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Facile synthesis of iridium nanoparticles with superior peroxidase-like activity for colorimetric determination of H_2O_2 and xanthine

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ABSTRACT

A facile and efficient method was developed for the preparation of iridium nanoparticles (Ir NPs) with superior peroxidase-like activity. Under the catalytic action of the Ir NPs, the peroxidase substrate 3,3,5,5-tetramathylbenzidine (TMB) can be oxidized by H_2O_2 to form a blue-colored production oxTMB. Kinetic analysis indicated that the catalytic behavior was in accordance with the typical Michaelis-Menten kinetics and the catalytic reaction followed the ping-pang mechanism. Electron Spin Resonance (ESR) experiment showed that no hydroxyl radical formed in the reaction process. Peroxide substrates with different electron withdrawing ability (benzoyl peroxide, H_2O_2 and artemisinin) were used to study the catalytic reactions and results indicated that H_2O_2 can oxidize TMB with much faster rate than artemisinin and slower rate than benzoyl peroxide. These results suggest an electron transfer mechanism was involved in the system of TMB-H_2O_2-Ir NPs. By using TMB as the colorimetric substrate, H_2O_2 can be rapidly determined and the method was extended for the determination of xanthine based on its production of H_2O_2 in the presence of xanthine oxidase. The prepared Ir NPs exhibit good stability in wide range of pH and temperature. The Ir NPs retained at least 90% of their initial catalytic activity after stored at ambient temperature for three months. The high specificity for H_2O_2 and the excellent stability of the peroxidase-like Ir NPs showed the great application potential in biotechnology field.

1. Introduction

Peroxidase mimics, including carbon nanomaterials [1–3], metallic oxides [4,5], metal chalcogenides [6] and metal nanomaterials [7], have attracted a great deal of research interest due to their tunable catalytic activity, excellent stability and biocompatibility. The intrinsic drawbacks of peroxidase, such as time-consuming and expensive production, sophisticated purification, and low stability to temperature variations can be largely avoided by using the nanomaterial based peroxidase mimics [8,9]. Peroxidase mimics of noble metal nanomaterials show potential application value in chemical analysis and biotechnology field. Such as Au [10], Pt [11] and Pd [12] nanomaterials were exploited as peroxidase-like labels for colorimetric determination of glucose. Mouse interleukin 2 (IL-2) was determined based on the peroxidase-like activity of Au@Pt nanomaterials through enzyme linked immunosorbent assay (ELISA) [13]. Colorimetric assay of human chronic myelogenous leukemia cell lines (K-562) was established using the Au@Pd nanoparticles with peroxidase-like activity as labels [14]. Polyvinylpyrrolidone-capped Ir NPs (PVP-Ir NPs) with peroxidase-like activity were prepared for the first time in 2015, which can protect cells from H_2O_2-induced oxidative damage [15]. The catalytic mechanism of the PVP-Ir NPs was suggested to be an electron transfer process. However, little experimental evidences were provided to highlight the pathways. Furthermore, their method for the Ir NPs preparation was time consuming and the use of polymer PVP as the stabilizer may also hinder the catalytic activity of the prepared nanoparticles [16].

Hence in present study, a small molecule tannic acid was used as stabilizer to prepare the Ir NPs. Tannic acid is one kind of typical green amphiphilic molecule obtained from plants. The multiple O—H sites of tannic acid could weakly stabilized Ir NPs, which is important for mimics to exert the catalytic performance [17]. Moreover, the good water-solubility is beneficial for peroxidase mimics used in aqueous media. The Ir NPs stabilized with tannin acid can rapidly catalyze the oxidation of TMB to form blue oxTMB in the presence of H_2O_2. Xanthine, one mild stimulant and bronchodilator, was used to treat asthma especially, which can produce H_2O_2 in the presence of xanthine oxidase. Based on the peroxidase-like activity of the Ir NPs, H_2O_2 and xanthine were analyzed using

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TMB as colorimetric substrate. Furthermore, the Michaelis-Menten behavior of the Ir NPs was investigated according to the steady-state kinetics analysis, and the catalytic mechanism of the Ir NPs was proposed.

2. Materials and methods

2.1. Materials

Iridium (III) chloride trihydrate ($IrCl_3{\cdot}3H_2O$), 3,3,5,5-tetramathylbenzidine (TMB), artemisinin and xanthine were purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). Xanthine oxidase was purchased from Sigma-Alorich (Shanghai, China). Horseradish peroxidase (HRP) was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO, 97%) was purchased from Energy Chemical Co., Ltd. (Shanghai, China). Tannic acid, sodium borohydride ($NaBH_4$), hydrogen peroxide (H_2O_2), ethanol (C_2H_6O), acetic acid (HAc), sodium acetate anhydrous (NaAc), o-Phenylenediamine (OPD) and pyrogallol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were of analytical grade and were used without further purification. Milli-Q ultrapure water (18.2 MΩ) was used throughout all experiments.

2.2. Instruments

Absorption spectra and peroxidase kinetics assay were carried out on a UV–vis spectrophotometer with a 1 cm quartz cuvette (UV-2700, Japan Shimadzu Co., Ltd). High resolution transmission electron microscopy (HRTEM, JEM-2100, Japan electron optics laboratory co., Ltd) was performed at 200 kV to visualize the prepared Ir NPs, and Energy dispersive X-ray (EDX) analysis of the Ir NPs were carried out with JEM-2100 (HR) microscope. The samples for TEM measurement were prepared by slow evaporation of the Ir NPs solution on a carbon film supported by 300-mesh Cu grids. The hydrodynamic diameter of the Ir NPs was measured by dynamic light scattering (DLS) and the Zeta potential was determined by using the Zeta potential analyzer (Brookhaven instruments Corporation). The oxidation state of iridium and the interaction with tannin acid were analyzed by X-ray photoelectron spectroscopy (XPS) using a PHI Quantum 2000 Scanning ESCA Microprobe. Phase identification of the Ir NPs were conducted with X-ray diffraction (XRD, D8, Bruker AXS Co., Ltd) using Cu-Kα radiation source (λ = 1.54051 Å) over the 2θ range of 3–90°. All Electron Spin Resonance (ESR) measurements were carried out using a Bruker EMXplus-10/12 spectrometer at ambient temperature. ESR parameter settings were as follows: modulation amplitude 1 G, scan range 100 G, Center Field 3500 G, StaticFieldMon 3450 G, modulation frequency 100 kHz and microwave power 20 mW for the determination of spin adducts using spin traps DMPO.

2.3. Preparation of the Ir NPs

The Ir NPs were prepared by a simple hydrothermal process using Iridium (III) chloride ($IrCl_3$) as precursor and tannic acid as stabilizer. In brief, $IrCl_3$ solution (5.00 mL, 2.0 mM) was added to tannic acid solution (5.00 mL, 2.0 mM) under vigorous stirring and the color of the mixed solution immediately changed into faint yellow, indicating the formation of Ir-tannic acid complex. After boiling the mixture solution for 30 min, freshly prepared $NaBH_4$ solution (1 mL, 0.5 M) was added and the mixture was stirred for 3 h. The color of the solution changed from faint yellow to deep brown gradually, indicating the formation of the colloid Ir NPs. Finally, the obtained Ir NPs was stored at ambient temperature for use.

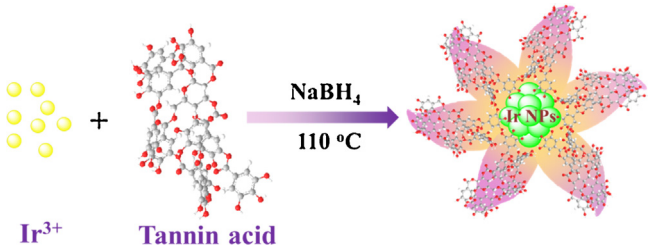

Fig. 1. Schematic of the Ir NPs preparation with one-step production.

2.4. Kinetic analysis

The Michaelis-Menten behavior of the Ir NPs was investigated by monitoring the absorbance of TMB at 652 nm with UV–vis spectrophotometer. The kinetic measurements were conducted in 5 mL NaAc buffer solution (20 mM, pH 4, 35 °C) containing 38.44 ng/mL Ir NPs, with various concentrations of TMB or H_2O_2. The Michaelis-Menten constant was calculated using Lineweaver-Burk plots, $1/v = (K_m/V_{max})(1/[S]) + 1/V_{max}$, where v represents the initial velocity, V_{max} stands for the maximal reaction velocity, [S] is the concentration of substrate. The K_m is the Michaelis constant, which is an indication for the affinity of the Ir NPs mimics with the substrate.

2.5. Catalytic mechanism study

2.5.1. Electron spin resonance

The catalytic mechanism of the Ir NPs was evaluated in the H_2O_2/UV/DMPO system in the presence and absence of Ir NPs. Samples containing 20 mM DMPO, 10 mM H_2O_2, and different concentrations of the Ir NPs (0, 38.44, 76.88, 153.76 ng/mL) were prepared in NaAc buffer (20 mM, pH 4). Each sample was UV-irradiated at 365 nm for 10 min, and then transferred to a quartz capillary tube and placed in the ESR cavity for spectra recording.

2.5.2. Catalytic performance comparison of the Ir NPs against benzoyl peroxide, H_2O_2 and artemisinin

Samples containing 100 μM TMB, 38.44 ng/mL Ir NPs, and different peroxides (benzoyl peroxide, H_2O_2 and artemisinin) were prepared in NaAc buffer (20 mM, pH 4), which were then incubated for various time in ambient temperature. The color changes and the absorbance spectra were recorded for subsequent analysis.

2.6. Determination of H_2O_2 and xanthine

For the determination of H_2O_2, an aliquot of 5 mL NaAc buffer solution (20 mM, pH 4) containing 250 μM TMB, 38.44 ng/mL Ir NPs and different concentrations of H_2O_2 was incubated at 35 °C for 10 min for UV–vis measurement.

For the determination of xanthine, 1.0 mL reaction solution containing 5×10^{-4} U xanthine oxidase and different concentrations of xanthine was incubated for 2 h under ambient temperature. Then, 10 μL 19.22 μg/mL Ir NPs, 250 μL 5 mM TMB and 1.0 mL 100 mM NaAc buffer were added. The reaction solutions were diluted to 5.0 mL with water and incubated at 35 °C for 10 min for UV–vis measurement.

3. Results and discussion

3.1. Preparation and characterization of the Ir NPs

The Ir NPs were prepared with one-pot reaction in aqueous solution using tannin acid as stabilizer (Fig. 1). Two adjacent hydroxyl groups in tannin acid could complex with Ir^{3+} to form five-member