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High Peroxidase-like Activity of Single Iron Atoms Anchored on N-doped Porous Carbon for the Colorimetric Detection of Biothiols

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Abstract: In this report, single iron atoms anchored on nitrogen-doped porous carbon (Fe-ISAs/CN) was prepared and shown to possess high peroxidase-like activity. Fe-ISAs/CN could efficiently catalyze the oxidation of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2, producing a blue color. In terms of the excellent peroxidase-like activity, single iron atoms anchored on nitrogen-doped porous carbon playing a critical role. Besides, an inhibition effect on the oxidation of TMB catalyzed by Fe-ISAs/CN was induced by biothiols in the presence of H_2O_2. Taking these advantages together, a simple colorimetric method for rapid and sensitive detection of biothiols was established.

Keywords: Fe-ISAs/CN; TMB; Colorimetric detection

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1. Ntroduction

In the past few years, nanomaterial-based peroxidase have attracted wide attention in the field of chemical industry, biological and biomedical fields owing to their superiority in catalytic efficiency.Single-atom catalysts (SACs) are a new concept and have attracted ever-increasing interest in the field of heterogeneous catalysis. SACs mean that obtaining maximum atom efficiency with the lowest size limit and exposing most of their abundant active sites in catalysis.[1] Compared with their bulky counterparts, atomically dispersed catalysts have a larger surface area and expose more active sites. Considering these advantages, the catalytic activity of single-atom based catalysts will be greatly facilitated and have potential applications in catalysis. Currently, most of the reported articles about SACs mainly focused on CO oxidation,[2] hydrogen evolution reaction,[3] and CO_2 reduction.[4] However, up to now, there is few report about employing SACs as peroxidase.

Herein, we demonstrate that single iron atoms anchored on N-doped porous carbon (Fe-ISAs/CN) possesses peroxidase-like activity. As a proof of concept, we investigated the peroxidase-like activity of Fe-ISAs/CN by catalyzing the peroxidase substrate TMB in the presence of H_2O_2. Additionally, an inhibition effect was induced by biothiols on the oxidation of TMB catalyzed by Fe-ISAs/CN in the presence of H_2O_2, resulting in a simple colorimetric method for the detection of biothiols. Our results indicate that the proposed method based on the peroxidase-like activity of Fe-ISAs/CN is simple, low cost, rapid, and with high sensitivity and selectivity for the detection of biothiols.

2. Experimental section

2.1 Synthesis of CN and Fe-ISAs/CN

CN and Fe-ISAs/CN were synthesized according to the reported method with little modifications.[5]

To obtain Fe-ISAs/CN, $Fe(acac)_3$@ZIF-8 was firstly prepared. Typically, 0.876 g 2-methylimidazole was dispersed in 10 ml methanol with stirring in beaker A. 0.793 g $Zn(NO_3)_2 \cdot 6H_2O$ and 0.094 g $Fe(acac)_3$ were dissolved in 20 ml methanol under ultrasound for 15 min to obtain a clear solution in beaker B. Afterwards, the resulting clear solution in beaker B was added into beaker A. After

stirring for 60 min, the obtained solution was placed into a Teflon-lined stainless-steel autoclave and maintained at 120 °C for 4 h.

2.2 Colorimetric detection of biothiols

16 μL of 1 mg/ml Fe-ISAs/CN, 16 μL of different concentrations of biothiols stock solution, 16 μL of H_2O_2 (2 M) and 16 μL of TMB (15 mM) were added sequentially into 1552 μL of acetate buffer solutions (0.1 M, pH 4.0). After incubated at 25 °C for 5 min, the absorbance spectroscopy was recorded at 652 nm.

3. Results and discussions

3.1 Peroxidase-like activity of Fe-ISAs/CN

Dodecahedral Fe-ISAs/CN was synthesized through a cage-encapsulated-precursor pyrolysis route. The ZIF-8 precursors were first prepared and employed as molecular-scale cages to separate and encapsulate the metal precursors $Fe(acac)_3$. And then mixed $Fe(acac)_3$ with ZIF-8 (denoted as $Fe(acac)_3$@ZIF-8). Lastly, pyrolysis $Fe(acac)_3$@ZIF-8 at 800 °C under the protection of nitrogen gas, resulting in the formation of single iron atoms anchored on nitrogen-doped porous carbon (Fe-ISAs/CN). The peroxidase-like activity of Fe-ISAs/CN was screened by employing the most used peroxidase substrates TMB. As shown in Fig. 2, Fe-ISAs/CN could catalyze the oxidation of TMB by H_2O_2, producing a typical blue color reaction. The maximum absorbance of the reaction mixture located at 652 nm, resulting from the oxidation products of TMB (oxTMB) (Fig. 2, curve b). N-doped porous carbon (CN), which did not have single iron atoms anchored, presented low peroxidase-like activity (Fig. 2, curve a), indicating that the CN lacked the ability of catalytic oxidation. However, when the Cys is added, the peroxidase-likeactivity of Fe-ISAs/CN was inhibited and the absorbance was decreased significantly (Fig. 1, curve c). All these observations supported that Fe-ISAs/CN behaved like peroxidase and could be used for colorimetric detection of biothiols.

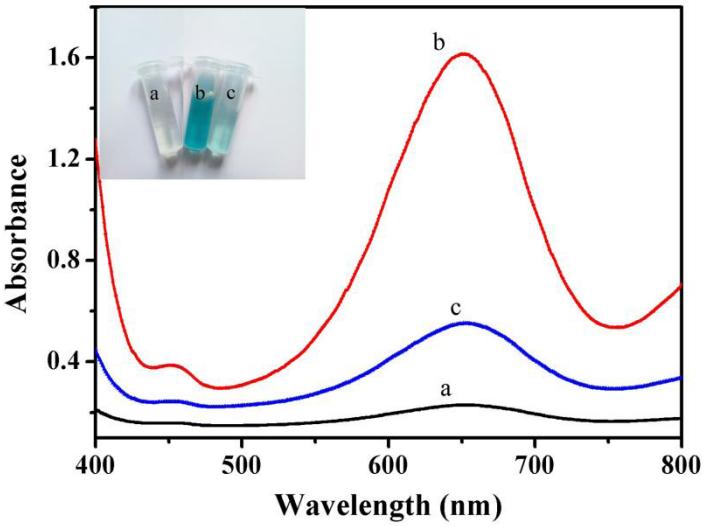

Fig. 1. UV-vis absorption spectra of (a) CN + TMB + H_2O_2, (b) Fe-ISAs/CN + TMB + H_2O_2, (c) Fe-ISAs/CN + TMB + H_2O_2 + Cys. Inset: the corresponding photographs of the colored products.

3.2 Colorimetric detection of biothiols

Biothiols are compounds with antioxidant properties and play major roles in many biological processes, therefore, monitoring the concentration of thiol compounds is crucial to the diagnosis of diseases. Fig. 2 showed that the addition of Cys to the Fe-ISAs/CN-TMB-H_2O_2 system leaded to the fading of the blue color (curve d), indicating an inhibition effect on the oxidation of TMB catalyzed by Fe-ISAs/CN was induced by Cys. In consequence, a colorimetric method was established subsequently using the Fe-ISAs/CN-TMB-H_2O_2 system to detect biothiols. Absorbance difference ΔA ($\Delta A=A_0-A$, A_0 and A are the absorbance at 652 nm without and with biothiols) was used as a function of the concentration of biothiols and the line was plotted in Fig. 4A. Notably, a good liner relationship between ΔA and the concentration of biothiols can be observed. The linear range for biothiols detection was 0.5 μM to 50 μM and the low detection limit (LOD) obtained for GSH, Cys and Hcy were 0.066 μM, 0.068 μM and 0.110 μM, respectively (S/N=3). The sensitivity of Fe-ISAs/CN peroxidase responding to biothiols decreased in the following order GSH > Cys > Hcy. As illustrated in Fig. 2B, the absorbance at 652 nm had a gradual diminution with the increasing concentrations of Cys. Fig. 2C showed the color changed from dark blue to colorless with the increasing concentration of Cys. Therefore, the proposed method can also be applied to visually detect biothiols with naked eye.

3.4 Selectivity of the colorimetric method

To verify the selectivity for biothiols detection, absorbance response to the Fe-ISAs/CN-TMB-H_2O_2 system using biothiols and other possible coexisting substances in human blood serum were investigated, respectively. As displayed in Fig. 3, only 50 μM biothiols could cause remarkable increase of ΔA_{652}, whereas the other coexisting substances (100 μM) gave a limited increase of ΔA_{652} as expected, which was negligible compared with biothiols. The results manifested that the presence of possible coexisting substances in human blood serum can be virtually ignored, and the Fe-ISAs/CN-TMB-H_2O_2 system had a high selectivity for the detection of biothiols.

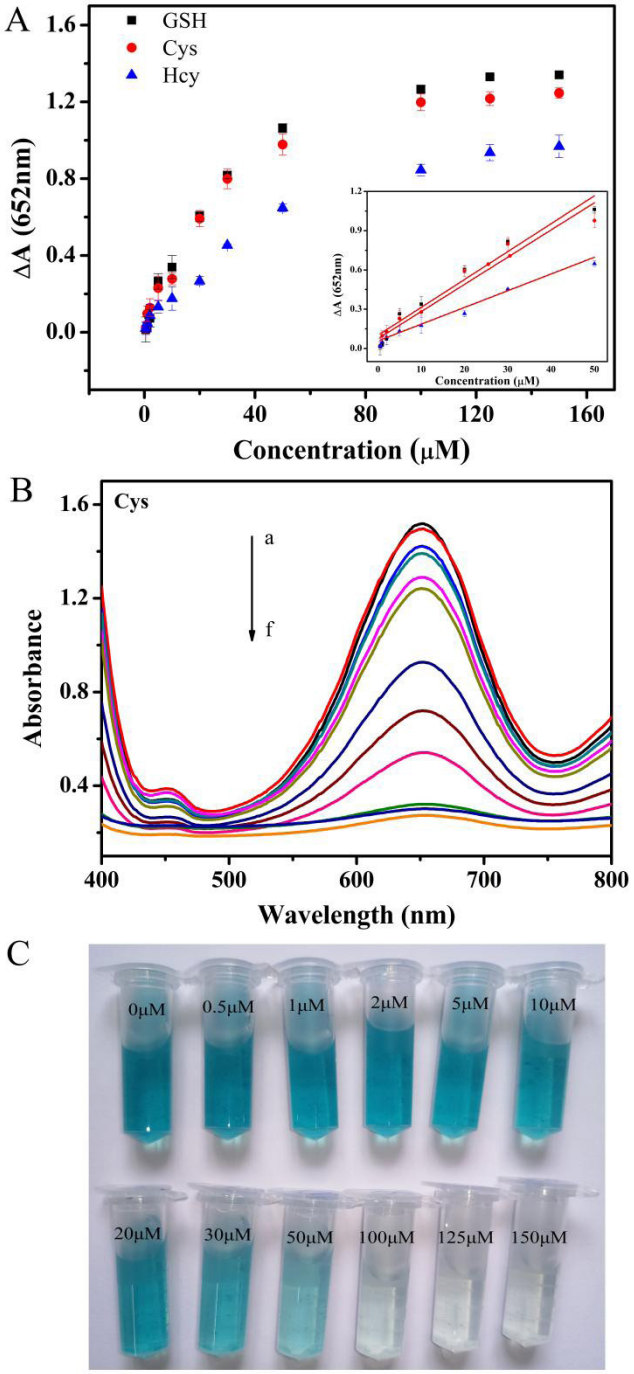

Fig.2. (A) Relationship between ΔA_{652} and the concentration of biothiols. Inset showed the linear calibration plots for detection of biothiols. (B) UV-vis absorption spectra of the Fe-ISAs/CN-TMB-H_2O_2 system in the presence of various concentrations of Cys. (C) The corresponding photographs of (B).

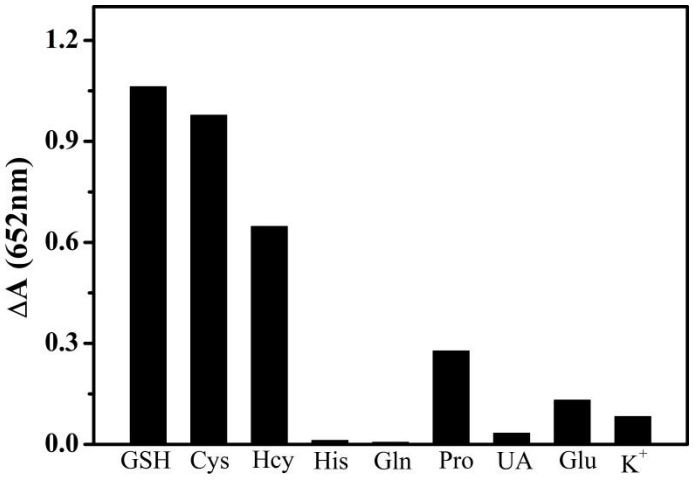

Fig.3. Effects of biothiols and other biomolecules on the peroxidase-like activity of Fe-ISAs/CN. Biothiols was 50 μM, and other biomolecules were 100 μM.

4. Conclusions

In summary, we found that Fe-ISAs/CN possessed excellent peroxidase-like activity for the first time and its catalytic activity depended strongly on the pH and temperature, which was similar to that of HRP. In terms of the excellent peroxidase activity of Fe-ISAs/CN, single iron atoms anchored on nitrogen species played an important role. According to kinetic analysis, Fe-ISAs/CN had a higher affinity to TMB than HRP. Moreover, in comparison with natural HRP, Fe-ISAs/CN had better stability and reusability. Since biothiols had an inhibition effect on the peroxidase-like activity of Fe-ISAs/CN, a simple, low cost and rapid colorimetric method for the detection of biothiols with high sensitivity and selectivity was developed. Take the above advantages together, we confidently believe that Fe-ISAs/CN has potential application in biosensing and medical diagnosis as peroxidase.

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