Synthesis of metal-2D metal-organic framework composite Nanosheets and Their Application in Cascade Catalytic Reactions

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Abstract. Cascade reactions have always had an irreplaceable position in biocatalysis. In this work, we are inspired by the cascade reactions catalyzed by natural enzymes and combining the peroxidase mimetic activity of metal organic frameworks (MOF) nanosheets and the glucose oxidation mimetic enzyme activity of gold nanoparticles (AuNPs) constitutes a dual nanozyme mimetic cascade reaction. AuNPs /Zn-TCPP nanosheets with dual mimetic enzyme activities were prepared by in situ reduction to generate AuNPs on the surface of 2D MOF. And used to further detect the content of hydrogen peroxide or glucose.

1. Introduction

Enzymes, which are mostly proteins, have significant applications in medicine, agriculture, chemical industry, and food processing because of their high substrate specificity and catalytic efficiency for biological reactions. However, after exposure to extreme pH and high temperature, the catalytic activity of enzymes is usually lost. Enzymes are also susceptible to digestion by proteases. These intrinsic drawbacks dramatically hinder the practical applications of enzymes. [1] Therefore, people vigorously develop and research mimic enzymes that can be used to replace natural enzymes. Since 2007, Yan et al discovered that Fe_3O_4 nanoparticles(NPs) have peroxidase activity,[2] nanozymes have achieved great development.

Ultrathin two-dimensional nanosheets have attracted more and more attention after Novoselvo and colleagues used mechanical cracking to separate graphene flakes from graphene in 2004.[3] Among them, some 2D nanomaterials, such as graphene oxide, [4] boron nitride (BN), [5] transition metal dichalcogenides (TMDs),[6] metal-organic frameworks (MOF), [7] etc., have simulated enzyme activities. Compared with bulk materials, 2D materials have a larger surface area, increase the diffusion rate of substrate molecules, reduce the diffusion barrier, and allow more substrate molecules to reach the catalytically active site.[8] Furthermore, compared with natural enzymes, they not only have stable biological activity and low requirements for action conditions, but also can flexibly adjust the activity of simulated enzymes by changing the physical properties of the material itself, such as size and thickness.

Among them, MOF and their derivatives are expected to become direct substitutes for natural enzymes in enzymatic reactions due to their tunability and versatility of metal nodes and linking units, and their modifiability after synthesis. As known, incorporating 2D MOF nanosheets with other functional materials is an efficient way to prepare 2D MOF-based hybrid nanomaterials.[9] And the sheet-like structure of the 2D MOF exhibit unique physical and chemical properties such as it can load more catalytically active sites and increase the interaction area with the substrate, effectively solving the problem of low catalytic activity of nanozymes. Then, Au NPs / Zn-TCPP nanosheets were synthesized,[10] The surface of tetrakis(4-carboxyphenyl) porphyrin (TCPP) has abundant carboxyl groups and a heme-like macroporphyrin hollow structure, which was used as a ligand and combined with different metal nodes to prepare nanosheets. Carboxyl group in the TCPP ligand assisted the decomposition of H_2O_2 to form ·OH and oxidized TMB,[11] and the porphyrin structure mimics the active center of natural peroxidase to improve the peroxidase activity of the nanosheets.[12] We used MOF as a substrate for mimicking enzymes, and combined it with gold nanoparticles to form an enzymatic cascade reaction. The catalytic properties of Au NPs have been reported.[13] Since then, several catalytic activities of Au NPs have been explored, such as glucose oxidase,[14] oxidase,[15] peroxidase,[16] superoxide dismutase,[17] etc. In this paper, we exploit the glucose oxidase activity of gold nanoparticles, which can catalyze the oxidation of glucose to gluconic acid. Then, the glucose oxidase activity of Au NPs and the peroxidase activity of metalloporphyrin nanosheets are combined to construct an enzyme-catalyzed cascade reaction. A series of 2D MOF nanosheets consisting of binuclear inorganic paddle wheel units (e.g., Zn^{2+}, Co^{2+}, and Cu^{2+}) and heme-like metalloporphyrin polydentate organic ligands (e.g., TCPP) were designed. Then, Au NPs were synthesized by in situ reduction on 2D MOF nanosheets to prepare hybrid nanosheets, named Au NPs/M-TCPP. Then, the intrinsic glucose oxidase activity and peroxidase activity exhibited

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by Au NPs and MOF nanosheets were used to form an enzymatic catalytic cascade reaction.

2. Experimental

2.1 Synthesis of Zn-TCPP nanosheets

Synthesis of Zn-TCPP nanosheets. Zn $(NO_3)_2 \cdot 6H_2O$ (4.5 mg, 0.015 mmol), pyrazine (0.8 mg, 0.01 mmol) and PVP (20.0 mg) in 12 mL of the mixture of DMF and ethanol (V: V=3:1) were dissolved in a 20 mL capped vial. Then TCPP (4.0 mg, 0.005 mmol) dissolved in 4 mL of the mixture of DMF and ethanol (V: V=3:1) were added dropwisely under stirring. After that, the solution was sonicated for 10 min. The vial was heated to 80°C and then kept the reaction for 24 h. The resulting purple nanosheets were washed twice with ethanol and collected by centrifuging at 8,000 r.p.m. for 10 min. Finally, the obtained Zn-TCPP nanosheets were redispersed in 10 mL of ethanol.

2.2 Synthesis of Au NPs/Zn-TCPP hybrid nanosheets

After 100 μL of $HAuCl_4$ (10 mM) was added into 10 mL aqueous solution of Zn-TCPP nanosheets (0.1 $mg \cdot mL^{-1}$), the mixture was stirred for 1 min, followed by the addition of 25 μL of tannic acid aqueous solution (0.1 M). Then the hybrid nanosheets were collected by centrifuge at 10,000 r.p.m for 10 min. Finally, the obtained Au NPs/Zn-TCPP hybrid nanosheets were redispersed in water.

2.3 TMB oxidation reaction

The experiments of TMB oxidation catalyzed by Au NPs/Zn-TCPP hybrid nanosheets were performed by mixing 50 μL of TMB (25 mM, used as substrate), 200 μL of H_2O_2 (5 mM) and 750 μL of the Au NPs/Zn-TCPP hybrid nanosheets (200 $\mu g \cdot mL^{-1}$). After incubation of mixed solution for 10 min, Au NPs/Zn-TCPP hybrid nanosheets were removed from the aforementioned solutions by centrifuge, and then the resulting supernatants were characterized by the UV-Vis spectroscopy.

2.4 An artificial enzymatic cascade reaction

750 μL of Au NPs/Zn-TCPP hybrid nanosheets (200 $\mu g \cdot mL^{-1}$) were mixed with 200 μL of glucose (1 M), and the mixed solution was incubated for 4 h. After that, 50 μL of TMB (25 mM) was added into the aforementioned solution, which was then incubated for different reaction time. Finally, Au NPs/Zn-TCPP hybrid nanosheets were removed from the aforementioned solutions by centrifuge, and then the resulting supernatants were characterized by the UV-Vis spectroscopy.

3. Results and Discussion

3.1 Synthesis of 2D MOF Nanosheets and Evaluation of Their Peroxidase-like Activities.

In this work, hybrid nanosheets were prepared using 2D MOF nanosheets as the matrix for in situ synthesis of Au NPs. The preparation process is shown in Figure 1a. We first synthesized a series of TCPP nanosheets using surfactant-assisted synthesis. Among them, PVP (polyvinylpyrrolidone) acts as a surfactant molecule attached to the surface of MOF, resulting in the anisotropic generation of MOF and the formation of ultrathin 2D nanosheets.[18]

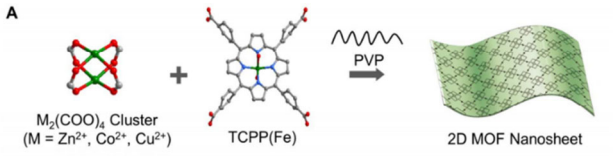
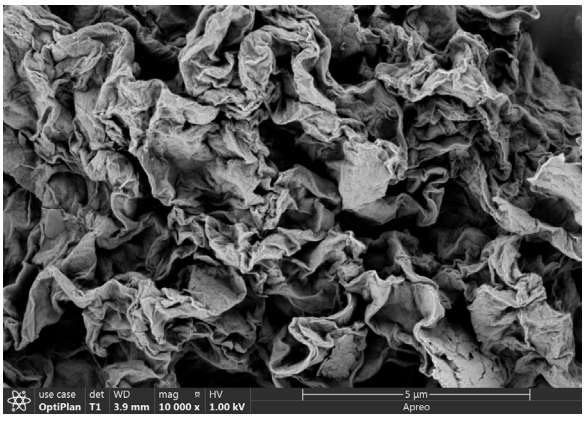


Fig. 1. Scheme showing the surfactant-assisted bottom-up synthesis of 2D MOF nanosheets.

The TEM and SEM images in Figure 3c show a typical 2D TCPP nanosheet. It is obvious that our synthesized TCPP nanosheet is a sheet-like structure. The lateral dimension is about 3-4μm, the larger the lateral size, the more gold nanoparticles can be supported, which is beneficial to improve the catalytic activity. The corresponding selected area electron diffraction (SAED) pattern (Figure) shows clear bright spots, which could be assigned to (100) and (110) planes of 2D Zn-TCPP nanosheet, confirming its crystal structure. Atomic force microscopy image indicates the thickness of 2D Zn-TCPP nanosheets is 4.1 ± 1.3 nm (Figure). X-ray diffraction (XRD) patterns further identify the crystalline nature of 2D Zn-TCPP nanosheets (Figure). Three diffraction peaks of 2D Zn-TCPP nanosheets are indexed as (110), (001), and (002), which are similar to those of bulk Zn-TCPP.



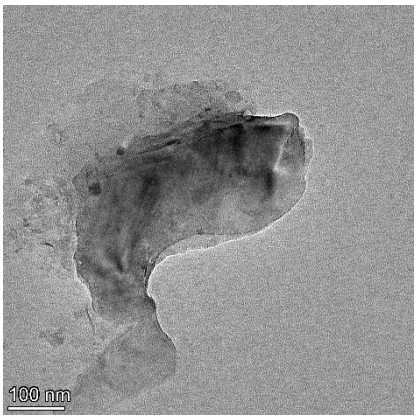


Fig 3. a) SEM image of randomly oriented Zn-TCPP nanosheets. b) TEM image of 2D Zn-TCPP nanosheets.

The Zn-TCPP nanosheets were analyzed by Fourier transform infrared (FTIR) spectroscopy. As shown in Figure 4, the stretching of the $\nu_{C=O}$ in PVP at 1669 cm^{-1} was transferred to 1676 cm^{-1} after mixing with Zn^{2+}, indicating that the C=O groups in PVP had stronger interactions with Zn^{2+}.[19] Therefore, after nucleation, PVP attaches to the surface of the nanosheets, leading to anisotropic growth of the nanosheets and finally forming thinner Zn-TCPP nanosheets.

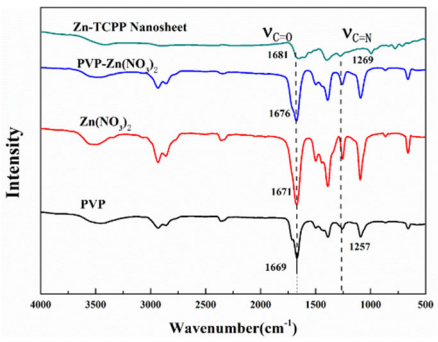


Fig 4 FTIR spectra of Zn-TCPP nanosheets, Zn $(NO_3)_2$, PVP and the mixture of Zn $(NO_3)_2$ and PVP with a mole ratio of 1:1.

3.2 Synthesis of 2D Au NPs/Zn-TCPP Nanosheets and Determination of Cascade Catalytic Activity

After that, the $HAuCl_4$ was in-situ reduced with tannic acid on the TCPP nanosheets to generate gold nanoparticles, and the Au NPs/Zn-TCPP mixed nanosheets were generated. Since the molecular formula of tannic acid contains more phenolic hydroxyl groups, it is very useful for reducing $HAuCl_4$ to Au NPs. As an effective and safe reducing agent, we chose to use tannic acid for the reduction of gold nanoparticles. Since the concentration of tannic acid is closely related to the size of the gold nanoparticles formed. Therefore, we first determined the most suitable tannic acid concentration and $HAuCl_4$ concentration through a series of reactions. As shown in Figure 5, when the concentration of $HAuCl_4$ was 50 mM, the catalytic activity of tannic acid at a concentration of 16.7 mM was the best.

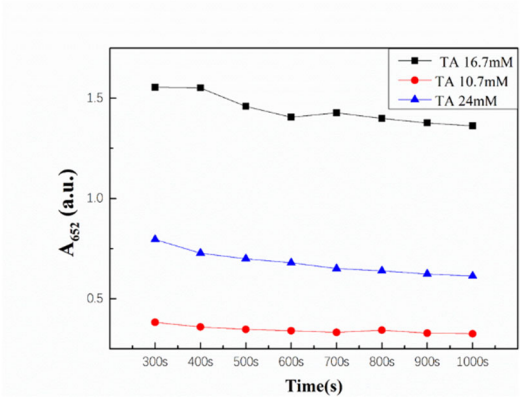


Fig 5 Activity of synthesized hybrid nanosheets by different concentrations of tannic acid changes with time.

Furthermore, in order to make the synthesized hybrid nanosheets thinner, the catalytic activity is better. 2D MOF can be separated using various methods, such as micromechanical exfoliation, surface assisted in situ growth, freeze-thaw method etc.[20] Among them, we chose the most convenient freeze-thaw method to exfoliate the 2D MOF nanosheets.[21] Water freezes and expands below 0 °C, exerting a strong squeezing force between the layers of the 2D material, and the volume expansion causes the breaking of van der Waals bonds in the layered material, which in turn promotes the exfoliation of the 2D nanosheets. Since the surface of the 2D Zn-TCPP nanosheets is rich in carboxyl and is hydrophilic, it is beneficial for water molecules to enter the interlayer. We disperse the fabricated two-dimensional metal-organic framework nanosheets in a solution with a ratio of water and ethanol of 3:1,[22] put them into an environment of minus 18 degrees Celsius, freeze them for 48 hours to 72 hours, and then take them out and wait for them to be thawed. Activity assay was then performed.

Acknowledgments

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