Biochemical and Kinetics Properties of MnO2 Nanozymes Toward n-Electron Irreversible Oxidation of 3,3'-Diaminobenzidine

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Article

Biochemical and Kinetics Properties of MnO_2 Nanozymes Toward n-Electron Irreversible Oxidation of 3,3′-Diaminobenzidine

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Abstract: MnO_2 nanoparticles are considered nanozymes with intrinsic peroxidase-like activity and there are several reports on their application in the field of nanozyme-based sensing and detection, however, up to now, there is no report on the investigation of their biochemical and kinetics properties toward enzyme-mediated oxidations. Hence, in this work, the MnO_2 nanozymes were synthesized and characterized with different characterization methods. Thereafter, their biochemical properties including pH stability, thermal stability, and salt stability as well as their kinetics perfromances were evaluated toward n-electron irreversible oxidation of 3,3′-diaminobezedine. The results showed that the as-prepared nanozymes reveal their maximal enzyme-like activity over a wide pH range of 3.0-6.0 at a temperature of 23-25 °C toward oxidation of 3,3′-diaminobezedine. Besides, the salt stability studies exhibited that the as-mentioned nanozymes can save their maximal activity over a wide range of high salt concentrations over 3-7 M of NaCl. Moreover, the kinetics studies revealed a K_m as low as 1.6 mM and a V_{max} as high as 47 nM sec^{-1} for the MnO_2 nanozymes toward irreversible oxidation of DAB to produce the brown-colored polyDAB.

Keywords: MnO_2 nanozyme; brown-colored polyDAB; pH stability of nanozymes; thermal stability of nanozymes; kinetics of nanozymes

1. Introduction

The fast development of nanoscience and material chemistry has increased interest in researching new and innovative synthesis methods to produce new nanomaterials with unique catalytic activity [1, 2], unique optical properties [3], high active area [4], antibacterial properties [5], and high biocompatibility [6]. The new field of nanozyme-based catalysis, which has been introduced as an alternative to enzyme-based catalysis, is called nanozyme chemistry. On the other hand, nanozymes are known as nanomaterials with high enzyme-like activity and can be used to simulate enzymatic reactions in harsh environmental conditions (for example, higher temperature or wider pH range) [7-10]. In fact, natural enzymes show several weaknesses as follows [11, 12]; (I) low stability (narrow thermal range and pH range), (II) difficulty in recovery, and (III) inability to reuse the enzyme in reactions, especially industrial reactions. Commonly, to overcome these problems, enzyme immobilization has been considered [13, 14]. Although enzyme immobilization can enhance enzyme stability, however, the immobilized enzymes reveal very lower activity than the native enzymes due to the enzyme inactivation during the immobilization process [15]. Hence to solve these difficulties, the design and development of low-cost nanozymes with high stability and quasi-enzymatic activity were considered an interesting way for performing enzyme-catalyzed reactions in harsh conditions [16-18]. Recently, nanozyme-based systems had been used for several applications in the field of catalysis [19, 20], biomedical imaging [21], tumor therapy [22, 23], and sensing and detection [24-26].

Manganese dioxide (MnO_2) has been utilized for developing different catalytic systems, for instance, chemical, electro-catalytical, and photocatalytic systems due to its higher catalytic activity than other transition metal oxides as well as its low cost, high stability, and nontoxicity [27, 28]. However, the MnO_2 nanoparticles are known for their high enzyme-like activity with dual oxidase- and peroxidase-like activity which make them suitable nanozymes for sensing applications [29-33].

Considering the above-mentioned literature, MnO_2 nanoparticles are considered nanozymes with intrinsic peroxidase-like activity and there are several reports on their application in the field of nanozyme-based sensing and detection, however, up to now, there is no report on the investigation of their biochemical and kinetics properties toward enzyme-mediated oxidations. Hence, in this work, the MnO_2 nanozymes were synthesized and their biochemical properties including pH stability, thermal stability, and salt stability were evaluated toward n-electron irreversible oxidation of 3,3'-diaminobezedine. Besides, the kinetics performances of the as-prepared nanozymes toward DAB oxidation were also investigated.

2. Methods

2.1. Synthesis of MnO_2 nanozymes

To synthesize MnO_2 nanozymes, 150.0 mg $KMnO_4$ was dissolved in 15.0 mL of deionized water. Then, 150.0 µL of 30% hydrogen peroxide and 75.0 µL of 80% hydrazinium hydroxide were introduced into the solution followed by 5 min stirring. After 2.0 min, the brown precipitate was collected and then washed five times with deionized water.

2.2. Nanozyme activity assay

A mixture of 40 µL hydrogen peroxide, 0.5 mL of DAB (final concentration of 2.8 mM), and 40 µL of MnO_2-nanozymes (final concentration of 0.015 mg mL^{-1} in the mixture) was added to 2.0 mL of 0.4 M acetate buffer (pH= 4.0). After 30 min, the UV-Vis spectra against a reagent blank were recorded at 460 nm. It should be noted that the specific activity of the as-mentioned nanozymes (nM s^{-1}) was calculated using the absorption coefficient of the oxidation product at 460 nm (ε=5500 M^{-1} cm^{-1}). Afterward, the relative activity of the MnO_2 nanozymes was calculated using equation 1 [34]:

$$\text{Relative activity} = \text{activity}/(\text{maximum activity}) \times 100 \quad (1)$$

2.3. pH and thermal effect

The effect of pH on the nanozyme activity was determined by probing their activity over a pH range of 2.0-9.0. Afterward, the relative activity was calculated for each pH using equation 1, and the plot of activity as a function of pH was used as an index for pH stability measurements. Besides, the thermal stability of the as-mentioned nanozymes was investigated by calculating their activity after incubation at different temperatures for 30.0 min.

2.4. Salt stability

The nanozyme stability against high salt concentrations as a serious problem of native enzymes was evaluated by recording their nanozymatic activity in reaction media with high salt concentration over 3-7 M. It is notable that NaCl was used as a model salt for this experiment.

2.5. Kinetics studies

The kinetics studies were performed by measuring the activity of the as-mentioned nanozymes as a function of DAB concentrations based on the Michaelis–Menten model. Afterward, the kinetic parameters, V_{max} and K_m were estimated by using the linear plot of Lineweaver–Burk.

3. Results and discussion

3.1. Characterization of MnO_2 nanozymes

The size and morphological properties of MnO_2 nanoparticles were determined using DLS and scanning electron microscopy imaging methods, respectively. In this regard, the SEM image of the prepared MnO_2 nanozymes was recorded. The results shown in Figure 1, revealed that the as-

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prepared MnO_2 nanozymes have uniform and small size particles. However, the SEM image cannot provide any useful information on their size distribution. Hence, for the estimation of size distribution and calculation of the average size of these nanozymes, the DLS analysis was performed. The results shown in Figure 2, revealed that the as-prepared nanozymes have a size distribution over 64-171 nm with an average size of 109 nm.

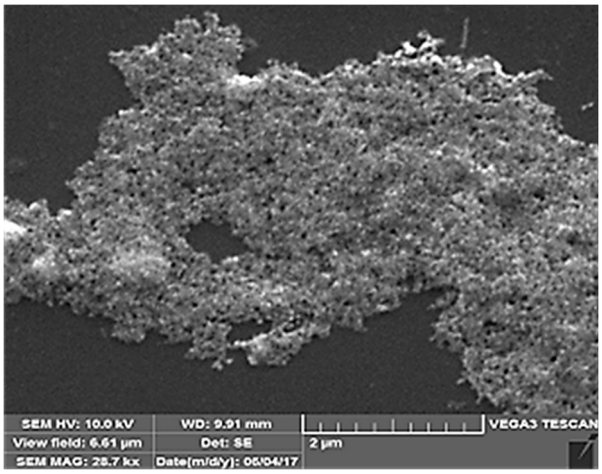

Figure 1. SEM image of the as-prepared MnO_2 nanozymes.

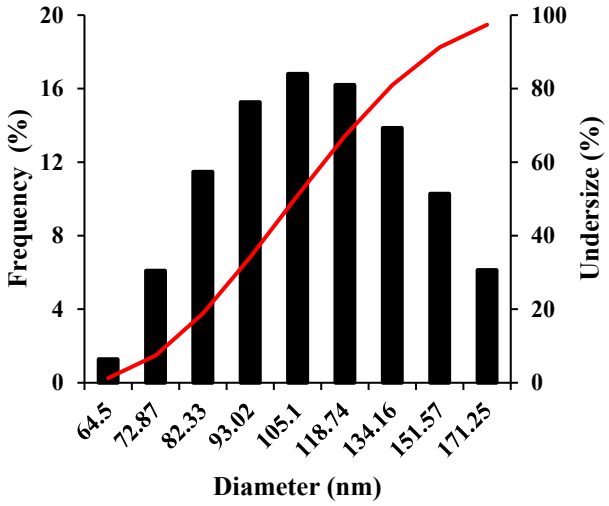

Figure 2. DLS results of the as-prepared MnO_2 nanozymes.

The crystalline properties of the synthesized MnO_2 nanozymes were investigated using XRD analysis. The results of this analysis are shown in Figure 3. As seen in this figure, the results of X-ray diffraction analysis indicate the presence of two characteristic peaks of MnO2 at the diffraction angles of 23.66 and 60.11 which are assigned to (101) and (312) plans of MnO_2, in order.

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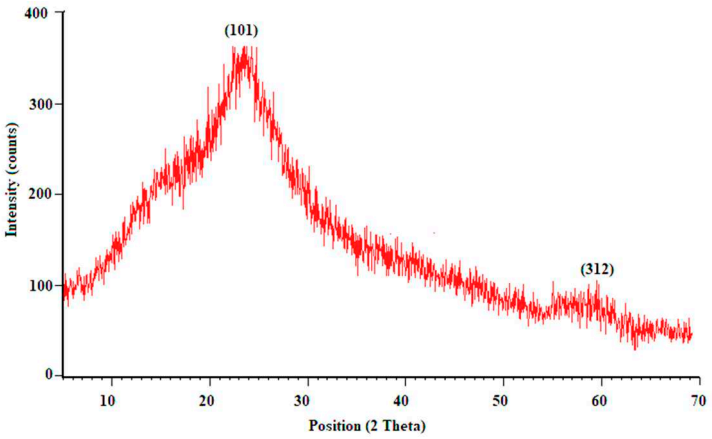

Figure 3. XRD pattern of the as-prepared MnO_2 nanozymes.

3.2. Investigation of nanozymatic behavior

The pseudo-peroxidase activity of MnO_2 nanoparticles was investigated using DAB as a peroxidase substrate and its brown-colored oxidation product (i.e. polyDAB) as an analytical probe system (Figure 4). As seen in Figure 4, in the presence of DAB, the synthesized MnO_2 nanozymes catalyze the oxidation process of DAB with hydrogen peroxide to form its corresponding brown-colored indamine polymer (polyDAB) with a maximum absorbance at 460 nm. In fact, during the oxidation of DAB, MnO_2 nanozymes act on hydrogen peroxide molecules and produce active hydroxyl radicals [24, 26, 31, 33]. Then the generated radicals react with DAB molecules to produce the DAB cation (DAB^+). The DAB^+ then reacts with a DAB molecule to produce a DAB dimer ($(DAB)_2$). By proceeding with this cycle, finally, an indamine polymer was produced as the final product of DAB oxidation, as reported [24, 26].

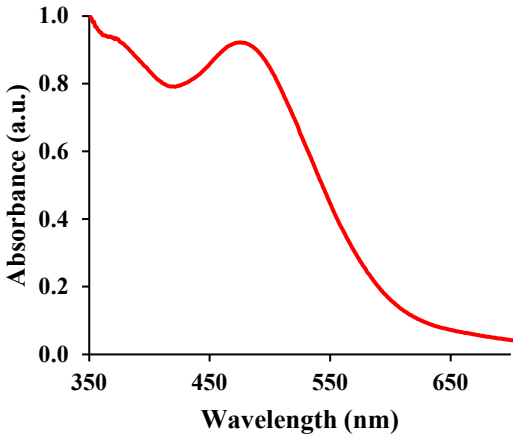

Figure 4. The UV-Visible spectrum of oxidation product ofMnO_2 nanozymes-mediated oxidation of DAB.

3.3. pH stability

The effect of pH on the nanozyme activity was determined by probing their activity over a pH range of 2.0-9.0. In fact, this experiment can provide insights into the stability of these nanozymes against environmental pH changes. The results are shown in Figure 5. According to these results, the maximum nanozyme activity of these nanozymes was estimated over a wide pH range of 3.0-6.0. It should be noted that at harsh acidic conditions (pH=2) and harsh basic conditions (pH=9.0), the as-

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mentioned nanozymes saved 82% and 71% of their maximal activity, in turn, pointing to their high pH stability.

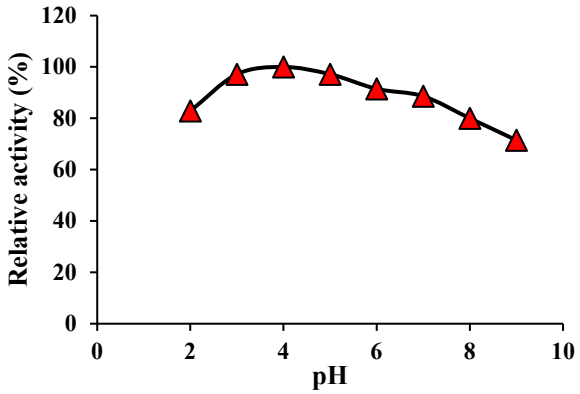

Figure 5. The pH stability of the as-prepared MnO_2 nanozymes.

3.4. Thermal stability

The thermal stability of the as-mentioned nanozymes was evaluated by measuring the relative activity of nanozymes over the temperature range of 23-40 °C. The results can obtain useful information about both the optimal temperature range of MnO_2 nanozymes and their stability against environmental temperature variations. The results are shown in Figure 6, according to this figure, the maximum nanozyme activity was estimated at a temperature range of 23-25 °C and then it was decreased by increasing the temperature.

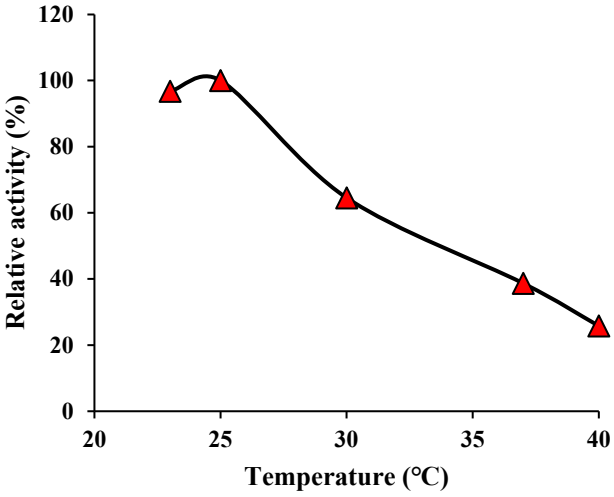

Figure 6. The Thermal stability of the as-prepared MnO_2 nanozymes.

3.5. Salt stability

The nanozyme stability against high salt concentrations as a serious problem of native enzymes was evaluated by recording their nanozymatic activity in reaction media with high salt concentration over 3-7 M of NaCl. The results shown in Figure 7, revealed that the as-mentioned nanozymes can save their maximal activity over a wide range of high salt concentrations over 3-7 M of NaCl. Based on the above results it can be concluded that the as-prepared MnO_2 nanozymes can be used for catalyzing the peroxidase-mediated oxidation reactions at high salt concentrations without any decrease in catalytic efficiency and nanozymatic activity instead of the unstable native peroxidase.

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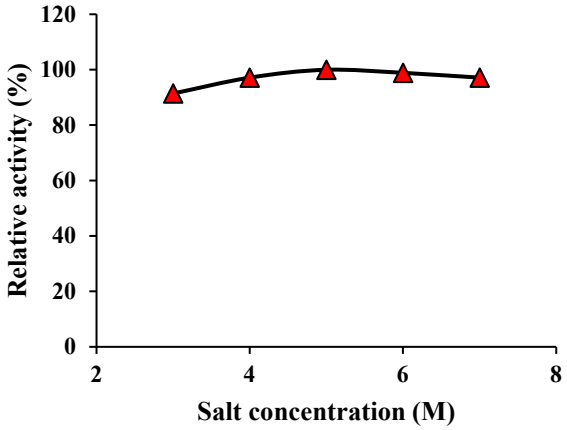

Figure 7. The salt stability of the as-prepared MnO_2 nanozymes.

3.6. Kinetics studies

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 enzyme/nanozyme-catalyzed reaction (i.e., catalytic efficiency) when all enzyme molecules or all nanozyme particles are saturated with the substrate [34, 35]. 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 [34, 35]. 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 and the Lineweaver-Burk linear plot for the as-mentioned nanozymes were shown in Figure 8. As seen in Figure 8A, the reaction rate was increased by increasing the DAB concentration and then reached a saturation state after a certain substrate concentration. Besides, Lineweaver-Burk linear plot (Figure 8B) provided a K_m as low as 1.6 mM and a V_{max} as high as 47 nM sec^{-1} for the MnO_2 nanozymes toward irreversible oxidation of DAB to produce brown-colored polyDAB.

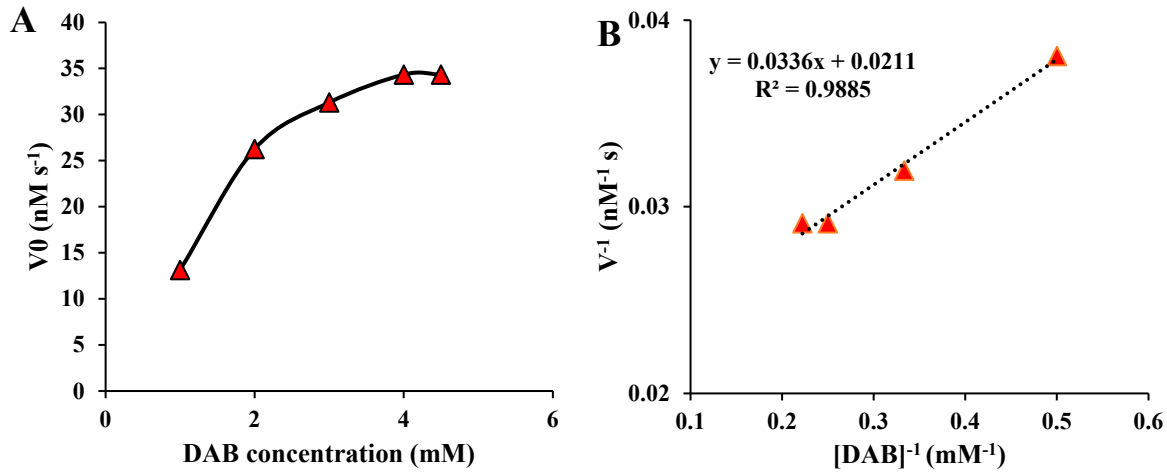

Figure 8. (A) Michaelis-Menten saturation curve and (B) Lineweaver-Burk linear plot for the MnO_2 nanozymes.

4. Conclusions

It is well-known that MnO_2 nanoparticles are nanozymes with intrinsic peroxidase-like activity. There are several reports on the application of these nanozymes for sensor development, however, up to now, there is no report on the investigation of their biochemical and kinetics properties toward enzyme-mediated oxidations. Hence, in this work, the MnO_2 nanozymes were synthesized and their biochemical properties including pH stability, thermal stability, and salt stability were evaluated. The results showed that the as-prepared nanozymes reveal their maximal enzyme-like activity over a wide pH range of 3.0-6.0 at a temperature of 23-25 ℃ toward oxidation of 3,3′-diaminobezedine as the peroxidase substrate. Besides, the salt stability studies exhibited that the as-mentioned nanozymes can save their maximal activity over a wide range of high salt concentrations over 3-7 M of NaCl. Moreover, the kinetics studies revealed a K_m as low as 1.6 mM and a V_{max} as high as 47 nM sec^{-1} for the MnO_2 nanozymes toward irreversible oxidation of DAB to produce brown-colored polyDAB.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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