents that scavenge active oxygen species (ROS), or the free radicals produced in biological systems. They play a crucial role in protecting the body by balancing oxidative stress. The utilization of nano-peroxidase mimic to determine antioxidant behavior has proved to be a simple and reliable method. Therefore, we presented a novel platform for evaluation of the antioxidant capability and antioxidant behaviors of ascorbic acid and glutathione using Ni/Co LDHs-HVA-H_2O_2 system.

2. Experiment section

2.1. Materials

30% H_2O_2, $Co(NO_3)_2{\cdot}6H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $NH_3{\cdot}H_2O$ were purchased from Tianjin Guangfu Chemical Reagent Factory (Tianjin, China). Glucose oxidase (GOx, 50 KU) and homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid, HVA) were purchased from Sigma-Aldrich. 3-(4-hydroxyphenyl) propionic acid (HPPA) was purchased from J&K Chemicals. Tyramine hydrochloride, glutathione, and ascorbic acid were purchased from Adamas. Glucose, fructose, lactose and maltose were purchased from Sangon Biotech (Shanghai).

All other chemicals were of analytical reagent grade and used without further purification. Deionized water was used throughout the experiment.

2.2. Apparatus and characterization

The LS-55 Fluorescence Spectrometer from PerkinElmer (U.K) was used to obtain fluorescence spectra and implement kinetic experiments. The surface morphology of Ni/Co LDHs was taken on a field emission scanning electron microscopy (FE-SEM Hitachi S-4800, Japan). Element determination was measured by a 4300 DV inductively coupled plasma–atomic emission spectrometry (ICP-AES) instrument (PerkinElmer, USA).

2.3. Preparation of Ni/Co LDHs

The Ni/Co LDHs microspheres were fabricated via a previously reported chemical coprecipitation method [32]. Briefly, 10 mL 0.1 mol L^{-1} $Co(NO_3)_2{\cdot}6H_2O$ and 20 mL 0.1 mol L^{-1} $Ni(NO_3)_2{\cdot}6H_2O$ were mixed, and 1 mL 35% $NH_3{\cdot}H_2O$ was added into the above mixed solution drop by drop. The precursor was sealed in a 50 mL glass bottle for 3 h at room temperature. The sample was collected and washed by centrifugation for several cycles and the as-prepared Ni/Co LDHs sample was obtained by deionized water and absolute ethanol washed for several times and dried in a vacuum oven at 60 °C overnight.

However, Ni/Co LDHs, as Co-Ni hydroxides, was dissolved in acidic solution [34]. The amount of leached cobalt and nickel ions in different pH was determined by ICP-AES (Fig. S1a). As shown in Fig. S1a, the cobalt and nickel ions could be leached from the Ni/Co LDHs in an acid reaction solution. While, when pH $\geq$ 9.0, the amount of leached cobalt and nickel ions was nearly disappeared. Therefore, as a model nano-peroxidase mimic, Ni/Co LDHs was selected using in basic solution.

The morphology and size of the synthesized Ni/Co LDHs was characterized by the transmission electron microscope (TEM) of Tecnai G^2 F30 instrument. Statistical analysis of TEM data revealed that the product consists of flower-like hierarchical architectures microsphere. The average particle diameter is found to be approximately 470 nm (Fig. S1b). The as-prepared Ni/Co LDHs nanoparticles, even without any surface modification, can well disperse in distilled water to form a transparent light yellow solution (Fig. S1c), which plays an important role in our luminescence system.

2.4. Comparison of fluorogenic substrates by kinetic analysis

The reaction kinetics for the catalytic oxidation of HVA, HPPA and tyramine with H_2O_2 by Ni/Co LDHs were carried out by LS-55 fluorescence spectrometer (PerkinElmer, U.K) at selected time intervals in scanning kinetics mode. Taking HVA as an example, the peroxidase-like activity was carried out at room-temperature using 10 μg mL^{-1} Ni/Co LDHs in a reaction volume of 3.0 mL Tris-HCl buffer solution (0.01 M, pH10.7) with HVA as substrate, and the concentration of H_2O_2 was fixed. The emission intensity at 425 nm (λ_{ex} = 315 nm) was used for quantitative analysis. The excitation and emission wavelength of HPPA and tyramine was 315 nm and 410 nm, respectively. The Michaelis-Menten constant was calculated using a Lineweaver-Burk plot:

$$1/v = K_m/V_{max}(1/[S] + 1/K_m) \quad (1)$$

where v is the initial velocity, V_{max} represents the maximal reaction velocity, and [S] is the substrate concentration. K_m is Michaelis-Menten constant, which is an indicator of enzyme affinity for its substrate.