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Tuning the oxidase mimics activity of manganese oxides via control of their growth conditions for highly sensitive detection of glutathione

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ABSTRACT

Different manganese oxides materials with various crystalline structures were prepared from $KMnO_4$ by using a cationic polymer branched polyethyleneimine (BPEI) as the reducing agent and structure directing agent through a hydrothermal process. By varying BPEI concentration, wire-like MnO_2, rod-like $MnOOH/Mn_3O_4$ and octahedral-like Mn_3O_4 nanoparticles were controllably synthesized, respectively. The as-prepared manganese oxides were characterized by XRD, FT-IR, TEM, SEM and nitrogen adsorption-desorption measurements. The intrinsic enzyme-like activity of the synthesized manganese oxides was evaluated by using 3,3′,5,5′-tetramethylbenzidine (TMB) as the substrate in the absence of H_2O_2. It was shown that the mesoporous wire-like MnO_2 was best-performing with a highly oxidase-like catalytic activity in the model reaction. The effect of synthetic conditions of mesoporous wire-like MnO_2 on its oxidase-like activity was further investigated. Based on the selective inhibition of glutathione (GSH) on the high oxidase-like activity of as-prepared MnO_2, a selective and sensitive colorimetric assay for GSH has been established. The limit of detection (LOD) for GSH was as low as 0.11 μM and the developed method was successfully applied to the detection of GSH in human serum samples.

1. Introduction

As alternatives for natural enzyme, nanozymes are a class of inorganic nanomaterials with enzyme-like activity and have been attracted tremendous attention in recent years. Nanozymes can compensate the shortcomings of natural enzymes such as low-cost, easy preparation, stability against harsh conditions, and tunability of catalytic activity [1,2]. However, as compared with natural enzymes, nanozymes usually exhibit the relatively low catalytic activity. So it is important to improve the catalytic activity of nanozymes to extend their applications [1]. Over the previous decades, a great deal of efforts has been made to explore reliable and efficient nanozymes for practical applications. For example, through using ionic liquid, graphene oxide, and nucleoside triphosphates as efficient modulators, Qu's group has succeed in enhancing the catalytic activity of Au/SiO_2 nanocomposites as peroxidase mimics [3], peroxidase-like activity of Au nanoclusters (NCs) [4], and the oxidase-like activity of nanoceria [5]. On the other hand, most of previous works are focused on the peroxidase-like activities of nanozymes [6–10]. The catalytic talent of these peroxidase mimics only performed in the presence of the strong oxidant hydrogen peroxide (H_2O_2), which limits their analytical applications because H_2O_2 is very sensitive to surrounding environment and may also damage some analytes [11]. Therefore, the development of oxidase-like nanozymes without using H_2O_2 has been attracted increasing interest. So far, many efforts have been made to explore oxidase-like materials with highly catalytic activity, especially the most concerned noble metal nanaoparticles (NPs) such as Au [12], Ag [13], Pt [14], or the noble metal alloy of Au@Pt [15], Au@Ag [16], Au@PdPt [17]. Although noble metals have outstanding oxidase-like activities, their high cost prohibits the real applications. Therefore, cheap non-noble metal oxides, especially transition-metal oxides with comparable activities are welcome [18–21].

It is well known that the catalytic property of a catalyst relies on its particle size, dimensionality, specific surface areas and morphology. The morphology of transition-metal oxides has a significant effect on their catalytic behaviors as well as their enzyme-like performances. For instance, Fe_3O_4 nanostructures with different morphologies exhibited distinct peroxidase-like activity following the order of sphere > triangular plates > octahedron [22], which was related to the exposure of catalytically active iron atoms or crystal planes. Wan et al. [23] compared the oxidase-like catalytic activity of MnO_2 with five variant morphologies (namely, nanosheet,

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nanosphere, nanowire, nanostick and nanocomplex) and found that MnO_2 nanowires and nanospheres exhibited the highest activity whereas the nanosticks showed the lowest, which proved the properties of materials changed by their size and the percentage of the surface atoms. In our early work, the effect of different structures of V_2O_5 nanomatrials on their peroxidase-like activities was investigated, and the peroxidase-like activity follows the order of nanowire > nanorod > nanoparticle > nanosheet > nanobelt [24], which is probably due to their surface facets and specific surface areas.

As a typical type of transition-metal oxides, manganese oxides have received great attention because of their promising applications, including in catalysis [25], energy storage [26], sensing [27], adsorption [28] and magnetic application [29]. Among which, the catalytic property of manganese oxides in oxidation-reduction reactions was excellent due to their different oxidation states, and/or various structures. Therefore, a great deal of works have been carried out for the controlled synthesis of manganese oxides nanomaterials with various composites and structures, and many methodologies have been developed based on the redox reaction of potassium permanganate ($KMnO_4$) and inorganic or organic agents, such as $S_2O_3{}^{2-}$ [30], ethanol [31], ethyl acetate [32], nonionic surfactants polyethylene glycol (PEG 400) [33] and tri-block copolymer P123 (PEG-PPG-PEG) [34] and so on. However, only limited works have been reported on the comparative study of oxidase-like manganese oxides with various morphologies and crystallographic forms, which directly affect the catalytic activity of enzyme mimics.

Herein, in this work, we constructed an efficient oxidase-mimicking nanozyme, based on the controllable synthesis of the desirable manganese oxides through the redox reactions between $KMnO_4$ and branched polyethyleneimine (BPEI). Through a simple manipulation of BPEI concentration, the wire-liked MnO_2 to octahedral-like Mn_3O_4 NPs could be controllably synthesized. The oxidase-like activity of the as-prepared samples was evaluated by using 3,3′,5,5′-tetramethylbenzidine (TMB) as the substrate. It was found that all the prepared manganese oxides samples showed oxidase-like activities to different extent, whereas the mesoporous wire-like MnO_2 showed the best performance. Taking advantage of the typical redox reaction of glutathione (GSH) and MnO_2 [35], a selective and sensitive colorimetric strategy for sensing GSH based on the inhibition of oxidase mimic activity of mesoporous wire-like MnO_2 was fabricated, and successfully applied in the detection of GSH in human serum samples. The present work offers a vivid example in exploring the relation between controllable synthesis of the desirable metal oxides and their novel oxidase-like catalytic properties for promising applications, including catalysts, and chem/biosensors.

2. Experimental

2.1. Reagents

All the reagents used in the experiments were analytical grade. $KMnO_4$ was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). BPEI ($M_w = \sim 25000$), TMB, *o*-phenylenediamine (OPD), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (Shanghai, Sigma-Aldrich, China). GSH was purchased from Aladdin reagent Co., Ltd. (Shanghai, China) and stored in a refrigerator at 4 °C. Human serum albumin (HSA) and immunoglobulin (IgG) were obtained from Solarbio Science & Technology Co. (Beijing, China). *o*-Phthalaldehyde (OPA) was purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). Ultra-pure water (Ω=18.2) was used all through the experiment.

2.2. Instrumentations

Absorption measurements were performed on a UV-2450 spectrophotometer (Shimadzu, Japan). The pH of the solution was measured by a Mettler Toledo FE20 pH meter (Mettler-Toledo Instruments Co. Ltd., Shanghai, China). The scanning electron microscopy (SEM) images were obtained from an S-4800 field emission scanning electron microscope (Hitachi, Japan). The transmission electron microscopy (TEM) was carried out on JEM 2100F field emission transmission electron microscopy (JEOL, Tokyo, Japan). The X-ray diffraction (XRD) patterns of the samples were obtained using a Bruker D8 Advance powder X-ray diffractometer (Bruker, Germany). Fourier transform infrared (FT-IR) spectra were acquired on a Tenson 27 Fourier transform infrared spectrometer (Bruker, Germany). The zeta potentials of the as-prepared manganese oxides were measured using a Zetasizer Nano-ZS instrument (Malvern, UK). The specific surface areas were determined by the Brunauer-Emmett-Teller (BET) method based on the N_2 adsorption-desorption isotherm data, which were obtained on an ASAP 2020 Micromeritics instrument (Maike, USA).

2.3. Synthesis of manganese oxides

The manganese oxides were prepared facilely by hydrothermal method and the detail synthesis process was as follows. 0.79 g of $KMnO_4$ was first added in 25 mL of ultra-pure water under magnetic stirring till the solid was dissolved completely, and then 25 mL of a certain concentration of BPEI aqueous solution was added quickly. The color of mixture changed from dark violet to brown immediately and a steady emulsion was formed. After vigorous stirring for 20 min, the mixture was transferred to a 100 mL Teflon-lined stainless steel autoclave, and heated at 160 °C for 8 h. The products were filtered through 0.45 μm pore size membranes, washed with ultrapure water and ethanol several times, and then dried at 60 °C for 12 h. The different manganese oxides products were obtained by varying the concentration of BPEI (25 mL of 0.25 wt%, 0.5 wt% and 1.0 wt% of BPEI, respectively) in the above synthesis process, and the samples were denoted as M-0.25, M-0.5 and M-1.0, respectively, with the yield from 46% to 68% as listed in Table 1.

2.4. Oxidase mimics activity of manganese oxides

In order to prove and compare the oxidase-like activity of the as-prepared manganese oxides, catalytic oxidation of TMB was performed at room temperature by mixing 0.25 mL of TMB (1 mM), 0.25 mL of manganese oxides (60 mg/L, ultrasonic dispersion for 2 min in advance) and 4.5 mL of 0.2 M NaAc-HAc buffer (pH 3.5). Then the UV–vis scanning data of the reaction system was recorded at 652 nm once per minute for 10 min right after all the reagents were mixed thoroughly. For evaluating the influence of factors on the oxidase-like activity of catalyst, the relative activity was used as criterion. That is, the maximum value in the curve was set as 100%, and the relative activity was the ratio of absorbance at target value to the maximum absorbance value at 652 nm.

2.5. General procedure for the colorimetric analysis of GSH in human serum samples

For colorimetric detection of GSH, firstly, 2 mg of catalyst was dispersed in the 50 mL of ultra pure water with ultrasonic method for 2 min. Then 0.25 mL of catalyst suspension was incubated with 0.50 mL GSH standard solution with a certain concentration, followed by addition of 0.25 mL of TMB (1 mM). Finally, 4.0 mL of NaAc-HAc buffer (0.2 M, pH 5.0) was added and mixed thoroughly. After incubation at room temperature for 10 min, the maximum