Comparison of nanozymatic behavior of protein-protected gold and SiO_2@gold nanoparticles

Saeed Reza Hormozi Jangi[1]*

[1]Hormozi Laboratory of Chemistry and Biochemistry, Zabol, 9861334367, Iran

*E-mail for correspondence: saeedrezahormozi@gmail.com (S.R. Hormozi Jangi)

Abstract

Herein, the nanozymatic behavior of protein-protected gold and SiO_2@gold nanoparticles were evaluated and their results compared with each other. The results showed that both protein-protected gold and SiO_2@gold nanoparticles reveal intrinsic peroxidase-like activity. Hence, to precise comparison of nanozymatic behavior of these nanozymes, the kinetic studies were performed using the Michaelis–Menten steady-state kinetics model and the velocity and affinity factors were calculated for both nanozyme and then utilized as a reliable way for comparison of nanozymatic behavior of these nanozymes. The results showed that the V_{max} of protein-protected gold nanoparticles was 12.0-fold higher than that of SiO_2@gold nanoparticles, revealing that the catalytic efficiency of protein-protected gold nanoparticles is 12.0-fold higher than SiO_2@AuNPs nanocomposite. Besides, the K_m value of SiO_2@gold nanoparticles was 2-order higher than that of protein-protected gold nanoparticles, indicating that the substrate affinity toward protein-protected gold nanoparticles is 2.0-order higher than the SiO_2@gold nanoparticles. Based on the results of this work it can be concluded that protein-protected gold nanoparticles are more efficient nanozymes than SiO_2@gold nanoparticles.

Keywords: Nanozyme; Artificial enzymes; Protein-protected gold nanoparticles; SiO_2@gold nanoparticle

1. Introduction

Importance and practical application of nanotechnology in modern life lead to design and synthesis of different nanomaterials with optical [1-3], catalytic [4, 5], anti-cancer features [6], medical [7], or anti-bacterial [8, 9] characteristics such as carbon and metal-based nanoparticles [10, 11], quantum dots [12, 13], metal oxide nanoparticle [14], magnetic nanoparticles [15], and metal-organic frameworks [16, 17], etc. Among various nanoparticles with different properties, recently, nanomaterials with enzyme-like properties, called nanozymes have been widely utilized for catalyzing industrial, clinical, and environmental enzyme-mediated reactions under harsh conditions [18-26]. The most significant advantage of these nanozymes compared to the native enzymes is their lower cost, higher efficiency, and especially, their high cycling stability and recyclability [19, 27-30]. Up to now, different nanoparticles with intrinsic peroxidase-like activity were designed and synthesized, for instance, Mn_3O_4 nanozymes [31], Cu-$CuFe_2O_4$ nanozymes [32], BSA-stabilized manganese dioxide nanoparticles [33], BSA-stabilized manganese phosphate nanoflower [34], carbon nanozymes [35], silica-coated-magnetic nanoparticles [36], MnO_2 nanoparticles [37], Fe_3O_4 nanozymes [38], self-cascade pyrite nanozymes [39], metal-organic frameworks [40], gold nanozymes [41-45], S/N codoped carbon nanozymes [46], and silver nanoparticles [47-52]. Among the different nanomaterials with excellent peroxidase-like activity, gold-based nanozymes have been widely for developing nanozyme-based sensors [53, 54], nanozyme-based cancer treatment [55], and nanozyme-mediated dye degradation [56]. Moreover, since the first report of patients infected with the new infection disease, COVID-19 in 2019 [57, 58], nanozyme-based methods have been developed for fast clinical diagnosis of this pandemic infection [59-62]. Hence, evaluation of their biochemical features and enzyme-like properties is important for developing nanozyme-based systems with better figures of merit. In this regard, the biochemical behavior of enzyme-like nanosilver was also investigated by our research group [63]. Besides, recently, our research group reported a research article on the investigation of biochemical behaviors of BSA-stabilized gold nanoparticles [64, 65].

Herein, the nanozymatic behavior of protein-protected gold and SiO_2@gold nanoparticles were evaluated and their results compared with each other. The results showed that both protein-protected gold and SiO_2@gold nanoparticles reveal intrinsic peroxidase-like activity. Hence, to precise comparison of nanozymatic behavior of these nanozymes, the kinetic studies were performed using the Michaelis–Menten steady-state kinetics model and the velocity and affinity factors were calculated for both nanozyme and then utilized as a reliable way for comparison of nanozymatic behavior of these nanozymes. Based on the results of this work it can be concluded that protein-protected gold nanoparticles are more efficient nanozymes than SiO_2@gold nanoparticles.

2. Experimental

2.1. Synthesis of nanomaterials

For the synthesis of protein-protected gold nanoparticles, 10.0 mM $HAuCl_4.4H_2O$ (5.0 mL) was introduced to 50 mg mL^{-1} bovine serum albumin (BSA; 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. For synthesis, the SiO_2/gold nanoparticles, in a typical experiment, 1.0 mL of $HAuCl_4$, 10.0 µL tetrakis(hydroxymethyl)phosphonium chloride, and 500.0 µL NaOH (2.0 M) were introduced in 50.0 mL deionized water. The resulting mixture was stirred for about 1.0 hour to prepare the colloidal gold solution. To synthesize the amino-functional SiO_2 NPs, 1.5 mL of TEOS was added into 40.0 mL of pure ethanol, followed by the addition of 3.0 mL of NH_4OH. The mixture was stirred for about 20.0 hours. Afterward, 20.0 mg of the resulting precipitate was treated with 600.0 µL of APTES for preparation of the final product. Afterward, 10.0 mg of the amino-functional SiO_2 NPs were incubated with a solution of colloidal gold (prepared in section 2.3) for about 12.0 hours. Afterward, the product was separated from the reaction media upon centrifuge at 10000 rpm for 30.0 min. The resulting product was dispersed in 1 mg mL^{-1} of PVP (stabilizer) aqueous

solution, followed by the addition of 60.0 μL of $HAuCl_4$ (10.0 mM) and 120.0 μL of 10.0 mM ascorbic acid (reducing agent) into the reaction media. The synthesis process was followed by stirring the above-mentioned mixture for about 5 min. Afterward, the resulting SiO_2/gold nanoparticles were collected, washed, and then dried at room temperature.

3. Results and discussion

3.1. Quantification of maxium velocity velocity and affinity factor

Kinetic studies were carried out to calculate the kinetic parameters of the as-prepared SiO_2/gold nanoparticles toward 2-electron reversible oxidation of TMB. In fact, the kinetic parameters of an enzyme were previously well defined with numeric values including affinity constant (K_m) and maximum enzymatic velocity (V_{max}) utilizing the Michaelis–Menten steady-state kinetics model. The V_{max} value reflects the intrinsic properties of an enzyme or nanozyme and is defined as the highest possible rate of the nanozyme-catalyzed reaction when all enzyme molecules or all nanozyme particles are saturated with the substrate which points to the catalytic efficiency of an enzyme or nanozyme. Hence, the higher value of V_{max} for an enzymatic/nanozymatic reaction can be assigned to the higher catalytic efficiency of the enzyme or nanozyme [66-71]. In contrast, the affinity of the substrate of an enzyme or nanozyme for interaction with its active site is represented by the K_m, as reported [66]. In fact, the lower values of K_m pointed to the higher affinity of the substrate for binding to the enzyme/nanozyme active site/nodes [17]. Therefore, to evaluate the kinetics parameters of the as-prepared nanocomposite, the Michaelis–Menten model was obtained. For accurate estimation of K_m and V_{max} of the SiO_2/gold nanoparticles-mediated oxidation reaction, the Lineweaver–Burk plot was also constructed for the SiO_2@gold nanocomposite-mediated reaction (Figure 1). The results revealed a V_{max} of 0.022 μM min^{-1} and a K_m as very low as 0.06 mM for the SiO_2@gold nanocomposite. Considering the low K_m value of the as-synthesized nanocomposite, it

can be concluded that the as-prepared nanocomposite shows high substrate affinity, as reported [17]. Besides, obtaining a V_{max} of 0.022 μM min^{-1}, revealed that the as-prepared nanocomposite has a very good catalytic efficiency.

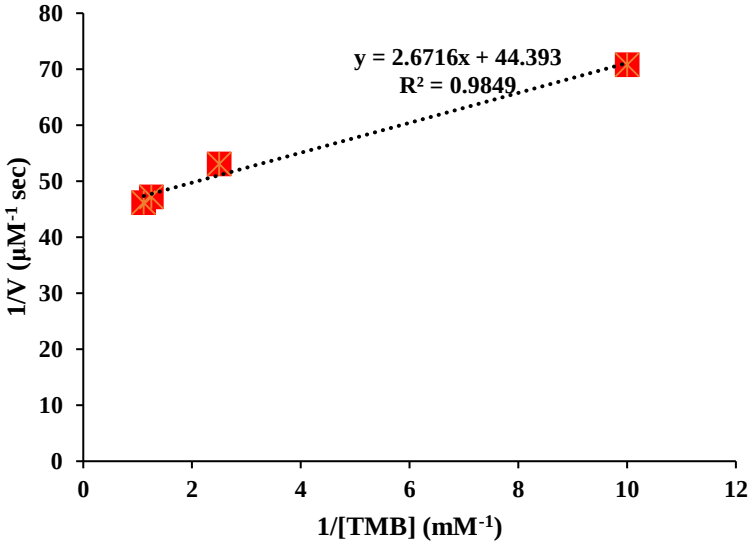

Figure 1. Lineweaver–Burk plot for the as-prepared SiO_2@gold nanoparticles to estimate the kinetic factors of nanozymatic reaction catalyzed by SiO_2@gold nanoparticles.

Besides, the kinetics studies for exploring more precise on reporting of the peroxidase-like activity and enzymatic power of the as-prepared protein-protected gold nanoparticles were carried out by estimating their activity as a function of substrate concentration and then, the standard Lineweaver–Burk plot was provided by plotting the inverse of the velocity of the nanozymatic reaction (V^{-1}) as a function of $[substrate]^{-1}$ for estimating the nanozymatic kinetics parameters. To estimate the kinetic parameters of gold nanozymes toward TMB oxidation, the Lineweaver–Burk plot was constructed

(Figure 2). Considering the results obtained in Figure 2, the V_{max} and K_m of the as-mentioned gold nanozymes were calculated at about 263 nM sec^{-1} and 0.03 mM, in order.

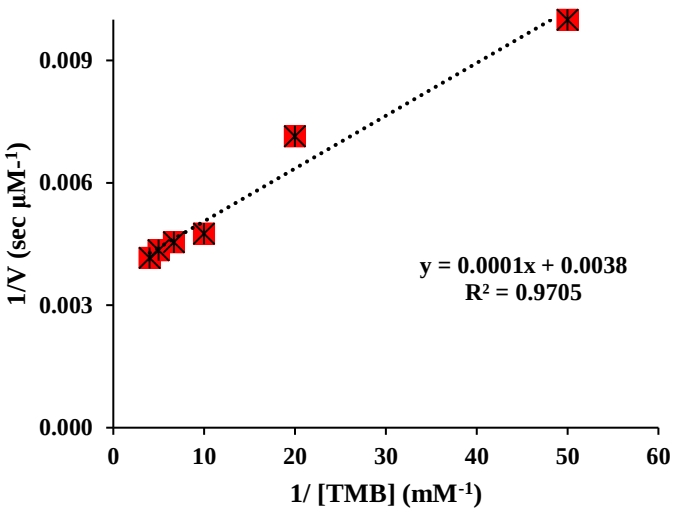

Figure 2. Lineweaver–Burk plot for the as-prepared protein-protected gold nanoparticles to estimate the kinetic factors of nanozymatic reaction catalyzed by protein-protected gold nanoparticles

2. Comparsion of nanozymatic behavior

According to the experimental results of this work, the V_{max} of gold nanoparticles was 12.0-fold higher than that of SiO_2/Au nanocomposite, revealing that the catalytic efficiency of BSA-stabilized gold nanoparticles is 12.0-fold higher than SiO_2/Au nanocomposite. Besides, the K_m value of SiO_2/Au nanocomposite was 2-order higher than that of gold nanoparticles, indicating that the substrate affinity toward gold nanoparticles is 2.0-order higher than the SiO_2/Au nanocomposite. Since, the active nodes of both nanozymes are the same (i.e., gold), the difference between their catalytic efficiency and

affinity can be assigned to their different sizes and the ability of the active nodes to bind the substrate. Based on the results of this work, small-size gold nanoparticles are characteristically more efficient peroxidase mimic materials than the SiO_2/Au nanocomposite.

4. Conclusions

Herein, the nanozymatic behavior of protein-protected gold and SiO_2@gold nanoparticles were evaluated and their results compared with each other. The results showed that both protein-protected gold and SiO_2@gold nanoparticles reveal intrinsic peroxidase-like activity. Hence, to precise comparison of nanozymatic behavior of these nanozymes, the kinetic studies were performed using the Michaelis–Menten steady-state kinetics model and the velocity and affinity factors were calculated for both nanozyme and then utilized as a reliable way for comparison of nanozymatic behavior of these nanozymes. The results showed that the V_{max} of protein-protected gold nanoparticles was 12.0-fold higher than that of SiO_2@gold nanoparticles, revealing that the catalytic efficiency of protein-protected gold nanoparticles is 12.0-fold higher than SiO_2@AuNPs nanocomposite. Besides, the K_m value of SiO_2@gold nanoparticles was 2-order higher than that of protein-protected gold nanoparticles, indicating that the substrate affinity toward protein-protected gold nanoparticles is 2.0-order higher than the SiO_2@gold nanoparticles. Based on the results of this work it can be concluded that protein-protected gold nanoparticles are more efficient nanozymes than SiO_2@gold nanoparticles.

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Conflict of interest

None.

5. References

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