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Research Article

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BSA-Stabilized Gold-Nanozymes Reveal 4-Order Higher Catalytic Efficiency and 2-Fold Higher Substrate Affinity than Mno_2-Nanozymes

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Abstract

In this study, the catalytic efficiency and substrate affinity of BSA-stabilized gold-nanozymes and MnO_2-nanozymes were quantified via kinetics studies. In this regard, BSA-stabilized gold-nanozymes and MnO_2-nanozymes were synthesized by simple and green methods and then characterized by TEM and DLS analysis. Both nanozymes revealed semi-special morphology, however, the BSA-stabilized gold-nanozymes showed a small mean size of 13.0 nm while the MnO_2-nanozymes had a mean size of 100.0 nm. The kinetic studies were carried out using the Michaelis–Menten kinetics model for both BSA-stabilized gold-nanozymes and MnO_2-nanozymes and then the kinetic parameters of nanozymes (K_m and V_{max}) were estimated using the linear plot of Lineweaver–Burk for both nanozymes. The results exhibited a V_{max} of 185 nM sec^{-1} and 47 nM sec^{-1} for the BSA-stabilized gold-nanozymes 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 BSA-stabilized gold-nanozymes and MnO_2-nanozymes, respectively. The K_m of MnO_2-nanozymes is 2.2-fold higher than that of BSA-stabilized gold-nanozymes, since the K_m 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 BSA-stabilized gold-nanozymes. Considering the above results, the as-prepared BSA-stabilized gold nanozymes are very stronger peroxidase-like mimics than the metal oxide MnO_2-nanozymes.

Keywords: MnO_2-Nanozymes; BSA-Stabilized Gold-Nanozymes; Kinetics Studies; Catalytic Efficiency; Substrate Affinity

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 the enzyme immobilization, the fast advancement of the field of material science and nanochemistry leads to develop novel nanoscale materials such as MOFs and ZSM-5@Al-MCM nanocatalysts carbon dots magnetic nanoparticles and silver nanoparticles [5-11]. Among these nanoparticles, a wide variety of the introduced nanomaterials reveal excellent enzyme-like activity for example Fe_2O_3/Au hybrid nanozyme silver nanoparticles Pt nanozyme Fe/Cu single-atom nanozymes NEQC-340 BiOI-NFs gold nanoclusters MnO_2 nanoparticles unmodified silver nanoparticles and SiO_2-Fe_3O_4 nanoparticles which had been used for analytical sensing and biosensing water treatment food analysis gold nanoparticles and organic dye degradation [12-29]. Recently, the excellent peroxidase-like activity of gold nanozymes attracted good attention for application as alternatives to natural peroxidase [20, 21, 28,30]. 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 nanozymes instead of the native enzymes in biocatalysis is strongly dependent on their catalytic performances which can be determined by kinetic studies. In this study, the catalytic efficiency and substrate affinity of BSA-stabilized gold-nanozymes and MnO_2-nanozymes were quantified via kinetics studies. In this regard, BSA-stabilized

gold-nanozymes and MnO_2-nanozymes were synthesized by simple and green methods and then characterized by TEM and DLS analysis. The kinetic studies were carried out using the Michaelis–Menten kinetics model for both BSA-stabilized gold-nanozymes and MnO_2-nanozymes and then the kinetic parameters of nanozymes (Km and Vmax) were estimated using the linear plot of Lineweaver–Burk for both nanozymes. Considering the results of this work, the as-prepared BSA-stabilized gold nanozymes are very stronger peroxidase-like mimics than the metal oxide MnO_2-nanozymes.

2. Experimental

2.1 Materials

Bovine serum albumin (BSA), phosphoric acid, $KMnO_4$, NaOH, and hydrazinium hydroxide were from Merck Company. $HAuCl4.4H_2O$ and 3,3'-diaminobenzidine were obtained from Sigma Aldrich company.

2.2 Instrumentation

All UV-Vis measurements for estimation of kinetics parameters of nanozymes were carried out using a CT Chorm Tech UV 3300 spectrophotometer. The TEM images of both BSA-stabilized nanozymes and MnO_2-nanozymes were recorded using a transmission electron microscope (Zeiss, model EL10C). The pH measurements for adjusting the pH of solutions to the desired values were carried out using an ATC portable pH meter.

2.3 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.

2.4 Synthesis of BSA-stabilized Gold-nanozymes

To do synthesis the BSA-stabilized 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.

2.5 Steady-State Kinetics Studies

The catalytic efficiency and substrate affinity of both BSA-stabilized nanozymes and MnO_2-nanozymes were evaluated via 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 molar extinction coefficient of ε=5500 mol_{-1} cm_{-1}.

3. Results and Discussion

3.1 Characterization of BSA-Stabilized Gold-nanozymes

The as-prepared BSA-stabilized gold-nanozymes were synthesized via a simple, high throughput and green method at physiological temperature and then characterized for their morphological properties and size by TEM imaging method and DLS analysis, in order. The morphological properties and the mean size of the as-prepared nanozymes were investigated by the TEM imaging method. To do this, the TEM image of the as-prepared nanozymes was recorded. The results are shown in Figure 1A, as shown in this figure, the as-prepared nanozymes show a semi-spherical morphology with uniform particles. It is mentionable that, the uniformity of the particles of the as-prepared nanozymes is a significant advantage from an enzymatic point of view because the uniform particles showed higher enzyme-like activity than the particles with low uniformity. Besides, the results showed that the as-prepared nanozymes have a narrow size distribution of 7.7-18.3 nm with a mean size of about 13.2 nm which makes them suitable for enzyme-mimicking applications because the size of nanozymes can strongly affect their enzyme-like activity. For exploring more precise size reporting of the as-prepared nanozymes, the DLS analysis was performed. The results are shown in Figure 1B, as shown in this Figure, the as-prepared nanozymes show a size distribution over 7.3-.31.3 nm with a mean diameter of 13.16 nm. However, the maximum of particles has a size in the range of 10.5-17.2 nm. Besides, the mode of the particles has a size of about 13.0 nm. Notably, the results of the DLS analysis (mean size of 13.16 nm) are close to those of the TEM imaging method (mean size of 13.2 nm).

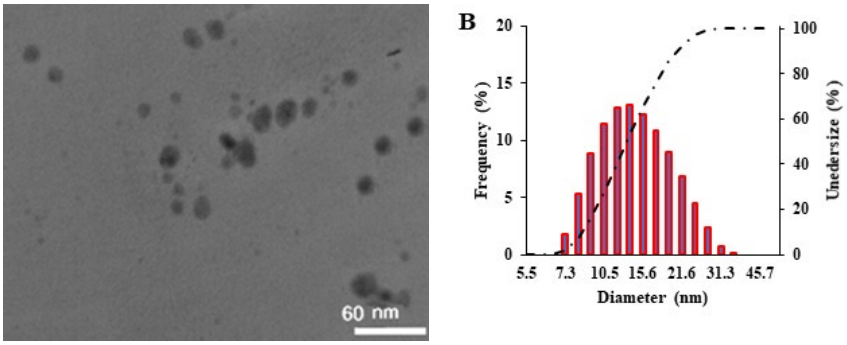

Figure 1: (A) TEM image and (B) DLS pattern of the as-prepared BSA-stabilized gold-nanozymes.

3.2 Characterization of MnO_2-nanozymes

The size and morphological properties of MnO2 nanoparticles were determined using DLS and TEM imaging methods, respectively. For the evaluation of the morphological properties and particle size of these nanozymes, the TEM imaging method was carried out. The results shown in Figure 2A revealed that the as-prepared MnO_2-nanozymes have a semi-spherical morphology along with an average size as small as 100.0 nm. Besides, for estimation of size distribution along with exploding more accuracy and precision about the calculation of the average size of the as-prepared MnO_2-nanozymes, the DLS analysis was also performed. The histogram of the particle size as a function of frequency and undersize is shown in Figure 2B. The results shown in Figure 2B revealed that the as-prepared nanozymes have a size distribution over 64-171 nm with an average size of 109 nm.

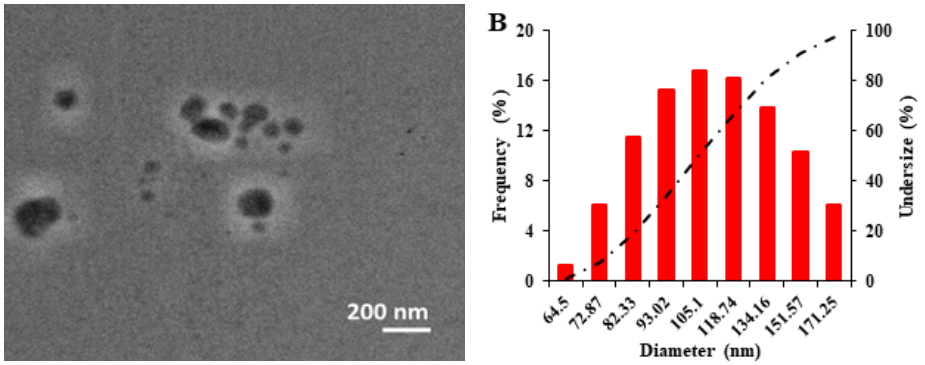

Figure 2: (A) TEM image and (B) DLS results of the as-synthesized MnO2-nanozymes.

3.3 Evaluation of Catalytic Efficiency and Substrate Affinity of Nanozymes via Kinetics Studies

Kinetic studies were carried out to estimate the kinetic parameters (i.e., Km and Vmax) of the as-prepared MnO_2-nanozymes and BSA-stabilized gold nanozymes as the pseudo-peroxidase nanoenzyme toward n-electron irreversible oxidation of 3,3'-diaminobezedine. It is well known that the Vmax 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 Vmax 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 Km value, the lower values indicate a higher affinity of the substrate for binding to the enzyme/nanozyme.

3.3.1 Kinetic Performances of MnO_2-Nanozymes

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 3A. As seen in Figure 3A, 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. Due to the inaccuracy of the results of non-linear saturation curves, to explore more precise on the estimation of 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 3B. Based on this plot, a Km of 1.6 mM and a Vmax of 47 nM s^{-1} were provided for MnO_2-nanozymes mediated reaction.

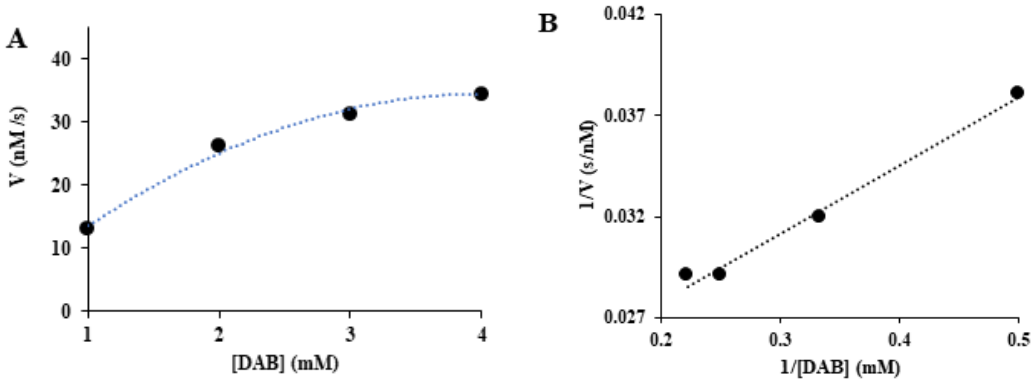

Figure 3: (A) Michaelis–Menten curve and (B) Lineweaver-Burk linear plot for MnO_2-nanozymes mediated reaction.

3.3.2 Kinetic Performances of BSA-stabilized Gold Nanozymes

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 4A. As seen in Figure 4A, 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 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 4B, exhibiting a *Vmax* of 185 nM s^{-1} and a K_m of 0.72 mM for gold-nanozymes mediated reaction.

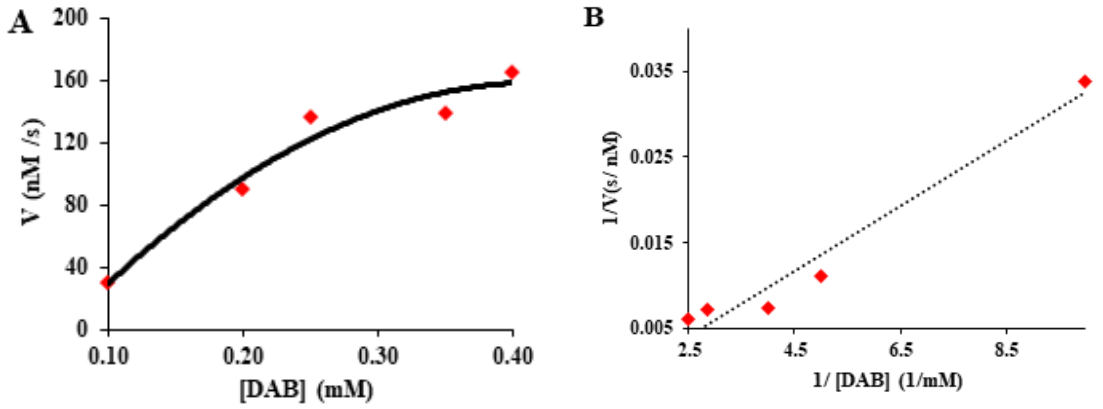

Figure 4: (A) Michaelis–Menten curve and (B) Lineweaver-Burk linear plot for BSA-stabilized gold-nanozymes-mediated reaction.

3.3.3 Comparison of Kinetic Features of Two Nanozymes

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 Km of MnO_2-nanozymes is 2.2-fold higher than that of gold nanozymes. Since the K_m 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.

4. Conclusions

In this study, the catalytic efficiency and substrate affinity of BSA-stabilized gold-nanozymes and MnO_2-nanozymes were quantified via kinetics studies. In this regard, BSA-stabilized gold-nanozymes and MnO_2-nanozymes were synthesized by simple and green methods and then characterized by TEM and DLS analysis. Both nanozymes revealed semi-special morphology, however, the BSA-stabilized gold-nanozymes showed a small mean size of 13.0 nm while the MnO_2-nanozymes had a mean size of 100.0 nm. The kinetic studies were carried out using the Michaelis–Menten kinetics model for both BSA-stabilized gold-nanozymes and MnO_2-nanozymes and then the kinetic parameters of nanozymes (K_m and V_{max}) were estimated using the linear plot of Lineweaver–Burk for both nanozymes. The results exhibited a Vmax of 185 nM sec^{-1} and 47 nM sec^{-1} for the BSA-stabilized gold-nanozymes 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 BSA-stabilized gold-nanozymes and MnO_2-nanozymes, respectively. The *Km* of MnO_2-nanozymes is 2.2-fold higher than that of BSA-stabilized 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 BSA-stabilized gold-nanozymes. Considering the above results, the as-prepared BSA-stabilized gold nanozymes are very stronger peroxidase-like mimics than the metal oxide MnO_2-nanozymes.

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Conflict of Interest

None.

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