The Detection of Hydrogen Peroxide by MOF-Based Nanoparticles with Micro Morphology Analysis by Transmission Electron Microscope

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Abstract. MOF materials are new types of nanozymes with catalytic activities. Herein, a nanozyme with peroxidase-like activities was synthesized and took on a cube-like morphology. Besides, the best environment and the detection limit of the nanozyme for hydrogen peroxide were also achieved through experiments.

Keywords: MOF, nanozyme, peroxidase, enzymatic activity.

1. Introduction
Enzymatic reactions are common under natural circumstances and are celebrated for the high catalytic efficiency and selectivity. However, due to the high price, instability and difficulty in recycling, these reactions cannot be widespread applied in industrial productions. In order to lower the price and improve the stability, scientists have long been trying to synthesize artificial enzymes using chemical synthesis since 1950 [1] and produced several kinds of materials such as porphyrin and crown ether.

Nanozyme is a new type of artificial enzyme. It refers to nanomaterial that possesses intrinsic catalytic abilities and has attracted the attention of the world owing to its outstanding performance such as high stability, low cost and adjustable catalytic abilities. Given these advantages, nanozyme possesses widespread use in diversified fields, including biosensing, catalysis, clinical medicine, environmental protection, etc [2-4]. Since the breakthrough discovery that Fe₃O₄ nanoparticles (NPs) have intrinsic horseradish peroxidase (HRP)-like activity [5], many kinds of inorganic nanomaterials as peroxidase mimics have emerged, including carbon-based nanomaterials [6], metal- and metal oxide-based nanomaterials [7, 8], MOF-based nanomaterials [9], and metal sulfides [10].

In this account, a nanozyme with intrinsic peroxidase-like activity has been synthesized. Then the most suitable environment and detection limit of this nanozyme has been found.

2. Materials and methods

2.1. Materials and equipments
MnSO₄·H₂O, PVP, ethanol, K₃[Fe(CN)₆], TMB, sodium acetate, scanning electron microscope, Transmission electron microscope.
2.2. Preparation of nanozyme

The nanozyme was fabricated through coprecipitation synthesis and dried at room temperature. In brief, 0.45g of MnSO$_4$·H$_2$O and 2g poly (vinylpyrrolidone) (PVP) were added into 100mL ethanol under vigorous stirring to get a homogeneous solution. Meanwhile, 0.7g K$_3$[Fe(CN)$_6$] was dissolved in 50mL distilled water. Then, the prepared K$_3$[Fe(CN)$_6$] solution was dropwise added into the above mixed solution in 10 minutes. The final mixture was agitated for 2 hours at room temperature. The resulting brown mixture was centrifuged, washed several times with ethanol and finally dried at room temperature.

2.3. Influence of pH of the solution

Firstly, 50mM sodium acetate buffer solution with pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 were prepared and each group was prepared 40ml. Next, 50mM 3, 3’, 5, 5’-tetramethylbenzidine (TMB) in DMSO and an aqueous solution of 100mM hydrogen peroxide were prepared. Take 1.0mL of pH 3-9 solution and place them in a 1.5mL centrifuge tube, then 50μL of nanoenzyme sample, add 20μL of TMB and 20μL of H$_2$O$_2$ were added into the centrifuge tube and observe the color change. After 10 minutes, measure the absorbance of the solution at 652nm to observe the effect of pH on the enzyme activity and determine the optimal reaction pH of the solution. Repeat 3 samples for each group.

2.4. Influence of reaction temperature

Take 1.0mL of the optimal pH value solution and place them in a 1.5mL centrifuge tube, and place them at 0, 10, 20, 30, 40, 50, 60, 70, and 80 degrees Celsius for 10 minutes, and then 50μL nanoenzyme sample, 20μL TMB and 20μL H$_2$O$_2$ were added into the centrifuge tube. Continue the constant temperature reaction and observe the color change. Measure the absorbance of the solution at 652nm to determine the best reaction temperature after 10 minutes’ reaction. Repeat 3 samples for each group.

2.5. Influence of TMB substrate concentration

Take 1.0mL of the optimal pH solution and place it in a 1.5mL centrifuge tube, incubate at the optimal temperature for 10min. Then 50μL of nanozyme sample, 0.5, 1.0, 1.5, 2, 3, 4, 5mM TMB (Final concentration), and 20μL H$_2$O$_2$ were added into the centrifuge tube. Observe the color change, and measure the absorbance of the solution at 652nm after 10 minutes. Repeat 3 samples for each group.

2.6. Influence of nanozyme concentration

Take 1.0mL of the optimal pH value solution and place it in a 1.5mL centrifuge tube, incubate at the optimal temperature for 10 minutes. Then the optimal concentration of TMB solution, the final concentration of 1, 2, 3, 4, 5, 6, 7 were added respectively. 7ug/mL nanozyme sample, then add 20μL H$_2$O$_2$. Observe the color change, and measure the absorbance of the solution at 652nm after 10 minutes. Repeat 3 samples for each group.

2.7. Detection of hydrogen peroxide

The experiment was carried out at the optimum pH value and optimum reaction temperature conditions. Take 1.0mL of the optimal pH solution and place it in a 1.5mL centrifuge tube, incubate at the optimal temperature for 10 min, add the optimal concentration of nanoenzyme sample and the optimal concentration of TMB solution, then different concentrations of hydrogen peroxide were added. Continue the constant temperature reaction, observe the color change, and measure the absorbance of the solution at 652nm after 10 minutes. Repeat 3 samples for each group. Determine the detection limit, detection range and linear detection range of hydrogen peroxide.
3. Results and discussion

3.1. The morphology of nanozyme
The structure and morphology of this nanozyme were identified by SEM, TEM. SEM and TEM images indicate that the particles exhibit a cube-like morphology and the length of the edge was approximately 490nm.

![SEM and TEM images of nanozyme](image)

**Figure 1.** The structure of nanozyme (a) the SEM and (b) TEM image of nanozyme.

3.2. Influence of pH to the enzymatic activity
The absorbance of TMB under different pH was shown in Fig 2. The absorbance showed an upward trend from pH=2 to pH=3.83 and reached its top at about 0.55 when pH was 3.83. Afterwards, the absorbance quickly declined to 0.05 when pH=10. Therefore, 3.83 is the pH when the nanozyme could reach its best catalytic activity.

![Absorbance spectrum at different pH](image)

**Figure 2.** (a) the absorbance of TMB under different pH (b) fitting result of pH.

3.3. Influence of temperature to the enzymatic activity
The absorbance spectrum at different temperatures was shown in Fig 3. The absorbance gradually rose to reached its top at about 0.7397 when the temperature was 56.97℃. Then, the absorbance significantly decreased to 0.35 when the temperature was 70℃. Therefore, 56.97℃ is the most suitable temperature for the nanozyme to reach its best catalytic activity.

![Absorbance spectrum at different temperature](image)
3.4. Influence of concentration of TMB to the enzymatic activity
The absorbance spectrum at different concentration of TMB was shown in Fig 4. The absorbance he absorbance showed an upward trend from 0.5mM to 2mM reached its top at about 0.975 when the concentration of TMB was 2.61mM. Then, the absorbance slowly declined to 0.95 when the concentration of TMB was 0.95. Therefore, 2.61mM is the most suitable TMB concentration for the nanozyme to reach its best catalytic activity.

3.5. Influence of concentration of nanozyme to the enzymatic activity
The absorbance spectrum at different concentration of TMB was shown in Fig 5. When the concentration was below 6ug/ml, the absorbance spectrum had linear relationship with the concentration of peroxidase and the formula is \( y = 0.202x + 1.001 \times 10^{-4} \). When the concentration was above 6ug/ml, the absorbance spectrum remained unchanged.
3.6. The concentration detection of H$_2$O$_2$

The absorbance spectrum at different concentration of H$_2$O$_2$ was shown in Fig 6. At the best the upper detection limit of H$_2$O$_2$ was 10mM. When the concentration was below 5mM, the absorbance spectrum had linear relationship with the concentration of H$_2$O$_2$ and the formula is $y=0.188x+0.349$.

Figure 6. The absorbance of different H$_2$O$_2$ concentration.

4. Conclusion

It was proved that the MOF synthesized in this experiment possessed the properties of peroxidase. The nanozyme exhibited a cube-like morphology and had the best catalytic activities when pH=3.83, T=56.97$^\circ$C and the concentration of TMB was 2.61mM. Apart from this, a method was developed for the detection of H$_2$O$_2$.

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