Peroxidase-Like Properties of Multiple Nano-Metallic Oxides under Various Conditions

Ting Liu,^{†,a} Kunfeng Zhao,^{†,b} Linyu Jin,^{†,a} Jun Zhu,^b Yamei Dong,^b
Yinan Yan,^b Ping Wang,^{*,b} and Dannong He^{*,a,b}

^a School of Materials Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

^b National Engineering Research Center for Nanotechnology, Shanghai 200241, China

Six types of different nano-metallic oxides were studied as peroxidase-like enzyme. By changing the amounts of nano-metallic oxides, pH value, the concentration of H₂O₂, reaction time, and so on, the peroxidase-like characteristics of all the six nano-metallic oxides were probed, and four of them (Co₃O₄, CrO_x, NiO, MnO_x) have the oxidase-like properties in the absence of H₂O₂, which is of great importance in the application of degrading phenolic pollutants in waste water.

Keywords nano-metallic oxides, peroxidase-like enzyme, H₂O₂

Introduction

Biological enzyme is a type of catalytic function of protein. It can accelerate reaction speed in small amounts and has a high catalytic efficiency, exclusive reaction, mild reactive conditions. However, it is difficult to purify the enzyme; moreover, it is easy to lose its activity and the costs of storage and use are high. The current issues inspire intense interests in the application of nanoparticles in the field of biomedicine and water contamination.^[1-4]

In 2007, Yan and coworkers^[5] reported that magnetic nanoparticles possess an intrinsic enzyme mimetic activity similar to that found in the natural peroxidase, which are widely used to oxidize organic substrates in the treatment of wastewater or as detection tools. Since then, many peroxides-like enzymes have been synthesized,^[5-17] such as multi-walled carbon nanotube loaded Fe₃O₄ nanoparticles,^[18] gold nanoparticle functionalized with graphene quantum dots (GQD@AuNPs), *etc.*^[19-30] Compared with the horse radish peroxidase (HRP), these nanoparticles with high stability can be repeatedly used and have high efficiency of peroxides-like activity, and also have potential applications in degrading phenolic pollutants in waste water.

Nowadays, researchers always concentrate on one metallic oxide, and have never explored multiple nano-metallic oxides. However, there are still questions of whether those nano-metallic oxides have commonness when acting as enzymes, how they react when put

in different conditions, and what are the factors that affect the peroxidase-like properties. In this paper, six types of different nano-metallic oxides were studied as peroxidase-like enzymes (Co₃O₄, CrO_x, NiO, MnO_x, CuO, Fe₃O₄). 3,3',5,5'-Tetramethylbenzidine (TMB), and 2,2'-azinobis[3-ethylbenzothiazoline-6-sulfonic-acid]-diammonium salt (ABTS) was used as substrates, which are typically employed as HRP substrates in various bioassays. By changing the amounts of metal-oxide nanoparticles, pH value, the concentration of H₂O₂, reaction time, and so on, peroxidase-like characteristics of all the six types of nano-metallic oxides were probed.

Experimental

Reagents and instruments

The commercial 3,3',5,5'-tetramethylbenzidine and 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) were purchased from Sigma-Aldrich. Hydrogen peroxide (H₂O₂), sodium acetate (NaAc), Na₂CO₃ and nitrates [Co(NO₃)₂, Cr(NO₃)₃, Ni(NO₃)₂, Mn(NO₃)₂, Cu(NO₃)₂ or Fe(NO₃)₂] were purchased from Sinopharm Chemical Reagent Company. All of the solvents were analytical reagent grade and were used without any pretreatment. Water used during the experiment was obtained from a Millipore water purification system (18.2 MΩ, Milli-Q, Millipore).

The absorbance value was monitored using Bio-red iMark spectrophotometer at 655 nm. X-ray diffraction

* E-mail: pingw2006@163.com, hdn_neracn@163.com; Tel.: 0086-021-34291286-8035; Fax: 0086-021-34291125
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†These authors contributed equally to this work.

(XRD) patterns were recorded on a D/max-2600 PC (Rigaku Corporation) diffractometer equipped with a Cu K α radiation source ($\lambda=0.15432$ nm), operating at 40 kV and 40 mA. Brunauer-Emmett-Teller (BET) surface areas of samples were measured by nitrogen adsorption at -196 °C using a Micromeritics ASAP 2020 apparatus. Transmission electron microscope (TEM) analyses were performed with a JEM-2100F microscope operated at 120 kV.

Preparation of metal oxides

At room temperature (25 °C), 20 mL aqueous solution of nitrate [Co(NO₃)₂, Cr(NO₃)₃, Ni(NO₃)₂, Mn(NO₃)₂, Cu(NO₃)₂ or Fe(NO₃)₂] was added drop wise to aqueous solution of Na₂CO₃ under vigorous stirring. The resulted suspension was heated to 50 °C and continuously stirred for 1 h. The precipitates were filtered, washed repeatedly with deionized water till neutrality, dried at 100 °C and calcined at 300 °C for 3 h. The concentration of aqueous solution of nitrate and Na₂CO₃ were both 1 mol/L, and the amount of Na₂CO₃ was that the transition metal nitrate was completely precipitated.

Influence of different amounts of metal oxides on reaction

100 μ L TMB (5 mg/mL) was mixed with a series amounts of metal oxides nanoparticles in centrifuge tubes. Different amount of Milli-Q water was used to make sure the final volume was 200 μ L. The absorbance value of the solution was measured by Bio-red iMark spectrophotometer after 30 min.

Influence of the amount of H₂O₂ and pH on reaction

Aliquots (50 μ L) of different metal oxides nanoparticles were mixed with 20 μ L TMB/ABTS (5 mg/mL) with a series concentration of H₂O₂ in centrifuge tubes. Different amount of sodium acetate buffer solution was used to make sure the final volume was 200 μ L. To assess the influence of pH value, we adjusted the pH value of the solution. Then, the absorbance value of the solution was measured by Bio-red iMark spectrophotometer at room temperature.

Results and Discussion

In order to determine the crystal phase composition and grain the size of oxide catalyst, the XRD characterization of the catalyst was carried out (Figure 1). XRD spectra show that the four oxides of Fe₃O₄, Co₃O₄, NiO, CuO are pure metallic oxides; manganese oxide are mixed of Mn₂O₃ and MnO₂, chromic oxide is amorphous oxide. According to the formula of Scherrer, the size of these metallic oxides was 10, 9, 4, 23, 6 nm (Table 1), and the size of amorphous chromium oxide was unable to calculate. As shown in Figure 2, the size of metallic oxides was basically the same with the calculated results. Besides this, specific surface is an impor-

tant factor affecting the activity of catalyst. Based on the BET physical adsorption, we obtained the specific surface of Fe₃O₄, Co₃O₄, NiO, CuO, MnO_x, CrO_x, respectively (Table 1).

Figure 1 XRD patterns of six types of metal oxides materials.

Table 1 Comparison of the properties of six metal oxides

Sample	SBET/ (m ² •g ⁻¹)	DNP ^a / (nm)	Specific rate ^b / (mol•m ⁻² •h ⁻¹)
Co ₃ O ₄	100	9	3.91×10^{-4}
CrO _x	40	—	1.20×10^{-3}
NiO	195	4	1.98×10^{-5}
MnO _x	156	6	2.84×10^{-4}
CuO	39	23	9.17×10^{-5}
Fe ₃ O ₄	89	10	3.22×10^{-5}

^a Measured by XRD; ^b Tested at 40 min.

The characteristics of the peroxidase-like enzymes of the six different metallic oxides were compared with each other. As shown in Figure 3, after 30 min, the catalytic activity of Co₃O₄, CrO, MnO_x is significantly better than the other three metallic oxides. In general, the catalytic activity was enhanced with the increase of the amounts of metal oxides. The catalytic activity of Co₃O₄, CrO, MnO_x reaches the highest value when the amounts is near 0.1 mg/mL. The solution has turned from blue to yellow when the amounts continue to increase, which is mainly due to excessive reaction.

In order to test the intrinsic activity of the catalysts, we obtained the specific activity per unit area of the catalyst according to the specific surface of metallic oxide (Table 1, Figure 3b). It shows that CrO_x has the highest specific activity, while Co₃O₄ and MnO_x have no obvious advantage despite their higher activity. That demonstrates that CrO_x has better catalytic activity than the other oxides. However, the catalytic efficiency of the metal oxide is generally lower than that of HRP (Figure 3c), whereas the metal oxide holds the advantages of low cost, simple synthesis, easy to store, and reusability, therefore, it is highly applicable in many aspects.

Figure 2 TEM images of (a) Co_3O_4 , (b) CrO_x , (c) MnO_x , (d) NiO , (e) CuO , and (f) Fe_3O_4 .

Figure 3 The peroxidase-like activity of the metal oxides is related to the amounts of the metal oxides. (a) Plot of metal oxides amounts *versus* the absorbance; (b) Plot of metal oxides amounts *versus* the absorbance in the specific surface area; (c) Plot of HRP concentration *versus* the absorbance.

The absorption value of most catalyzed system increases along with the increase of H_2O_2 concentration except CrO_x (Figure 4). We suspect that this is because the catalytic activity of CrO_x is too strong to be affected

by the H_2O_2 concentration. For MnO_x , the catalytic performance is higher with lower H_2O_2 concentration. This is because the addition of H_2O_2 makes the reaction too intense, and thus the color reaction changed from blue to colorless. To prove that, we studied the reaction law of these six kinds of metal oxides with time (Figure 5). The absorption values of Co_3O_4 , CrO_x and MnO_x decreased rapidly within 40–100 min, and those of Fe_3O_4 , CuO and NiO changed little during the test time (Figure 6). Actually, the reaction solutions of Co_3O_4 , CrO_x and MnO_x changed from blue to colorless when the reaction time was long enough (>4 d). Moreover, the reaction systems of Fe_3O_4 , CuO and NiO also changed from blue to colorless after more than 10 d.

Figure 4 Effects of H_2O_2 concentration on the catalytic reaction of TMB.

Figure 5 Effects of time on the catalytic reaction.

Figure 6 Effects of H_2O_2 concentration on the catalytic reaction of ABTS.

In addition to the TMB, we also verified the catalytic effect of the ABTS as the substrate. The catalytic ability of MnO_x is much higher than that of the other kinds of nanoparticles. Similarly, the absorbance decreases as the H_2O_2 concentration increases for the same reason. The absorbance value of CuO and Fe_3O_4 still increase along with the increase of H_2O_2 concentration, whereas the catalytic property of NiO , CrO_x and Co_3O_4 is different from that of TMB as the substrate. To explore the reason for the above, the influence of pH value was studied (Figure 7). Under the acid conditions, the metal oxides show catalytic properties, and the absorbance increases along with the decrease of pH value. In addition, NiO has the most suitable pH value in pH 5. This may introduce the difference of the catalytic properties of the peroxidase-like enzymes between TMB and ABTS.

Figure 7 Effects of pH on the catalytic reaction of ABTS.

In the experiment, we found that Co_3O_4 , CrO_x , MnO_x , NiO could still effectively catalyze TMB and ABTS in the absence of H_2O_2 . As shown in Figure 8, these four metal oxides have oxidase-like enzyme properties and this phenomenon is worth to be studied in detail as the next subject.

Conclusions

In this paper, the peroxidase-like properties of six types of nano metal oxides (Co_3O_4 , CrO_x , NiO , MnO_x , CuO , Fe_3O_4) were studied. The effects of TMB, ABTS, amounts, time, pH, *etc.* on the catalytic reaction were probed. At the same time, we found that Co_3O_4 , CrO_x , NiO , MnO_x have the oxidase-like properties. This re-

Figure 8 Color changes after the catalytic reaction in the absence of H_2O_2 .

search has potential applications in biomedical detection and the degradation of the phenolic pollutants in waste water.

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