A Comparative Study on the Kinetics Performances of Gold- and MnO_2- Nanozymes

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Posted Date: 5 July 2023

doi: 10.20944/preprints202307.0258.v1

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Article

A Comparative Study on the Kinetics Performances of Gold- and MnO_2- Nanozymes

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Abstract: Gold- and MnO_2- nanozymes are well-known for their enzyme-like activity. In this regard, initially, gold- and MnO_2- nanozymes were synthesized by simple and green methods. Afterward, the kinetic studies were performed using the Michaelis–Menten model for both gold- and MnO_2- nanozymes. The kinetic parameters including K_m and V_{max} were calculated via the construction of the linear plot of Lineweaver–Burk for both nanozymes. The results showed a V_{max} and K_m of 185 nM sec^{-1} and 47 nM sec^{-1} for the gold- and MnO_2-nanozymes, in order. The ratio of V_{max}(gold)/V_{max}(MnO_2) was found to be about 4.0 which pointed that the catalytic efficiency of gold-nanozymes is 4.0-fold higher than the catalytic efficiency of MnO_2- nanozymes. The K_m value was found to be 0.72 mM and 1.6 mM for the as-prepared gold- and MnO_2- nanozymes, respectively, and the K_m of MnO_2-nanozymes is 2.2-fold higher than that of gold nanozymes. Since the Km shows the affinity of substrate for binding to nanozyme active nodes (lower K_m=higher affinity), it is consultable that the substrate affinity toward MnO_2-nanozymes is 2.2-fold lower than that of the gold-nanozymes. Considering the above results, the as-prepared gold nanozymes are very stronger peroxidase-like mimics than the metal oxide MnO_2-nanozymes.

Keywords: MnO_2-nanozymes; gold-nanozymes; kinetic parameters; comparative study

1. Introduction

Although the enzymes exhibit very high specificity and selectivity toward their substrates along with high catalytic performance, they suffer several disadvantages such as low stability (narrow pH and thermal range); difficult recovery, and no reusability, as reported [1]. To overcome these drawbacks, the enzyme immobilization process has been developed to enhance the enzyme stability against environmental changes and make them reusable [2]. As already we mentioned enzyme immobilization permits the possible increase in stability, however, the specific and relative activities of the most immobilized enzymes are found to be lower than the free enzymes which can be explained by the effect of immobilization on enzymes' conformational transition after their immobilization [3,4]. Besides, enzyme immobilization, the fast advancement of the field of material science and nanochemistry leads to develop novel nanoscale materials such as MOFs (e.g., NEQC-340) [5] and carbon dots [15], ZSM-5@ Al-MCM nanocatalysts [7], gold nanoparticles [26], and silver nanoparticles [8]. Among these nanoparticles, a wide variety of the introduced nanomaterials reveal excellent enzyme-like activity [6] for example Fe_2O_3/Au hybrid nanozyme [9], silver nanoparticles [10,11], Pt nanozyme [12], Fe/Cu single-atom nanozymes [13], NEQC-340 [14], unmodified silver nanoparticles [20], MnO_2 nanoparticles [19], BiOI-NFs [16], gold nanoclusters [17,18], and SiO_2-Fe_3O_4 nanoparticles [21] which had been used for analytical sensing and biosensing [22], water treatment [23], food analysis [24], and organic dye degradation [21]. Recently, the excellent peroxidase-like activity of gold nanozymes attracted good attention for application as alternatives to natural peroxidase [17,18]. Besides, the metal oxide, manganese dioxide (MnO_2) reveals high oxidase- and peroxidase-like activity. The significance of MnO_2 nanoparticles compared to gold nanozymes is their dual oxidase- and peroxidase-like activity while the gold nanozymes show only peroxidase-like activity. However, it is well-known that the applicability of the nanozmyes instead of the native enzymes in biocatalysis is strongly dependent on their catalytic performances which can be determined by kinetic studies. Hence, in this study, a comparative study on the kinetics performances

 doi:10.20944/preprints202307.0258.v1

of gold- and MnO_2- nanozymes was performed. In this regard, initially, gold- and MnO_2- nanozymes were synthesized by simple and green methods. Afterward, the kinetic studies were performed using the Michaelis–Menten model for both gold- and MnO_2- nanozymes. The kinetic parameters including K_m and V_{max} were calculated via the construction of the linear plot of Lineweaver–Burk for both nanozymes.

2. Experimental

2.1. Synthesis of MnO_2-nanozymes

150.0 mg $KMnO_4$ was dissolved in 15.0 mL of deionized water, followed by the addition of 150.0 µL of 30% hydrogen peroxide and 75.0 µL of 80% hydrazinium hydroxide under 5 min stirring. Afterward, nanozymes were collected, washed, and dried at room temperature.

2.2. Synthesis of gold-nanozymes

To do synthesis the BSA-protected nanozymes, 10.0 mM $HAuCl_4.4H_2O$ (5.0 mL) was introduced to 50 mg mL^{-1} bovine serum albumin (5.0 mL), followed by stirring at 37 °C and adding 1.0 M NaOH to adjust pH. The solution was incubated at 37 °C for 12 hours to complete the synthesis process.

2.3. Steady-state kinetics studies

The kinetic parameters of the as-prepared nanozymes were calculated based on Michaelis–Menten equation and the Lineweaver–Burk method as a function of the concentration of 3,3'-diaminobenzidine (DAB; nanozyme substrate). The nanozyme activity (nM sec^{-1}) was measured by probing the absorbance of the resulting colored product at 460 nm considering a molecular extinction coefficient ε=5500 molar cm^{-1}.

3. Results and discussion

Kinetic studies were carried out to estimate the kinetic parameters (i.e., K_m and V_{max}) of the as-prepared MnO_2 nanozyme as pseudo-peroxidase nanoenzyme toward n-electron irreversible oxidation of 3,3′-diaminobezedine. It is well known that the V_{max} value reflects the intrinsic properties of the enzyme/nanozyme and is defined as the highest possible rate of the nanozyme-catalyzed reaction (i.e., catalytic efficiency) when all enzyme molecules or all nanozyme particles are saturated with the substrate. The higher value of V_{max} is assigned to the higher catalytic efficiency of the enzyme/nanozyme. In contrast, the affinity of the substrate of an enzyme/nanozyme to interact with its active site is represented by the K_m value, the lower values indicate a higher affinity of the substrate for binding to the enzyme/nanozyme.

3.1. Steady-state saturation plots of the Michaelis-Menten model

The estimation of the kinetic parameters of MnO_2 nanozymes was performed by measuring the initial velocity of the nanozyme-mediated reaction as a function of the DAB concentration. The Michaelis-Menten saturation curve for the as-mentioned MnO_2-nanozymes was shown in Figure 1. As seen in Figure 1, the MnO_2-nanozymes mediated reaction rate was increased by increasing the DAB concentration and then reached a saturation state after a certain substrate concentration.

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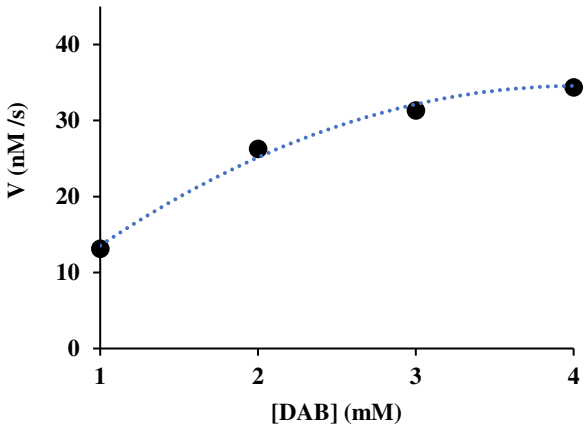

Figure 1. Michaelis–Menten plot for MnO_2-nanozymes mediated reaction.

Besides, to evaluate the kinetics performances of the as-prepared gold-nanozymes, the Michaelis–Menten plot was constructed by plotting the velocity of the nanozymatic reaction as a function of DAB concertation. The results are shown in Figure 2. As seen in Figure 2, the rate of gold-nanozyme-mediated oxidation reaction was increased by increasing the substrate concertation and then leveling off. In comparison to the MnO_2-nanozymes, the gold-nanozymes can oxidize lower concentrations of DAB at a very higher reaction rate which pointed to their higher peroxidase-like activity compared to the MnO_2-nanozymes

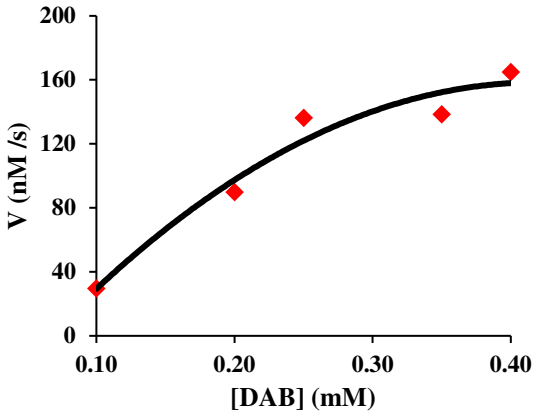

Figure 2. Michaelis–Menten plot for gold-nanozymes mediated reaction.

3.2. Quantification of kinetic parameters utilizing Lineweaver-Burk linear model

Due to the inaccuracy of the results of non-linear saturation curves, to explore more precise on the kinetic performances of the as-prepared nanozymes, their kinetic parameters were quantified utilizing Lineweaver-Burk linear model. The Lineweaver-Burk linear plot for MnO_2-nanozymes mediated reaction is represented in Figure 3. Based on this plot, a K_m of 1.6 mM and a V_{max} of 47 nM s^{-1} were provided for MnO_2-nanozymes mediated reaction.

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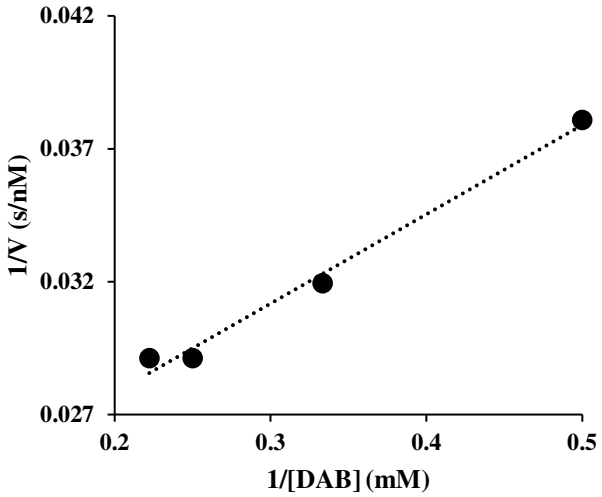

Figure 3. Lineweaver-Burk linear plot for MnO_2-nanozymes mediated reaction.

Besides, to explore more precise on the kinetic performances of gold-nanozymes toward DAB oxidation, the Lineweaver–Burk plot was also constructed for gold-nanozymes mediated reaction for accurate estimation of K_m and V_{max} of the gold enzymes-mediated oxidation reaction. The results are shown in Figure 4, exhibiting a V_{max} of 185 nM s^{-1} and a K_m of 0.72 mM for gold-nanozymes mediated reaction. The ratio of V_{max}(gold)/V_{max}(MnO_2) was found to be about 4.0 which pointed that the catalytic efficiency of gold-nanozymes is 4.0-fold higher than the catalytic efficiency of MnO_2-nanozymes. The K_m value was found to be 0.72 mM and 1.6 mM for the as-prepared gold- and MnO_2-nanozymes, respectively, and the K_m of MnO_2-nanozymes is 2.2-fold higher than that of gold nanozymes. Since, the Km shows the affinity of substrate for binding to nanozyme active nodes (lower K_m=higher affinity), it is consultable that the substrate affinity toward MnO_2-nanozymes is 2.2-fold lower than that of the gold-nanozymes.

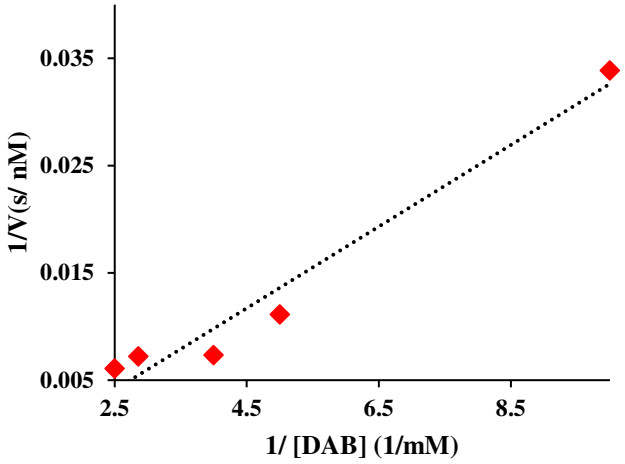

Figure 4. Lineweaver-Burk linear plot for gold-nanozymes mediated reaction.

4. Conclusions

In this study, a comparative study on the kinetics performances of gold- and MnO_2- nanozymes. The kinetic studies were performed using the Michaelis–Menten model for both gold- and MnO_2-nanozymes. The kinetic parameters including K_m and V_{max} were calculated via the construction of the linear plot of Lineweaver–Burk for both nanozymes. The results showed a V_{max} and K_m of 185 nM sec-

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Acknowledgments: The authors gratefully thank the Hormozi Laboratory of Chemistry and Biochemistry for the support of this work.

Conflict of interest: None.

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