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Co_3O_4/CuO hollow nanocage hybrids with high oxidase-like activity for biosensing of dopamine

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ABSTRACT

Here, we report the synthesis, characterization, and oxidase-like activity of Co_3O_4/CuO hollow nanocage (HNC) nanocomposites. The Co_3O_4/CuO HNCs were successfully prepared by hydrothermal treatment of ZIF-67 in copper nitrate solution, followed by carbonization in air. The obtained nanocomposites were characterized by scanning electron microscopy, transmission electron microscopy, X-ray photoelectron spectroscopy, and X-ray diffraction. The Co_3O_4/CuO HNCs possess enhanced oxidase-like activity compared with pure Co_3O_4 and CuO, and they can catalytically oxidize the 3,3′,5,5′-tetramethylbenzidine (TMB) substrate in the absence of H_2O_2 to produce an intense blue product. Reactive oxygen species (ROS) measurements indicate that $^1O_2$ and $O_2\cdot^-$ radicals are the major ROS in catalytic oxidation of TMB by dissolved oxygen in the TMB–Co_3O_4/CuO HNC system. We then developed a simple, sensitive, visual, and colorimetric biosensing method for dopamine (DA) based on its inhibiting effect on TMB oxidation. The proposed method allows for DA detection with a limit of detection of 0.027 μM and a dynamic range of 0.05–8 μM. This new colorimetric method was successfully used to detect DA in biological samples. The present work demonstrates a simple strategy to fabricate an efficient oxidase mimic with potential applications in bioanalysis and clinical diagnosis.

1. Introduction

Since the first work on the intrinsic peroxidase-like activity of ferromagnetic nanoparticles (NPs) in 2007 [1], nanomaterial-based enzyme mimics (nanozymes) have continued to attract interest for applications in various fields, including bioanalysis, environmental analysis, and medical treatment [2–5]. This is because they can overcome some of the shortcomings of natural enzymes, such as the high cost of preparation and purification, and difficulties in recovery and recycling. Various nanozymes have been developed, including carbon-based nanomaterials [6,7], selenide and sulfide nanomaterials [8–10], metal nanomaterials [11,12], and metal oxide nanomaterials [13–16]. However, the current research in the field of nanozymes is mostly concentrated on peroxidase mimics, and little attention is being paid to oxidase mimics [3]. It is well known that H_2O_2 is needed as an electron acceptor for peroxidase-like catalyzed reactions. However, because of its strong oxidizing property, H_2O_2 can destroy some targets [17]. Furthermore, H_2O_2 readily decomposes and loses its oxidation capacity. Unlike peroxidase mimics, oxidase mimics can catalyze oxidation of some organic substrates without H_2O_2. This unique advantage makes oxidase mimics an appealing alternative for constructing sensors with simple operation, good compatibility, and high reliability.

The superior catalytic properties of noble-metal-based nanostructures make them promising oxidase mimic candidates for applications in biocatalysis. For example, some noble metal NPs have been shown to exhibit oxidase-like activity, including Au NPs [18], Ag NPs [19], Pt NPs [20], and the noble metal alloys Au@Pt NPs [21], Au@Ag NPs [22], and Au@PdPt NPs [23]. Although noble metals offer superior catalytic activity, they are expensive and a limited resource, which inevitably hinders their wide application. Therefore, low cost and available non-noble metal oxides, particularly transition-metal oxides with comparable activities, are good candidates for oxidase mimics [13,24–28].

Metal–organic frameworks (MOFs), a unique type of emerging material, have received considerable attention in various fields, such as gas storage [29], catalysis [30], energy storage systems [31], and electrochemical sensors [32]. They also can act as self-sacrificial templates to synthesize hollow and porous metal oxides, which possess

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innumerable reaction sites, high porosity, and easy gas accessibility [33]. Among these metal oxides, Co_3O_4 nanostructures derived from MOFs have received great attention because of their excellent catalytic activity, ease of preparation, good response stability, and low cost [34]. For instance, hollow Co_3O_4 derived from ZIF-67 exhibits high catalytic activity and stability for CO oxidation [35], it can be used as the anode material for Li-ion battery [36], and it is an excellent supercapacitor electrode material [37]. Mu et al. [38] showed that Co_3O_4 NPs with different morphologies exhibit varied catalytic activities as catalase mimics. Ding et al. [39] reported that perylene-diimide-modified Co_3O_4 NPs exhibit enhanced peroxidase-like activity. To further improve the catalytic activity, hybrid nanocomposites have been designed, and they exhibit superior catalytic performance to their monometallic counterparts because of the electronic structure effect and synergistic effect [40]. For example, Koo and co-workers [33] found that PdO-functionalized Co_3O_4 hollow nanocages (HNCs) exhibit a higher response to acetone than single Co_3O_4 nanomaterials. Zhu et al. [41] reported that MOF-derived ZnO/Co_3O_4 nanocomposites show enhanced electrochemical performance compared with the single Co_3O_4 and ZnO NPs. Li et al. [42] synthesized double-shelled Fe_2O_3/Co_3O_4 hollow microcubes with improved electrochemical performance owing to their unique microstructure and the synergistic effect between Fe_2O_3 and Co_3O_4. These examples demonstrate that constructing metal oxide nanocomposites is an effective way to obtain high-performance catalysts.

In this study, based on the excellent properties of Co_3O_4 HNCs, CuO NPs and Co_3O_4 HNCs were integrated into nanocomposites by hydrothermal treatment of ZIF-67 in $Cu(NO_3)_2{\cdot}3H_2O$, followed by carbonization in air. CuO is selected because it is a typical p-type semiconductor and a promising catalyst with good catalytic activity in sensor development [43], degradation of organic pollutants [44], and so forth. The as-prepared Co_3O_4/CuO HNCs were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), and X-ray diffraction (XRD). For comparison, single Co_3O_4 HNCs and CuO NPs were also prepared, and their structural characteristics and oxidase-like activities were evaluated. The reaction kinetics and mechanism were investigated. Finally, a new and facile colorimetric method was developed with the Co_3O_4/CuO HNC composite as an oxidase mimic for detection of dopamine (DA) in human serum and DA hydrochloride injection. This is the first report of using MOFs as starting template materials to produce bimetallic oxide nanostructures with excellent oxidase mimic activity for sensing DA.

2. Experimental section

2.1. Chemicals

All of the chemicals were of analytical reagent grade and used as received without any further purification. Cobalt nitrate hexahydrate ($Co(NO_3)_2{\cdot}6H_2O$) and copper nitrate trihydrate ($Cu(NO_3)_2{\cdot}3H_2O$) were purchased from Kelong Chemical Reagent Co., Ltd. (Chengdu, China). 2-Methylimidazole (MIM), DA hydrochloride (DA), and *p*-benzoquinone were obtained from Aladdin (Shanghai, China). 3,3′,5,5′-Tetramethylbenzidine (TMB) and superoxide dismutase (SOD) were purchased from Sigma-Aldrich (Shanghai, China). Acetic acid (HAc), sodium acetate (NaAc), ethanol, and methanol were supplied by Chongqing Taixin Chemical Co., Ltd. (Chongqing, China). Glucose and lactose were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycine was purchased from Chengdu Chemical Reagents Company (Sichuan, China).

2.2. Instrumentation

The SEM images were recorded with a Hitachi model S-4800 field emission electron microscope (Hitachi, Japan) with an accelerating voltage of 25 kV. The XRD patterns of the as-prepared products were recorded with a TD-3500 X-ray diffractometer (Dandong, China) under the conditions of nickel-filtered Cu K_α radiation ($\lambda = 0.15406$ nm), a current of 20 mA, a voltage of 30 kV, and a scanning rate of 4° min^{-1} in the angle range 5–80° (2θ). A transmission electron microscope (Tecnai G2 F20) equipped with an energy dispersed X-ray detector (EDX) operating at an accelerating voltage of 200 kV was used for detailed microstructure and composition analysis of the prepared samples. The XPS and X-ray excited auger electron spectroscopy (XE-AES) measurements were performed with a Thermo ESCALAB 250XI multifunctional imaging electron spectrometer (Thermo Fisher Scientific Inc.) equipped with an Al Kα radiation source (1486.6 eV). The Co_3O_4/CuO HNC samples for the XPS and XE-AES measurements were prepared in a spherical shape with a diameter of about 500 μm. The working pressure was 8×10^{-10} Pa. The Co 2p, Cu 2p, O 1s, C 1s, Cu LMM, and Co LMM spectra were recorded under the following conditions: pass energy 30 eV, 0.1 eV $step^{-1}$, working voltage 12.5 kV, and filament current 16 mA. All of the binding energies were calibrated by referencing to the C 1s binding energy (285.0 eV). The adsorption measurements were performed with a UV-2450 spectrophotometer (Shimadzu, Suzhou).

2.3. Synthesis of the Co_3O_4/CuO HNCs

First, ZIF-67 was synthesized according to a previously reported method [45]. In brief, 498 mg of cobalt nitrate hexahydrate was dissolved in 50 mL of methanol, and 656 mg of 2-methylimidazole was dissolved in 50 mL methanol. The latter solution was poured into the former pink solution with vigorous stirring for 30 min. The mixture was then incubated at room temperature without stirring. After 24 h, a dark purple solid was collected by centrifugation and washed several times with methanol, and the obtained ZIF-67 was then dried at 60 °C overnight. To prepare the Co_3O_4/CuO HNCs, 80 mg of as-prepared ZIF-67 was dissolved in 50 mL of ethanol. After ultrasonic treatment of the solution for 1 min, 40 mg of $Cu(NO_3)_2{\cdot}3H_2O$ was added and the mixture was then heated to 90 °C and kept at this temperature for 3 h. The product was collected by centrifugation and dried at 60 °C to obtain ZIF-67/Cu hydroxide (HD), which was carbonized at 360 °C for 2 h in air to obtain Co_3O_4/CuO HNCs. For comparison, we also synthesized Co_3O_4 and CuO by direct carbonation of ZIF-67 in a muffle furnace at 360 °C for 2 h in air and a rapid-precipitation method, respectively (see the Supporting information).

2.4. Catalytic activity of the Co_3O_4/CuO HNCs and kinetic analysis

The catalytic activity of the prepared Co_3O_4/CuO HNCs was evaluated based on the TMB color reaction. In brief, 0.5 mL of 200 mg/L Co_3O_4/CuO HNCs and 0.25 mL of 2 mM TMB were added to 4.25 mL of 0.2 M NaAc–HAc buffer (pH = 4.0). The mixture was incubated at 40 °C for 20 min, and then used for ultraviolet–visible absorption spectroscopy measurements.

The kinetic measurements were performed in time course mode by monitoring the absorbance change at 652 nm in 1 min intervals. The Michaelis–Menten constant was calculated using Lineweaver–Burk plots of the double reciprocal of the Michaelis-Menten equation: $1/\nu = K_m/V_{max}\cdot(1/[S] + 1/K_m)$, where ν is the initial velocity, V_{max} is the maximal reaction velocity, and [S] is the concentration of the substrate. K_m is the Michaelis–Menten constant, which indicates the enzyme affinity for the substrate.

2.5. Application of the Co_3O_4/CuO HNCs as oxidase mimetics for DA detection

For DA analysis, the calibration curve for DA was firstly obtained. The absorption spectra of mixtures containing Co_3O_4/CuO HNCs (0.5 mL, 200 mg/L), 0.25 mL DA with various concentrations from 0 to 2 mM, TMB (0.25 mL, 2 mM), and NaAc-HAc buffer (4.0 mL, 0.2 M, pH 4.0) were recorded from 500 to 800 nm after 20 min reaction at