Review Article

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Oxygen Vacancies and Hydroxyl Radicals are Contributed in Catalytic Oxidation of 3,3',5,5'-Tetramethylbenzidine Over NH₂-MiL-88 (Fe, Ni) as the Main Reactive Oxygen Species

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Abstract

Herein, the reactive oxygen species contributed in catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH_2 -MiL-88 (Fe, Ni) were identified by investigating the effect of different scavengers on the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine. The possible contribution of O_{xx} , hydroxyl radicals, and 1O_2 in the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH2-MiL-88 (Fe, Ni) were investigated. The results revealed that oxygen vacancies (O_{xx}) and hydroxyl radicals are significantly contributed in nanozymatic reaction while by presence of 1O2, the catalytic oxidation reaction is not affected. Considering the results, hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine were adsorbed over the nanozymes and then the active metal center of the nanozymes affected on hydrogen peroxide to generate 0OH or O_2 -. Afterward, the oxidation of 3,3',5,5'-tetramethylbenzidine to its corresponding colored product was occurred by the reactive oxygen species.

Keywords: Peroxidase-Like Nanozyme, NH₂-MiL-88 (Fe, Ni), Reactive Oxygen Species, 3,3',5,5'-Tetramethylbenzidine

1. Introduction

Native enzymes suffer some disadvantages such as instability in harsh reaction conditions, difficult recovery, etc. However, despite their disadvantages, high catalytic performances, specificity, and selectivity are their characteristic advantages over the artificial catalysts [1-10]. There two approach for solving the disadvantages of these native enzymes. The common way is immobilization of enzymes, however immobilization leads to some decrease in the enzyme activity [11-15]. Nanomaterials were introduced by unique spectral, optical, catalytic, and stability as a result of fast development of nanoscience in the recent years which some of them reveal significant enzyme-like activity especially peroxidaselike properties with significant advantages over native enzymes for example, high pH and thermal stability, excellent reusability, and high storage stability [16-44]. Up to now nanozymes had been utilized organic dye biodegradation, battery development, sensor and biosensor design, especially after first report of COVID-19, they applied for its clinical sensing [45-70]. In this field proving the catalytic mechanism of the reaction is an attractive research topic. Hence, herein, catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH2-MiL-88 (Fe, Ni) as the peroxidase-like nanozyme was

performed, revealing high catalytic activity of the as-prepared nanozymes. The reactive oxygen species contributed in catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni) were identified by investigating the effect of different scavengers on the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine.

2. Experimental

2.1. Synthesis of NH,-MiL-88 (Fe, Ni)

For the preparation of graphene oxide/gold nanoparticles, the gold nanoparticle and graphene oxide dispersions were mixed with a 1:1 ratio. The reaction mixture was then ultra-sonicated for about 30 minutes to produce the graphene oxide/gold nanoparticles. The obtained graphene oxide/gold nanoparticles dispersion was then stored in dark for further use.

2.2. Nanozyme Assay

TMB assay was used for determining the enzyme-like activity of peroxidase-like graphene oxide/gold nanoparticles as a standard method. Briefly, 50.0 μ L of nanozyme was introduced to 6.8 mL of acetate buffer (20 mM; pH 3.5) containing 100 μ L TMB and 50 μ L H₂O₂, incubated at 40.0 °C. After reaction is completed, the

absorbance of the colored product was measured at 650 nm.

3. Results and Discussion

The reactive oxygen species contributed in catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH_2 -MiL-88 (Fe, Ni) were identified by investigating the effect of different scavengers on the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine. The possible contribution of O_{vs} , hydroxyl radicals, and 1O_2 in the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH_2 -MiL-88 (Fe, Ni) were investigated.

3.1. Possible Contribution of Hydroxyl Radicals (0OH)

It is well-known that different types of reactive oxygen species were produced in nanozyme-mediated reactions for instance catalytic oxidation of 3,3′,5,5′-tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni). Hence, to identify the possible contribution of different kinds of the reactive oxygen species in the catalytic reactions, the free radical scavenging experiments were designed. In this regard, thiourea was selected as the scavenger of hydroxyl radicals (°OH). The results of inhibition of the catalytic reaction by thiourea were shown in Figure 1. As can be seen from this figure, the absorbance of the resulted colored product of the oxidation of 3,3′,5,5′-tetramethylbenzidine was decreased by increasing the concentration of thiourea, revealing the contribution of hydroxyl radicals in the catalytic oxidation of 3,3′,5,5′-tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni). 1,4-benzoquinone (PBQ) and NaN3 scavenges superoxide anion (O2.-) and singlet oxygen (1O2), and EDTA scavenges oxygen vacancies (O_{ve})

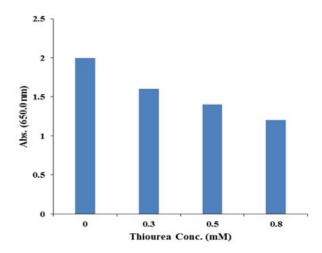


Figure 1: Possible Contribution of Hydroxyl Radicals (⁰OH) on the Catalytic Oxidation of 3,3′,5,5′-Tetramethylbenzidine Over NH₂-MiL-88 (Fe, Ni) Investigated by Effect of Thiourea as Scavenging Agent

3.2. Possible Contribution of Oxygen Vacancies (O_{vs})

The effect of oxygen vacancies (Ovs) on the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni) was evaluated. In this regard, EDTA was selected as the scavenger of oxygen vacancies (O_{vs}). The results of inhibition of the catalytic reaction by EDTA were shown in Figure 2. As can be seen from

this figure, the absorbance of the resulted colored product of the oxidation of 3,3',5,5'-tetramethylbenzidine was completely disappeared by introducing EDTA into the reaction media, revealing the contribution of oxygen vacancies (O_{vs}) in the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH_2 -MiL-88 (Fe, Ni).

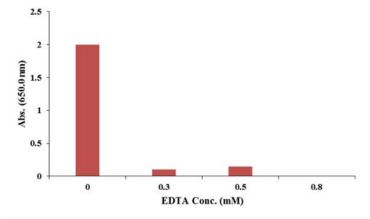


Figure 2: Possible Contribution of Oxygen Vacancies (O_{vs}) on the Catalytic Oxidation of 3,3',5,5'-Tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni) Investigated by Effect of EDTA as Scavenging Agent

3.3. Possible Contribution of Singlet Oxygen (10,)

The effect of singlet oxygen ($^{1}O_{2}$) on the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni) was evaluated. In this regard, NaN3 was selected as the scavenger of singlet oxygen ($^{1}O_{2}$). The results of inhibition of the catalytic reaction by NaN3 were shown in Figure 2. As can be seen from

this figure, the absorbance of the resulted colored product of the oxidation of 3,3',5,5'-tetramethylbenzidine was not changed by introducing NaN₃ into the reaction media. Hence, it can be concluded that by presence of ¹O₂, the catalytic oxidation reaction is not affected.

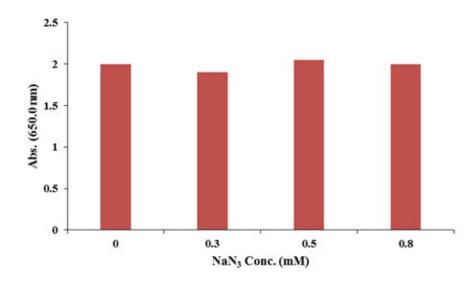


Figure 3: Possible Contribution of Singlet Oxygen (${}^{1}O_{2}$) on the Catalytic Oxidation of 3,3′,5,5′-Tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni). NaN₂ was Selected as the Scavenger of Singlet Oxygen (${}^{1}O_{2}$)

4. Conclusion

Herein, the reactive oxygen species contributed in catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni) were identified by investigating the effect of different scavengers on the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine. The possible contribution of Ovs, hydroxyl radicals, and ¹O₂ in the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine over NH₂-MiL-88 (Fe, Ni) were investigated. The results revealed that oxygen vacancies (O_{vs}) and hydroxyl radicals are significantly contributed in nanozymatic reaction while by presence of ¹O₂, the catalytic oxidation reaction is not affected. Considering the results, hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine were adsorbed over the nanozymes and then the active metal center of the nanozymes affected on hydrogen peroxide to generate ${}^{0}O_{H}$ or O₂-. Afterward, the oxidation of 3,3',5,5'-tetramethylbenzidine to its corresponding colored product was occurred by the reactive oxygen species.

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