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Co_3O_4 nanoparticles as an efficient catalase mimic: Properties, mechanism and its electrocatalytic sensing application for hydrogen peroxide

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ABSTRACT

Nanomaterial-based enzyme mimics have recently attracted considerable interest due to their easy preparation, low cost, high stability and so on. Herein Co_3O_4 nanoparticles (NPs) were used as a catalase mimic, catalyzing the decomposition of hydrogen peroxide to oxygen. The catalytic activity of Co_3O_4 NPs increased dramatically by adjusting the pH from acid to neutral and alkaline conditions. The catalytic activities and the mechanisms were investigated using the procedures of thermodynamics, steady-state kinetics and hydroxyl radical detection. The activation energy of Co_3O_4 NPs was determined to be 43.3 $kJ\,mol^{-1}$ which was similar to 42.8 $kJ\,mol^{-1}$ of catalase. The catalytic behaviour of Co_3O_4 NPs showed a typical Michaelis–Menten kinetics and good affinity to H_2O_2. The turnover number and specificity constant of Co_3O_4 NPs were very close to those of catalase. Based on the above results, Co_3O_4 NPs were an efficient catalase mimic. A catalytic mechanism was proposed where hydroxyl radicals took part in the catalytic recycles. Co_3O_4 NPs had better stability than natural catalase when they were exposed to solutions with different pH values and temperatures. As an efficient and stable catalase mimic, Co_3O_4 NPs were used as the amperometric sensor for the detection of hydrogen peroxide.

1. Introduction

Catalases are present in all aerobic organisms and many anaerobic organisms [1]. They are protective enzymes responsible for the decomposition of hydrogen peroxide to molecular oxygen and water (Eq. (1)) [2]. They have biomedical application against oxidative stress which has been linked to a variety of diseases and disorders [3], and can also be used commonly for the determination of hydrogen peroxide released from some substances which are the target of the general clinical analyses [4,5].

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O \qquad (1)$$

As biological catalyst, catalases have remarkable advantages such as high efficient and selective catalysis under mild conditions. However the natural enzyme is not stable because of its denaturation under harsh environmental conditions. Additionally, their preparation and purification are usually expensive and time-consuming. Therefore, attempt to develop stable and efficient mimics of catalase is becoming a significant field for various applications [6,7]. For instance, the lifespan of the *Caenorhabditis elegans* extended largely by treating with synthetic catalase mimics [8].

According to the structural differences of catalytic centre, catalase can be divided into heme catalase and manganese catalase. Therefore, so far most reported models of catalase mimics are compounds with redox-active metal centres containing either iron (Fe) or manganese (Mn) in homogenous media [9–11]. However, the cobalt-based catalase mimics were rarely reported. Recently, nanomaterials have received increasing attention in enzyme mimetic research because of their several distinct properties, such as high surface area to volume ratio, abundance of reactive groups on their surfaces for further functionalization and more catalytic sites on their surface than their bulk counterparts. The enzyme mimics of nanomaterials reported are mainly peroxidase mimics [12–20], however, the nanomaterials with catalase-like activity were rarely studied. Miyamoto and coworkers first reported that small Pt NPs exhibited both superoxide dismutase-like (SOD) and catalase-like activity [21]. And then cerium nanoparticles has been found to exhibit catalase-like activity which correlated with a reduced level of cerium in the +3 state [22]. Recently, Fe_3O_4 nanoparticles and RuO_2 nanoparticles were also found with catalase-like activity [23,24]. With the development in the field, the new properties and functions of nanomaterials as catalase mimics make them potentially useful in bioassays and biomedicine fields. However, there have been few attempts to investigate the catalytic kinetics and mechanisms of nanomaterial-based catalase mimics, and little is known about the catalase-like properties of Co_3O_4 NPs.

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Not long ago, we have firstly found the Co_3O_4 NPs exhibited high peroxidase-like activity in acid conditions, meanwhile showed weak catalase-like activity in the same conditions [16]. This initial result inspired us to improve the catalase-like activity of Co_3O_4 NPs. In the present work, we demonstrate that the catalase-like activity of Co_3O_4 NPs could be tunable by adjusting the pH from acid to neutral and alkaline conditions. The properties and mechanism of the catalase-like activity of Co_3O_4 NPs were investigated in depth and detail. The kinetic parameters showed Co_3O_4 NPs had efficient catalytic activity in comparison to natural catalase. The catalytic activity of Co_3O_4 NPs showed better stability than catalase in exposure to extreme pH values and high temperature. And based on the reduction of H_2O_2 by Co_3O_4 NPs, a fast, sensitive and low cost H_2O_2 sensor was developed. The good stability, easy preparation and special properties of Co_3O_4 NPs make them the promising catalase mimics.

2. Experimental

2.1. Materials

Catalase from bovine liver (EC 1. 11. 1.6, 2000–5000 U/mg) was purchased from sigma (USA). H_2O_2, $Co(COOH)_2 \cdot 4H_2O$, 25% ammonia solution, ethanol and other regents were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). The chemicals were of analytical reagent grade and used as received.

2.2. Preparation of Co_3O_4 NPs

The Co_3O_4 NPs were prepared according to the method reported by Dong et al. [25]. Briefly, 0.50 g of $Co(CH_3COO)_2 \cdot 4H_2O$ was dissolved in solution of 15 mL ethanol and 10 mL water, and 2.5 mL of 25% ammonia was added drop by drop under vigorous stirring. The mixture was stirred in air for about 10 min to form a homogeneous fuscous slurry. Then the suspension was transferred into a 48 mL autoclave, sealed and maintained at 150 °C for 3 h. After this, the autoclave was cooled to ambient temperature naturally. The resulting black solid products were separated by centrifugation and washed with water three times, dried at 60 °C under vacuum for 4 h, and collected for characterization.

2.3. Preparation of Co_3O_4 NPs modified electrode

Prior to the surface modification, the glassy carbon electrodes (GCE, 3.0 mm in diameter) were firstly polished with 0.3 and 0.05 mm alumina slurry, and then cleaned ultrasonically in distilled water and ethanol successively. The Co_3O_4 NPs (30 mg) was dispersed into distilled water (10 mL) by ultrasonic dispersion to obtain a suspension of Co_3O_4 NPs (3 mg mL^{-1}). 100 μL of the obtained suspension with 5 μL of 3% PTFE solution to produce a colloidal solution. The colloidal solution (5 μL) was then dropped on the pretreated GCE surface and allowed to dry under ambient conditions for 3 h to obtain Co_3O_4 NPs modified electrodes.

2.4. Apparatus and procedures

The composition and phase of the product were identified by powder X-ray diffraction (XRD) on an *D*/max-rB X-ray diffractometer (Rigaku, Japan) using Cu Kα radiation ($\lambda = 1.5418$ Å). The morphology and size of the product were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM images were taken with a SU8000 scanning electron microscope (Hitachi, Japan). TEM measurements were performed on a Hitachi H-800 transmission electron microscope (Hitachi, Japan) at an accelerating voltage of 200 kV. The N_2 adsorption and desorption isotherm was obtained at 77 K using ASAP 2020 Physisorption Analyzer (Micromeritics, USA). FT-IR spectra were measured by a Spectrum 100 Frontier IR Spectrometer (Perkin Elmer, USA). XPS measurements were on a PHI 5700 ESCA System X-ray photoelectron spectrometer (Physical Electronics, USA) with an Al Kα excitation source (1486.6 eV).

Oxygen production was measured with a Clark-type electrode coupled to a Hansatech Oxygraph (Hansatech, U.K.). The reaction medium (3 mL total volume) contained H_2O_2 was started by the addition of Co_3O_4 NPs or catalase. Apparent steady state reaction rates at different concentrations of H_2O_2 were obtained by calculating the slopes of initial changes with time. Catalytic parameters were determined by fitting the reaction rates to the Michaelis–Menten equation.

Hydroxyl radical measurement was recorded on a LS55 fluorescence spectrometer (Perkin Elmer, American). 100 mM H_2O_2, 5 mM terephthalic acid and different concentrations of the Co_3O_4 NPs were first incubated in 100 mM NaH_2PO_4–NaOH buffer (pH 10.0) for 30 min. After centrifugation, the solutions were used for fluorometric measurement.

Cyclic voltammetric and amperometric measurements were performed on CHI 660D (Chenhua, China). A three-electrode system comprising a platinum plate as auxiliary, a Ag/AgCl electrode as reference and the Co_3O_4 NPs-modified electrode as working electrodes was used for all electrochemical experiments. All experimental solutions were deoxygenated by bubbling highly pure nitrogen for at least 20 min and maintained under nitrogen atmosphere during the measurements.

3. Results and discussion

3.1. Characterization of Co_3O_4 NPs

The Co_3O_4 NPs were prepared using a simple hydrothermal method in the absence of surfactants [25]. As a result, the produced particles are free of organic species, which might influence their catalytic activity. The crystal structures of the synthesized products were characterized by X-ray powder diffraction (XRD). The XRD pattern of the as synthesized product in Fig. 1a can be readily indexed to a pure face-centred cubic phase (space group Fd3m (227)) of spinel cobalt oxide with lattice constants $a = 8.083$ Å (JCPDS 42-1467) [26]. No impurity peaks were observed, which indicates the high purity of the final products. The morphology of the Co_3O_4 NPs was examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Fig. 1b showed the Co_3O_4 NPs were uniform and had narrow size distributions. The nanoparticles in the TEM image (Fig. 1c) were squares, indicating that the nanoparticles were nanocubes. And the average edge length was about 19 nm. The standard deviation for Co_3O_4 particle size was ≈4%, excluding agglomerate particles (Fig. 1d). The N_2 adsorption–desorption isotherm was shown in Fig. S1. The surface area of the Co_3O_4 NPs using the Brunauer–Emmett–Teller (BET) method was calculated to about 57.7 m^2 g^{-1}.

The chemical structure of the Co_3O_4 NPs was investigated by FT-IR spectrum, shown in Fig. 2a. The IR spectrum displays two sharp bands at 578 cm^{-1} (ν_1) and 666 cm^{-1} (ν_2), which originate from the stretching vibrations of the metal–oxygen bond [26]. The ν_1 band at 578 cm^{-1} is characteristic of Co^{3+} vibration in the octahedral hole, and ν_2 band at 666 cm^{-1} is attributable to Co^{2+} vibration in tetrahedral hole in the spinel lattice, confirming the formation of Co_3O_4 spinel oxide. The broad band at 3409 cm^{-1} is assigned to the O—H stretching vibration due to the adsorbed H_2O molecules [27]. Information about the surface composition of the products can be further provided by X-ray photoelectron spectroscopy (XPS) measurements, as shown in Fig. 2(b–d). The binding energies obtained in the XPS measurement were corrected for specimen charging by