A Comparative Study on Kinetics Performances of BSA-gold Nanozymes for Nanozyme-Mediated Oxidation of 3,3',5,5'-Tetramethylbenzidine and 3,3'-Diaminobenzidine

Saeed Reza Hormozi Jangi *

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Article

A Comparative Study on Kinetics Performances of BSA-gold Nanozymes for Nanozyme-Mediated Oxidation of 3,3′,5,5′-Tetramethylbenzidine and 3,3′-Diaminobenzidine

Saeed Reza Hormozi Jangi

Hormozi Laboratory of Chemistry and Biochemistry, Zabol 9861334367, Iran; saeedrezahormozi@gmail.com

Abstract: It is well-known that the peroxidase enzyme catalyzes the oxidation of different substrates such as 3,3′,5,5′-thetramethylbenzidine, and 3,3′-diaminobenzidine, however, the affinity of different substrates to the enzyme and consequently and catalytic performances of the enzyme toward different substrates is not same, resulting in different kinetics indexes. As the BSA-gold nanozymes are considered artificial peroxidase enzymes, hence, the kinetics indexes of these nanozymes should also be varied by varying their substrate. Hence, in this work, a comparative study was performed on the kinetics performances of BSA-gold nanozymes for enzyme-mediated oxidations of 3,3′,5,5′-thetramethylbenzidine, and 3,3′-diaminobenzidine. The results showed that the Km value of BSA-gold nanozymes was 0.03 mM and 0.72 mM toward TMB and DAB, in order, to reveal the higher affinity (lower Km) of TMB for binding to nanozyme active nodes compared to its alternative substrate, DAB. In contrast, the Vmax was found to be 263 nM sec^{-1} and 185 nM sec^{-1} for nanozmye-mediated oxidation of TMB and DAB, respectively. The higher Vmax of the nanozyme-mediated oxidation of TMB revealed that the catalytic efficiency of BSA-Au nanozymes toward TMB oxidation is higher (about 1.5-fold) than that of the DAB oxidation. The difference between the kinetic indexes of TMB and DAB may be related to their different oxidation pathways and their different reactivity. In fact, the DAB oxidizes via an n-electron irreversible oxidation pathway to produce an indamine polymer. While TMB nanozyme-mediated oxidation has occurred upon a 2-electron reversible mechanism for the production of a cation radical. These different pathways resulted in different kinetic performances.

Keywords: kinetics performances; BSA-gold nanozymes; 3,3′,5,5′-thetramethylbenzidine; 3,3′-diaminobenzidine

1. Introduction

Natural enzymes show several disadvantages as follows [1]; (I) low stability (thermal and narrow pH range) (II) difficulty in recovery, and (III) no reusability of the enzyme. Commonly, for overcoming these drawbacks, the enzyme immobilization process has been developed [2–4]. Another approach for overcome to these difficulties is utilizing highly stable nanoparticles with high enzyme-like activity in the enzyme-catalyzed reactions [1,5]. In fact, the recent progress in nanochemistry [17] and material science open a new door for developing high-performance nano-supports such as MOFs [6], catalytic materials [7,8], and nanoparticles with enzyme-like activity [5,9–13].

Nanozymes or nanoparticles with excellent enzyme-like activity are attracted much attention due to their stability and higher efficiency compared to natural enzymes [1,5]. Recently, nanozymes had been used for different applications for instance, analytical sensing of species, biocatalysis of reactions instead of natural enzymes, dye degradation [14], water treatment [15], food quality checking [16] and etc.. Up to now a wide range of these nanomaterials, for example, MnO2 nanoparticles [18], BiOI-NFs [19], silver nanoparticles [20], BSA-Au nanoclusters [21,22], SiO2-Fe3O4 NPs [23], and some MOFs, e.g., NEQC-340 [24], have been used as nanozymes with high pseudo-peroxidation activity. Since nanozymes can catalyze the oxidation of peroxidase substrates to their corresponding colored products, they have been used for analytical purposes [15,16]. For example,

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spectrophotometric measurement of several analytes such as cysteine [25], hydrogen peroxide [26], mercury ions [27], triacetone triperoxide [18], glucose [28], and glutathione [24] was performed by different nanozyme-based sensors. Recently catalytic property of gold nanozymes and their excellent peroxidase-like activity brought them up as safe, green, and high throughput alternatives of natural peroxidase enzymes to developed enzyme-mediated sensors [21,22,27]. Usually 3,3',5,5'-tetramethylbenzidine (TMB), and 3,3'-diaminobenzidine (DAB) substrates have been used as the peroxidase substrates, and their corresponding oxidation products were utilized as the analytical probes for sensing aims [9,11,13,21–24]. However, it is well-known that the affinity of these substrates for binding to enzyme/nanozyme is not the same, resulting in different kinetics indexes. As the BSA-gold nanozymes are considered artificial peroxidase enzymes, hence, the kinetics indexes of these nanozymes should also be varied by varying their substrate.

Hence, in this work, a comparative study was performed on the kinetics performances of BSA-gold nanozymes for enzyme-mediated oxidations of 3,3′,5,5′-thetramethylbenzidine, and 3,3′-diaminobenzidine. To do this, initially, BSA-gold nanozymes were synthesized via a protein-directed method utilizing bovine serum albumin (BSA) as both reductant and stabilizer. Thereafter, the as-prepared nanozymes were characterized by recording their TEM image. Afterward, the Michaelis–Menten model and the Lineweaver–Burk method were utilized for the estimation of kinetic parameters of BSA-gold nanozymes for both DAB and TMB.

2. Experimental Section

2.1. Materials

DAB was obtained from Sigma Aldrich Company. Deionized water was obtained from Zolal Teb chemical company (Iran). Other materials were obtained from Merck Company in their analytical or synthesis grades.

2.2. Instrumentations

A Metrohm 827 pH lab pH meter equipped with a combined glass electrode was applied for buffer preparation. An Ultrospec 4000 UV-Vis spectrophotometer manufactured by Pharmacia Biotech (Biochrom) Ltd. equipped with SWIFT Software was utilized for spectrophotometric assay of nanozyme activity toward oxidation of both DAB and TMB. The size and morphology of the as-prepared BSA-Au nanozymes were characterized by the TEM imaging method utilizing a transmission electron microscope (Zeiss, model EL10C) operated at an accelerating voltage of 80 kV.

2.3. Synthesis of BSA-gold nanozymes

BSA-gold nanozymes were synthesized based on the previously reported method [21], briefly, 5.0 mL of 10.0 mM $HAuCl_4.4H_2O$ solution was introduced to 5.0 mL 50 mg mL^{-1} bovine serum albumin (BSA), followed by stirring at 37 °C and adding 500 µL of 1.0 M NaOH. The solution was incubated at 37 °C for 12 h to synthesize the BSA-gold nanozymes.

2.4. Synthesis of BSA-gold nanozymes

For the DAB assay, 80.0 µL BSA- gold nanozymes were added into 1.3 mL of phosphate buffer solution (pH 7.0, 0.4 M) containing 200.0 µL of DAB (different concentrations) and 40.0 µL of HP (with a final concentration of 0.24 M) and thoroughly mixed at ambient temperature. The oxidation was followed for 25.0 min to complete the production of the corresponding indamine polymer (i.e., polyDAB). Thereafter, the nanozyme activity (nM sec^{-1}) was measured by probing the absorbance of the resulting polyDAB at 460 nm considering a molecular extinction coefficient ε=5500 molar cm^{-1}.

Regarding the TMB assay, 40 µL hydrogen peroxide solution (final concentrations of 0.24 M), 200 µL of TMB (different concentrations), and 80 µL of BSA-gold nanozymes were introduced to 1.3 mL of acetate buffer (0.3 M; pH, 0.4), followed by incubation for about 10 minutes at ambient temperature. After that, the absorbance of the blue-colored TMB-ox at 658 nm was used for nanozyme

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activity (nM sec^{-1}) calculation considering a ε of 39000 M cm^{-1}. Finally, the kinetic parameters of the as-prepared BSA-gold nanozymes for both DAB and TMB were investigated based on Michaelis–Menten equation and the Lineweaver–Burk method as the standard models for evaluation of the enzyme kinetics performances.

3. Results and discussion

3.1. Characterization of BSA-gold nanozymes

BSA-gold nanozymes were synthesized via a protein-directed method utilizing bovine serum albumin (BSA) as both reductant and stabilizer. Thereafter, the as-prepared nanozymes were characterized by recording their TEM image (Figure 1). The results showed that the as-prepared BSA-gold nanozymes have a size distribution of 7.7-18.3 nm with a mean size of about 13.2 nm. As can be seen from Figure 1, the as-prepared BSA-gold nanozymes reveal semi-spherical morphological properties with an approximately narrow size distribution which makes them suitable for enzyme-mimicking applications, considering this fact that the size of nanozymes can strongly affect their enzyme-like activity [21].

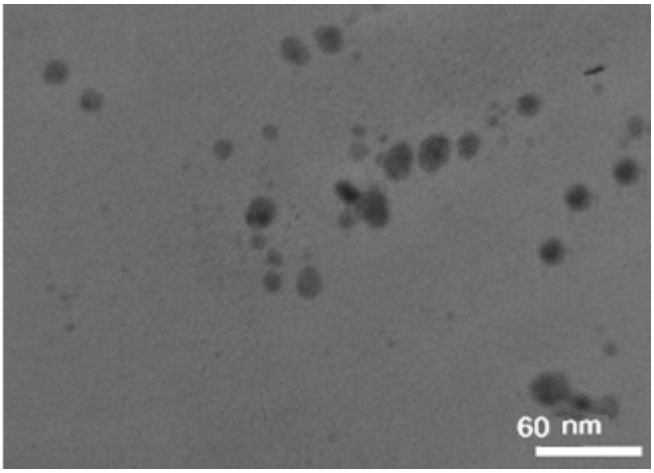

Figure 1. TEM image of as-prepared BSA-gold nanozymes.

3.2. Kinetics parameters of BSA-gold nanozyme for TMB oxidation

The kinetics studies of the as-prepared BSA-gold nanozymes were performed by measuring their activity as a function of DAB or TMB (i.e., enzyme-substrate) concentrations. The kinetic parameter, Vmax and Km was then calculated by using Michaelis–Menten and the linear plot of Lineweaver–Burk for both substrates. The Michaelis–Menten plot for oxidation of TMB catalyzed by BSA-gold nanozymes was shown in Figure 2A, revealing that the rate of nanozyme-mediated oxidation of TMB was increased by increasing the substrate concertation and then leveled off. To estimate the kinetic parameters of BSA-gold nanozymes toward TMB oxidation, the Lineweaver–Burk plot was constructed (Figure 2B). Based on Figure 3B, the V_{max} and K_m of the as-prepared nanoymes toward TMB oxidation were calculated at about 263 nM sec^{-1} and 0.03 mM, in order.

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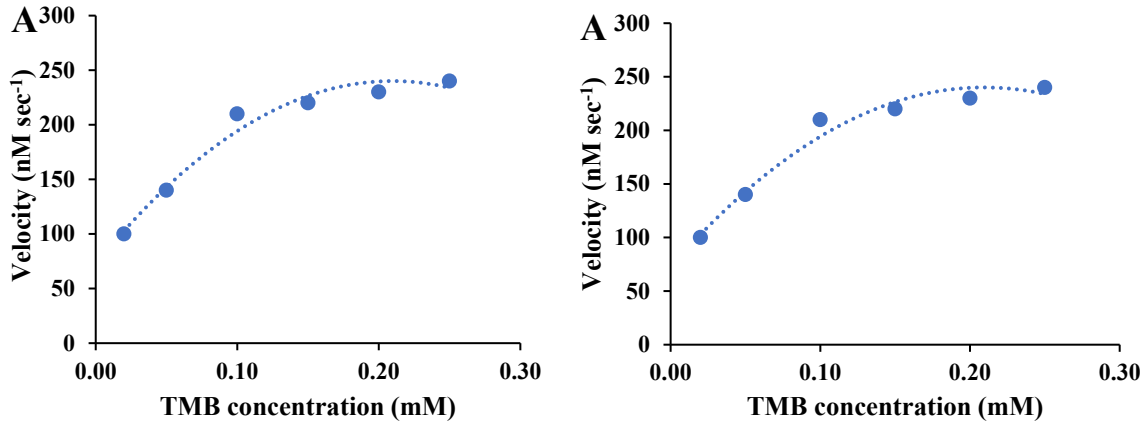

Figure 2. Kinetic performances of BSA-Au nanozymes toward TMB oxidation, (A) Michaelis–Menten plot and (B) Lineweaver–Burk plot.

3.3. Kinetics parameters of BSA-gold nanozyme for DAB oxidation

To evaluate the kinetics performances of the as-prepared BSA-gold nanozymes for DAB oxidation, the Michaelis–Menten plot for DAB oxidation by hydrogen peroxide in the presence of the BSA-gold nanozymes as the peroxidase-mimicking agents were obtained, and the results are shown in Figure 3A, revealed that the rate of nanozyme-mediated DAB oxidation was increased by increasing the substrate concertation and then leveled off which is same of the TMB oxidation but the TMB oxidation rate was found to be higher than that of the DAB oxidation by BSA-gold nanozymes. To explore more precise the kinetic performances of BSA-gold nanozymes toward DAB oxidation, the Lineweaver–Burk plot was constructed for estimation of Km and Vmax of BSA-gold enzymes-mediated oxidation of DAB (Figure 3B), revealing a Vmax and Km of 185 nM sec^{-1} and 0.72 mM, in order, for DAB oxidation.

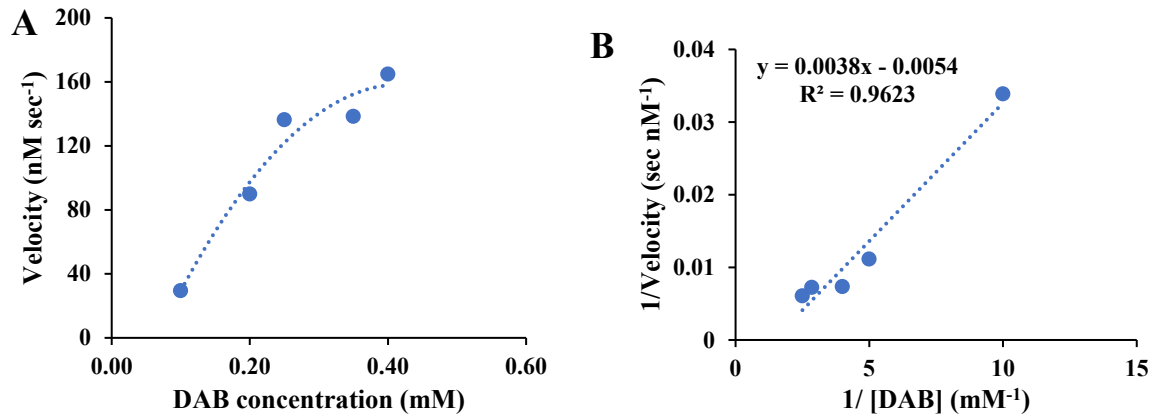

Figure 3. Kinetic performances of BSA-Au nanozymes toward DAB oxidation, (A) Michaelis–Menten plot and (B) Lineweaver–Burk plot.

3.4. Comparison of kinetic performances of BSA-gold nanozymes for different substrates

Table 1 shows the kinetic parameters of the as-prepared BSA-gold nanozymes for both DAB and TMB. As seen in this table, the V_{max} of DAB oxidation was found to be lower than that of the TMB oxidation which pointed to the fact that the catalytic efficiency of the as-prepared BSA-gold nanozymes toward TMB is significantly higher than their efficiency for the DAB. The ratio of V_{max} (TMB)/V_{max} (DAB) was calculated as 1.42, revealing that the catalytic efficiency of the as-prepared BSA-gold nanozymes for TMB oxidation is about 1.5-fold higher than their catalytic performances for DAB oxidation. The difference between the kinetic indexes of TMB and DAB may be related to their different oxidation pathways and their different reactivity. In fact, the DAB oxidizes via an n-

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electron irreversible oxidation pathway to produce an indamine polymer. While TMB nanozyme-mediated oxidation has occurred upon a 2-electron reversible mechanism for the production of a cation radical. These different pathways resulted in different kinetic performances. Besides, the K_m value for DAB was found to be 24-fold higher than that for TMB. It is well-known that the K_m shows the affinity of an enzyme to its substrate which its lower value assigned to a higher affinity [2–4]. Hence, the very higher K_m for DAB reveals the very lower affinity of DAB for binding to BSA-gold nanozyme active nodes compared to its alternative substrate, TMB. This difference can be related to the different reactivity of DAB and TMB.

Table 1. Kinetic parameters of BSA-gold nanozymes for TMB oxidation compared to those for DAB oxidation.

Substrate	Oxidation pathway	K_m (mM)	V_{max} (nM sec^{-1})
DAB	n-electron Irreversible	0.72	185
TMB	2-electron reversible	0.03	263

4. Conclusions

Herein, a comparative study was performed on the kinetics performances of BSA-gold nanozymes for enzyme-mediated oxidations of 3,3′,5,5′-thetramethylbenzidine, and 3,3′-diaminobenzidine. The results showed that the Km value of BSA-gold nanozymes was 0.03 mM and 0.72 mM toward TMB and DAB, in order, to reveal the higher affinity (lower Km) of TMB for binding to nanozyme active nodes compared to its alternative substrate, DAB. In contrast, the Vmax was found to be 263 nM sec^{-1} and 185 nM sec^{-1} for nanozmye-mediated oxidation of TMB and DAB, respectively. The higher Vmax of the nanozyme-mediated oxidation of TMB revealed that the catalytic efficiency of BSA-Au nanozymes toward TMB oxidation is higher (about 1.5-fold) than that of the DAB oxidation. The difference between the kinetic indexes of TMB and DAB may be related to their different oxidation pathways and their different reactivity. In fact, the DAB oxidizes via an n-electron irreversible oxidation pathway to produce an indamine polymer. While TMB nanozyme-mediated oxidation has occurred upon a 2-electron reversible mechanism for the production of a cation radical. These different pathways resulted in different kinetic performances.

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