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Determining kinetics parameters of bovine serum albumin-protected gold nanozymes toward different substrates

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Abstract

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, V_{\max} and K_m was then calculated by using Michaelis–Menten and the linear plot of Lineweaver–Burk for both substrates. 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. Besides, the K_m value for DAB was found to be very higher than that for TMB. This difference can be related to the different reactivity of DAB and TMB. as well as their different oxidation mechanism.

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Introduction

It is well known that although native enzymes reveal high specificity toward their substrates and high catalytic performance, they show several disadvantages such as low stability (narrow pH and thermal range); difficult recovery, and no reusability [1]. For overcoming these drawbacks, the enzyme immobilization process has been developed [2]. The enzyme immobilization permits. possible increase in stability (pH, thermal, storage, and solvent performance), easy to recover and handle via simple enzyme separation from the mixtures of reactants and products. Despite great advantages, the specific and relative activities of the most immobilized enzymes are found to be lower than the free enzymes. This decrease in enzyme activity 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 [5] and carbon dots [6], ZSM-5@ Al-MCM nanocatalysts [7], and silver nanoparticles [8]. Among these nanoparticles, a wide variety of the introduced nanomaterials reveal excellent enzyme-like activity [9] for example Fe₂O₃/Au hybrid nanozyme [10], silver nanoparticles [11][12], Pt nanozyme [13], Fe/Cu single-atom nanozymes [14], and unmodified silver nanoparticles [15], MnO₂ nanoparticles [16], BSA-Au nanoclusters [17][18], BiOI-NFs [19], NEQC-340 [20], and SiO₂-Fe₃O₄ nanoparticles [21]. Hence, considering this high enzyme-like activity, the researchers aimed to use these nanomaterials as enzyme alternatives to overcome the difficulties of natural enzymes [1][9]. Recently, nanozymes had been utilized for analytical sensing and biosensing [22], water treatment [23], food analysis [24], and organic dye degradation [21].

Recently excellent peroxidase-like activity of gold nanozymes attracted good attention for application as highly powerful alternatives to natural peroxidase [17][18]. Notably, the most common peroxidase substrates, 3,3',5,5'-tetramethylbenzidine (TMB), and 3,3'-diaminobenzidine (DAB) have been commonly used for quantification of their activity [9]. However, the affinity of different substrates for binding to nanozyme active sites is different from each other, leading to different kinetic performances of a nanozyme toward different substrates.

Herein, the kinetics performances of BSA-gold nanozymes were calculated toward oxidations of both TMB and DAB.

2. Experimental Section

2.1. Materials and instrumentations

All materials were obtained from Merck Company except DAB which provides by Sigma. spectrophotometric assay for kinetics studies was performed using an Ultrospec 4000 UV-Vis spectrophotometer manufactured by Pharmacia Biotech (Biochrom) Ltd. A transmission electron microscope (Zeiss, model EL10C) operated at an accelerating voltage of 80 kV was used for the characterization of the as-prepared nanozymes.

2.2. Nanozyme synthesis protocol

To do synthesis the BSA-protected nanozymes, 10.0 mM HAuCl₄·4H₂O (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. Kinetics measurements

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. 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 activity (nM sec^{-1}) calculation considering a ϵ of 39000 M cm^{-1} . 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 $\epsilon=5500 \text{ molar cm}^{-1}$.

3. Results and discussion

3.1. Nanozyme characterization

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.

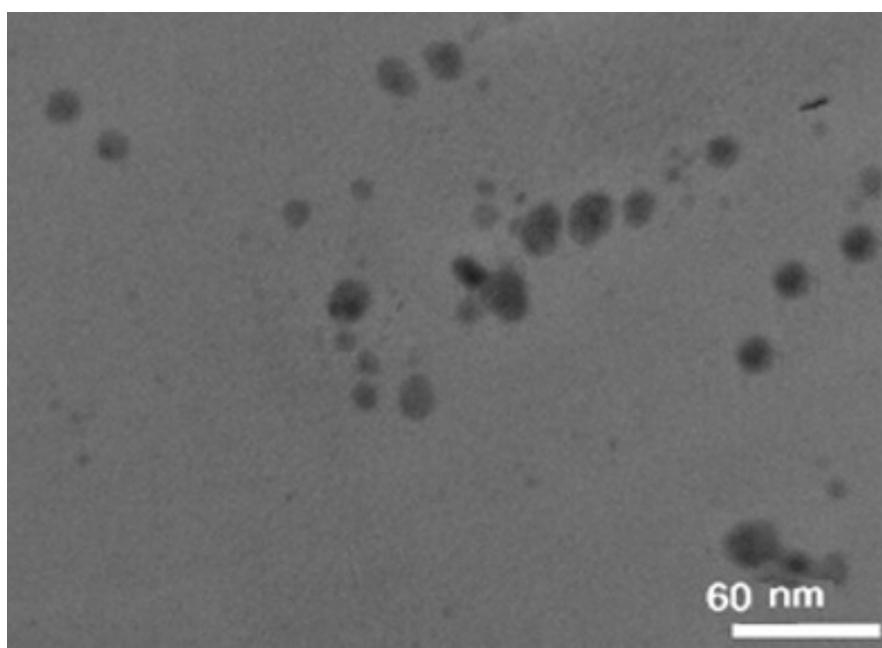


Figure 1. TEM image of the as-prepared BSA-protected nanozymes.

3.2. Kinetics Studies

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, V_{max} and K_m 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 concentration and then leveled off. Besides, 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 2B, revealed that the rate of nanozyme-mediated DAB oxidation was increased by increasing the substrate concentration 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.

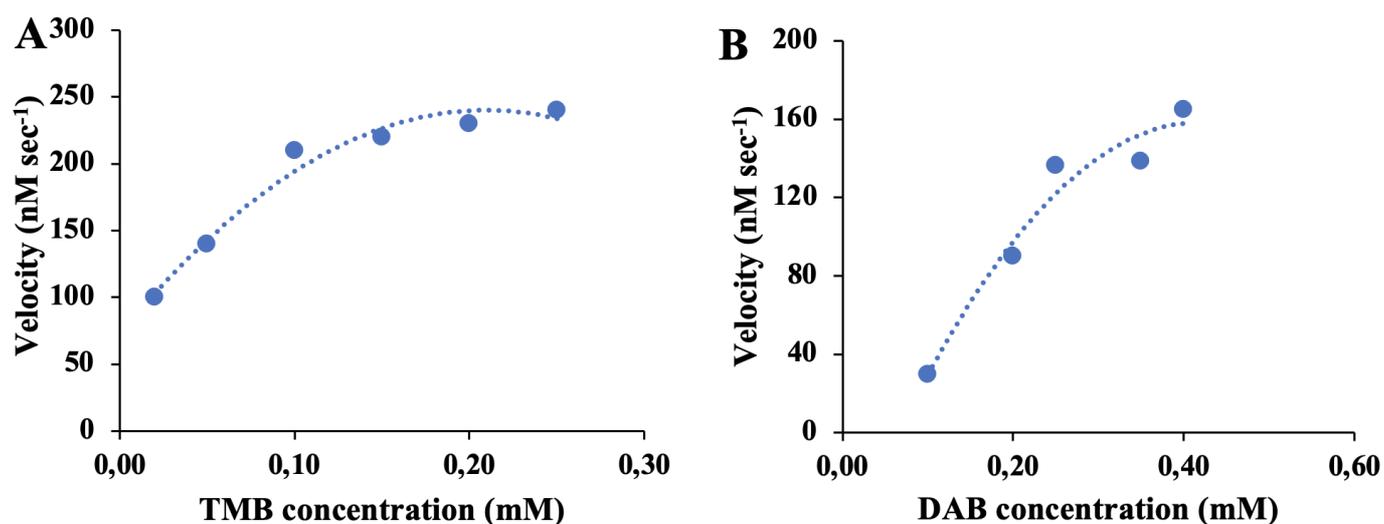


Figure 2. Michaelis–Menten plots for (A) TMB and (B) DAB oxidation.

To explore more precise the kinetic performances of BSA-gold nanozymes toward DAB oxidation, the Lineweaver–Burk plot was constructed for estimation of K_m and V_{max} of BSA-gold enzymes-mediated oxidation of DAB (Figure 3A), revealing a V_{max} and K_m of 185 nM sec^{-1} and 0.72 mM , in order, for DAB oxidation. To estimate the kinetic parameters of BSA-gold nanozymes toward TMB oxidation, the Lineweaver–Burk plot was constructed (Figure 3B). Based on Figure 3B, the V_{max} and K_m of the as-prepared nanozymes toward TMB oxidation were calculated at about 263 nM sec^{-1} and 0.03 mM , in order. The V_{max} of DAB oxidation was found to be lower than that of the TMB oxidation, revealing that the catalytic efficiency of the as-prepared BSA-gold nanozymes toward TMB is higher than that for the DAB. The K_m value for DAB was found to be very higher than that for TMB, revealing a higher affinity of TMB for binding to BSA-protected gold nanozymes than DAB. This difference can be related to the different reactivity of DAB and TMB as well as their different oxidation mechanisms.

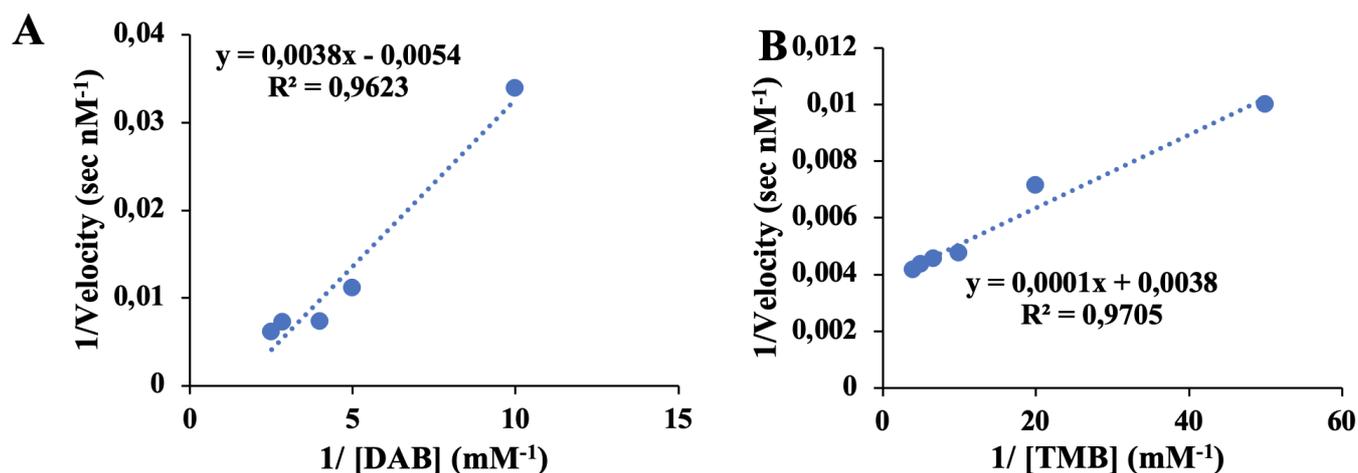


Figure 3. Lineweaver–Burk plots for (A) DAB and (B) TMB oxidation.

4. Conclusions

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, V_{\max} and K_m was then calculated by using Michaelis–Menten and the linear plot of Lineweaver–Burk for both substrates. 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. Besides, the K_m value for DAB was found to be very higher than that for TMB. This difference can be related to the different reactivity of DAB and TMB, as well as their different oxidation mechanisms.

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Conflict of interest

None.

Other References

- Li, T., Wang, Y., Liu, W., Fei, H., Guo, C., & Wei, H. (2023). Nanoconfinement-Guided Construction of Nanozymes for Determining H₂O₂ Produced by Sonication. *Angewandte Chemie International Edition*, 62(12), e202212438.

References

- ^{a, b} Zhou, Y., Liu, B., Yang, R., & Liu, J. (2017). Filling in the gaps between nanozymes and enzymes: challenges and opportunities. *Bioconjugate chemistry*, 28(12), 2903-2909.
- [^] Hormozi Jangi, S. R., & Akhond, M. (2021). High throughput urease immobilization onto a new metal-organic framework called nanosized electroactive quasi-coral-340 (NEQC-340) for water treatment and safe blood cleaning. *Process Biochemistry*, 105, 79-90.
- [^] Hormozi Jangi, S. R., & Akhond, M. (2022). Introducing a covalent thiol-based protected immobilized acetylcholinesterase with enhanced enzymatic performances for biosynthesis of esters. *Process Biochemistry*, 120, 138-155.
- [^] Hormozi Jangi, S. R., Akhond, M., & Dehghani, Z. (2020). High throughput covalent immobilization process for improvement of shelf-life, operational cycles, relative activity in organic media and enzymatic kinetics of urease and its application for urea removal from water samples. *Process Biochemistry*, 90, 102-112.
- [^] Hormozi Jangi, S. R. (2023). Synthesis and characterization of magnesium-based metal-organic frameworks and investigating the effect of coordination solvent on their biocompatibility. *Chemical Research and Nanomaterials*, 1(4), 1-9.
- [^] Hormozi Jangi, S. R., & Akhond, M. (2021). Ultrasensitive label-free enantioselective quantification of d-/l-leucine enantiomers with a novel detection mechanism using an ultra-small high-quantum yield N-doped CDs prepared by a novel highly fast solvent-free method. *Sensors and Actuators B: Chemical*, 339, 129901.
- [^] Hormozi Jangi, S. R. (2023). Low-temperature destructive hydrodechlorination of long-chain chlorinated paraffins to diesel and gasoline range hydrocarbons over a novel low-cost reusable ZSM-5@ Al-MCM nanocatalyst: a new approach toward reuse instead of common mineralization. *Chemical Papers*, 1-15.
- [^] Hormozi Jangi S. R.; Akhond M. (2020). High throughput green reduction of tris (p-nitrophenyl) amine at ambient temperature over homogenous AgNPs as H-transfer catalyst. *Journal of Chemical Sciences*, 132, 1-8.
- ^{a, b, c} Hormozi Jangi, A. R., Hormozi Jangi, M. R., & Hormozi Jangi, S. R. (2020). Detection mechanism and classification of design principles of peroxidase mimic based colorimetric sensors: A brief overview. *Chinese Journal of Chemical Engineering*, 28(6), 1492-1503.
- [^] Zeng, X., Ruan, Y., Chen, Q., Yan, S., & Huang, W. (2023). Biocatalytic cascade in tumor microenvironment with a Fe₂O₃/Au hybrid nanozyme for synergistic treatment of triple negative breast cancer. *Chemical Engineering Journal*, 452, 138422.
- [^] Hormozi Jangi, S. R., & Dehghani, Z. (2023). Spectrophotometric quantification of hydrogen peroxide utilizing silver nanozyme. *Chemical Research and Nanomaterials*. https://crn.shiraz.iau.ir/article_701960.html?lang=en.
- [^] Hormozi Jangi, S.R. (2023) Evaluating the Effect of Shelf-Storage, Daylight, and Air Oxygen on the Peroxidase-like Activity of Unmodified Silver Nanoparticles. *Preprints.org*, 2023052106. <https://doi.org/10.20944/preprints202305.2106.v1>
- [^] Yang, X., Xiang, J., Su, W., Guo, J., Deng, J., Tang, L.,... & Zhao, J. (2023). Modulating Pt nanozyme by using isolated cobalt atoms to enhance catalytic activity for alleviating osteoarthritis. *Nano Today*, 49, 101809.

14. [^]Chen, X., Wang, Y., Feng, M., Deng, D., Xie, X., Deng, C.,... & Yang, X. (2023). Dual-active-site Fe/Cu single-atom nanozymes with multifunctional specific peroxidase-like properties for S₂- detection and dye degradation. *Chinese Chemical Letters*, 34(6), 107969.
15. [^]Hormozi Jangi, S. R. (2023). Effect of daylight and air oxygen on nanozymatic activity of unmodified silver nanoparticles: Shelf-stability. *Qeios*. doi:10.32388/9RWVYI.3.
16. [^]Hormozi Jangi, S. R., Akhond, M., & Absalan, G. (2020). A field-applicable colorimetric assay for notorious explosive triacetone triperoxide through nanozyme-catalyzed irreversible oxidation of 3, 3'-diaminobenzidine. *Microchimica Acta*, 187, 431.
17. ^{a, b}Akhond, M., Hormozi Jangi, S. R., Barzegar, S., & Absalan, G. (2020). Introducing a nanozyme-based sensor for selective and sensitive detection of mercury (II) using its inhibiting effect on production of an indamine polymer through a stable n-electron irreversible system. *Chemical Papers*, 74, 1321-1330.
18. ^{a, b}Hormozi Jangi, S. R., Akhond, M., & Absalan, G. (2020). A novel selective and sensitive multinanozyme colorimetric method for glutathione detection by using an indamine polymer. *Analytica Chimica Acta*, 1127, 1-8.
19. [^]Ahmadi-Leilakouhi, B., Hormozi Jangi, S. R., & Khorshidi, A. (2023). Introducing a novel photo-induced nanozymatic method for high throughput reusable biodegradation of organic dyes. *Chemical Papers*, 77(2), 1033-1046.
20. [^]Hormozi Jangi, S. R., & Akhond, M. (2020). Synthesis and characterization of a novel metal-organic framework called nanosized electroactive quasi-coral-340 (NEQC-340) and its application for constructing a reusable nanozyme-based sensor for selective and sensitive glutathione quantification. *Microchemical Journal*, 158, 105328.
21. ^{a, b}Hormozi Jangi, S. R., Davoudli, H. K., Delshad, Y., Hormozi Jangi, M. R., & Hormozi Jangi, A. R. H. (2020). A novel and reusable multinanozyme system for sensitive and selective quantification of hydrogen peroxide and highly efficient degradation of organic dye. *Surfaces and Interfaces*, 21, 100771.
22. [^]Singh, A. K., Bijalwan, K., Kaushal, N., Kumari, A., Saha, A., & Indra, A. (2023). Oxidase-like Nanozyme Activity of Manganese Metal–Organic Framework Nanosheets for Colorimetric and Fluorescence Sensing of L-Cysteine. *ACS Applied Nano Materials*.
23. [^]Bittencourt, G. A., de Souza Vandenberghe, L. P., Martínez-Burgos, W. J., Valladares-Diestra, K. K., de Mello, A. F. M., Maske, B. L.,... & Soccol, C. R. (2023). Emerging contaminants bioremediation by enzyme and nanozyme-based processes—a review. *Iscience*.
24. [^]Li, Z., Hu, J., Zhan, Y., Shao, Z., Gao, M., Yao, Q.,... & Wang, L. (2023). Coupling Bifunctional Nanozyme-Mediated Catalytic Signal Amplification and Label-Free SERS with Immunoassays for Ultrasensitive Detection of Pathogens in Milk Samples. *Analytical Chemistry*, 95(15), 6417-6424.