Glutathione significantly inhibits the nanozymatic activity of N-doped carbon dots

Saeed Reza Hormozi Jangi[1]*

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

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

Abstract

Herein, the effect of the glutathione molecules (a possible inhibitor) on the peroxidase-like activity of the enzyme-like N-doped carbon dots was evaluated. To do this investigation, the enzyme-like activity of N-doped carbon dots was evaluated toward peroxidase-mediated oxidation of TMB as a common peroxidase substrate, revealing high intrinsic peroxidase-like activity of N-doped carbon dots. However, the intrinsic peroxidase-like activity of the N-doped carbon dot was significantly inhibited by the introduction of the glutathione molecules in the reaction media. Hence, the inhibitory effect of glutathione molecules on their nanozymatic activity was evaluated as a function of enzyme-like residual activity in the presence of glutathione molecules. The results reveal that the relative activity of nanozymes was inhibited by increasing the glutathione concentration in the reaction media and reached its minimum value (45%) when the glutathione concentration was 14 μM and then leveled off.

Keywords: N-doped carbon dots; nanozymes inhibitors; glutathione

Introduction

In recent years, a wide variety of nanoscale materials as artificial enzymes had been synthesized and utilized for different type of applications, for instance, sensing and detection, catalysis, biomedical, and imaging purposes. Among different types of nanoscale artificial enzymes, metal oxides such as manganese dioxide, Co_3O_4 and Fe3O4 nanoparticles, noble metal-based nanoparticles, for example, gold and silver nanoparticles, metal-organic frameworks, e.g., NEQC-340, and metallic nanoclusters for instance, BSA-AuNCs, and AgNCs attracted an attention for application in real word due to their high catalytic efficiency and biocompatibility [1-12]. It is notable that the nanoscale artificial enzymes are known as "nanozymes" due to their nanoscale particles. Up to now different nanozymes with oxidase-like, peroxidase-like, urease-like, and hydrolase-like activity were synthesized and applied for different applications, however, most nanozymes reveal significant peroxidase-like activity and can cleavage the peroxide bonds to produce active oxygen species such as hydroxyl radicals [13-18]. The resulting radicals can react with peroxidase substrates such as TMB, DAB, and OPD and proceed their oxidation to producing the colored products. It is notable that the product of oxidation of TMB and OPD is a colored cation radical while DAB oxidation resulted in production a colored indamine polymer via an n-electron irreversible oxidation pathway. Probing the absorbance of the colored product via spectrophotometric assay or analyzing the color intensity of the product via color analyzers can be utilized for estimation of enzyme-like activity of nanozymes as well as can be used as analytical response for

quantification of several analytes [19-30]. The wide application of nanozymes compared of the natural enzymes is maybe due to the characteristic limitations of native enzymes for instance pH and temperature instability, short storage time, no reusability, and highly expensive production methods. Notably, to fix drawbacks of native enzymes, there is two protocols; (i) immobilization of enzymes [31-33] and (ii) developing nanozymes. However, in the case of enzyme immobilization, the reduction of initial enzyme activity is a routine drawback and nanozymes development can be considered as a right solution for overcoming this drawback [28]. In fact, the deep development of nanoscience leads to synthesis of new nanomaterials with unique catalytic activity [34, 35], unique optical properties [36-38], high active area [39], antibacterial properties [40], and high biocompatibility [41]. Some of new nanomaterials with unique catalytic activity show enzyme-like activity with high stability, leading to their application in different fields, in developing sensing assays of amino acids, glutathione (GSH), tetracycline, metal cations, glucose, H_2O_2, explosives, malathion [42-48], and new SARS-CoV-2 [49] as after the first report of COVID-19 [50, 51]. However, the researches focusing on the inhibitory effect of inhibitors on nanozymes activity are limited to a few reports. Herein, the effect of the glutathione molecules (a possible inhibitor) on the peroxidase-like activity of the enzyme-like N-doped carbon dots was evaluated. To do this investigation, the enzyme-like activity of N-doped carbon dots was evaluated toward peroxidase-mediated oxidation of TMB as a common peroxidase substrate, revealing high intrinsic peroxidase-like activity

of N-doped carbon dots. However, the intrinsic peroxidase-like activity of the N-doped carbon dot was significantly inhibited by the introduction of the glutathione molecules in the reaction media. Hence, the inhibitory effect of glutathione molecules on their nanozymatic activity was evaluated as a function of enzyme-like residual activity in the presence of glutathione molecules. The results reveal that the relative activity of nanozymes was inhibited by increasing the glutathione concentration in the reaction media.

2. Experimental

2.1. Synthesis of N-doped carbon dots

300 mg ethylenediaminetetraacetic acid was directly heated at 400 ℃ for about 2 hours. Afterward, the CDs were dissolved in acetone and centrifuged to remove the residual solid particles. The solvent was then evaporated and the results CDs were collected and dissolved in water for next use.

2.2. Inhibitory experiments

In a typical test, different concentrations of inhibitor were introduced into 2.0 mL acetate buffer (pH, 4.0; 0.1 M) containing 90 μg mL^{-1} nanozymes, 60 μM of TMB, and 1.0 mM hydrogen peroxide. The mixture was incubated for about 5.0 min to complete the oxidation process. Afterward, the absorbance of the oxidation product was calculated at 663 nm. The residual activity of the nanozymes in the presence and the absence of the

inhibitor molecules was calculated by dividing the activity of the nanozymes by the activity of control (i.e., activity in the absence of inhibitor).

3. Results and discussion

The inhibitory effect of glutathione (GSH) molecules on the nanozymatic activity of peroxidase-like N-doped carbon dots was evaluated by calculating their nanozymatic activity in the presence and absence of glutathione molecules. The UV-visible spectra of the oxidation product of TMB in the presence and the absence of glutathione molecules as an inhibitor are shown in Figure 1, revealing that the absorbance at 662 nm was significantly reduced by introducing GSH into the reaction media. Based on this investigation it can be concluded that N-doped CDs reveal high intrinsic peroxidase-like activity of N-doped carbon dots, however, this intrinsic peroxidase-like activity significantly inhibit by glutathione molecules.

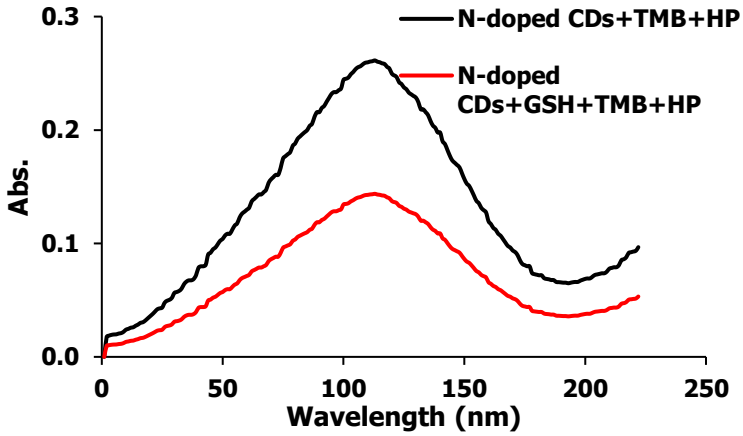

Figure 1. UV-visible spectra of the TMB-ox in the presence and the absence of glutathione molecules as an inhibitor

However, to provide a better view of the inhibitory effect of glutathione molecules on the nanozyme's activity, the inhibitory effect of glutathione molecules on their nanozymatic activity was evaluated as a function of enzyme-like residual activity in the presence of glutathione molecules. The results (Figure 2) reveal that the relative activity of nanozymes was inhibited by increasing the glutathione concentration in the reaction media and reached its minimum value (45%) when glutathione concentration is 14 µM and then leveled off, revealing a strong inhibitory effect of glutathione molecules on the nanozymatic behavior of peroxidase-like N-doped carbon dots.

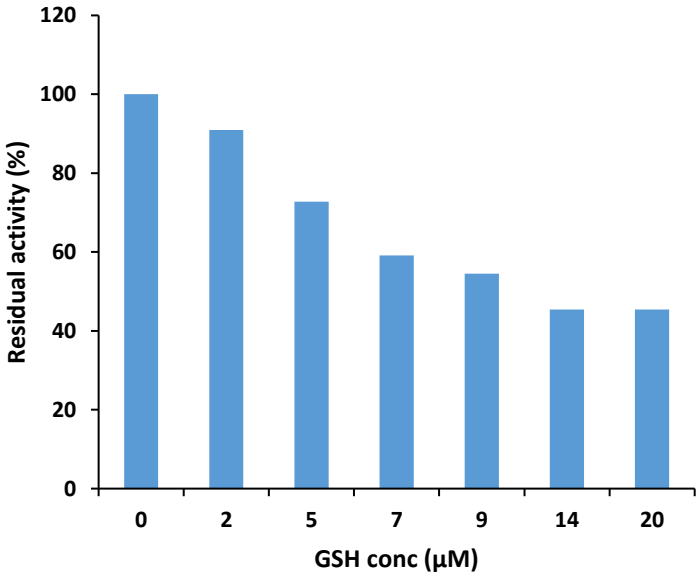

Figure 2. Residual activity of as-prepared peroxidase-like nanozymes in the presence of different concentrations of glutathione as a typical inhibitor.

4. Conclusions

Herein, the effect of the glutathione molecules (a possible inhibitor) on the peroxidase-like activity of the enzyme-like N-doped carbon dots was evaluated. To do this investigation, the enzyme-like activity of N-doped carbon dots was evaluated toward peroxidase-mediated oxidation of TMB as a common peroxidase substrate, revealing high intrinsic peroxidase-like activity of N-doped carbon dots. However, the intrinsic peroxidase-like activity of the N-doped carbon dot was significantly inhibited by the introduction of the glutathione molecules in the reaction media. Hence, the inhibitory effect of glutathione molecules on their nanozymatic activity was evaluated as a function of enzyme-like residual activity in the presence of glutathione molecules. The results reveal that the relative activity of nanozymes was inhibited by increasing the glutathione concentration in the reaction media and reached its minimum value (45%) when the glutathione concentration was 14 μM and then leveled off.

Acknowledgments

The authors gratefully thank the Hormozi Laboratory of Chemistry and Biochemistry (Zabol, Iran) for the support of this work.

Conflict of interest

There is no conflict of interest.

5. References

1. Vafabakhsh, M., Dadmehr, M., Noureini, S. K., Es' haghi, Z., Malekkiani, M., & Hosseini, M. (2023). Colorimetric detection of COVID-19 using aptasenor based on biomimetic peroxidase like activity of ChF/ZnO/CNT nano-hybrid.
2. Hormozi Jangi, S. R. (2023). Time course evaluation of nanozyme-mediated reversible/irreversible oxidation reactions over silver nanoparticles as peroxidase alternatives, Modern Chemistry & Applications, 11 (4) 426.
3. Jangi, S. R. H. (2023). Experimental evaluation of kinetics and biochemical characteristics of MnO2 nanoparticles as high throughput peroxidase-mimetic nanomaterials. Micromaterials and Interfaces, 1(1).
4. Hormozi Jangi, S. R. (2023). A Brief Overview of Nanozyme-Based Colorimetric and Fluorometric Sensors for Early Diagnosis of COVID-19. Trans Med OA, 1(2), 76-84.
5. Hermosilla, E., Seabra, A. B., Lourenço, I. M., Ferreira, F. F., Tortella, G., & Rubilar, O. (2021). Highly sensitive oxidation of MBTH/DMAB by MnFe2O4 nanoparticles as a promising method for nanozyme-based sensor development. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 621, 126585.
6. Dong, S., Dong, Y., Jia, T., Liu, S., Liu, J., Yang, D., ... & Lin, J. (2020). GSH-depleted nanozymes with hyperthermia-enhanced dual enzyme-mimic activities for tumor nanocatalytic therapy. Advanced Materials, 32(42), 2002439.

7. Hormozi Jangi, S. R. (2023). Biochemical characterization of enzyme-like silver nanoparticles toward nanozyme-catalysed oxidation reactions, Micromaterials and Interfaces 1 (1), 2170.
8. Jangi, S. R. H. (2023). Introducing a High Throughput Nanozymatic Method for Eco-Friendly Nanozyme-Mediated Degradation of Methylene Blue in Real Water Media. Sustainable Chemical Engineering, 90-99.
9. Wang, Q., Wei, H., Zhang, Z., Wang, E., & Dong, S. (2018). Nanozyme: An emerging alternative to natural enzyme for biosensing and immunoassay. TrAC Trends in Analytical Chemistry, 105, 218-224.
10. 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.
11. Hormozi Jangi, S. R. (2023). A Comparative Study on Kinetics Performances of BSA-gold Nanozymes for Nanozyme-mediated Oxidation of 3,3',5,5'-Tetramethylbenzidine and 3,3'-Diaminobenzidine. Biochemistry & Molecular Biology Journal 9(3), 21.
12. Jangi, S. R. H., & 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.

13. Shen, Y., Wei, Y., Liu, Z., Nie, C., & Ye, Y. (2022). Engineering of 2D artificial nanozyme-based blocking effect-triggered colorimetric sensor for onsite visual assay of residual tetracycline in milk. Microchimica Acta, 189(6), 233.
14. Hormozi Jangi, S. R., & Dehghani, Z. (2023). Spectrophotometric quantification of hydrogen peroxide utilizing silver nanozyme. Chemical Research and Nanomaterials, 2(1), 15-23.
15. Lu, Y., Ye, W., Yang, Q., Yu, J., Wang, Q., Zhou, P., ... & Zhao, S. (2016). Three-dimensional hierarchical porous PtCu dendrites: a highly efficient peroxidase nanozyme for colorimetric detection of H2O2. Sensors and Actuators B: Chemical, 230, 721-730.
16. Hormozi Jangi, S. R. (2023). Evaluation of Biochemical Behavior and Stability of Gold Nanoparticles with High Intrinsic Peroxidase-Like Activity. Petro Chem Indus Intern, 6(4), 234-239.
17. Hormozi Jangi, S. R., & Dehghani, Z. (2023). Kinetics and biochemical characterization of silver nanozymes and investigating impact of storage conditions on their activity and shelf-life. Chemical Research and Nanomaterials, 1(4), 25-33.

18. Jangi, S. R. H. (2023). Determining kinetics parameters of bovine serum albumin-protected gold nanozymes toward different substrates. Qeios.
19. Huang, Y., Ren, J., & Qu, X. (2019). Nanozymes: classification, catalytic mechanisms, activity regulation, and applications. Chemical reviews, 119(6), 4357-4412.
20. Wu, J., Wang, X., Wang, Q., Lou, Z., Li, S., Zhu, Y., ... & Wei, H. (2019). Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). Chemical Society Reviews, 48(4), 1004-1076.
21. Hormozi Jangi, S. R. (2023). Experimental Evaluation of Kinetic Characteristics of SiO2@ AuNPs Nanocom-posite and BSA-stabilized gold Nanoparticles toward Peroxidase-Mediated Reactions. Adv Nanoscie Nanotec, 7(1), 01-11.
22. Hormozi Jangi, S. R. (2023). Detection mechanism and principles of the multinanozyme systems as the new generation of nanozyme-mediated sensing assays: A critical review. Petro Chem Indus Intern, 6(5), 349-357.
23. Hormozi Jangi, S. R. (2023). An Experimental study on the kinetics characteristics and biochemical behaviour of peroxidase mimic core@shell silicone dioxide@gold nanocomposite. Nano Tech Nano Sci Ind J, 17(3).
24. Kang, K., Wang, B., Ji, X., Liu, Y., Zhao, W., Du, Y., ... & Ren, J. (2021). Hemin-doped metal–organic frameworks based nanozyme electrochemical sensor with

high stability and sensitivity for dopamine detection. RSC advances, 11(4), 2446-2452.

25. 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
26. Lu, W., Guo, Y., Zhang, J., Yue, Y., Fan, L., Li, F., ... & Shuang, S. (2022). A High Catalytic Activity Nanozyme Based on Cobalt-Doped Carbon Dots for Biosensor and Anticancer Cell Effect. ACS Applied Materials & Interfaces, 14(51), 57206-57214.
27. Ren, X., Chen, D., Wang, Y., Li, H., Zhang, Y., Chen, H., ... & Huo, M. (2022). Nanozymes-recent development and biomedical applications. Journal of Nanobiotechnology, 20(1), 92.
28. Jangi, A. R. H., Jangi, M. R. H., & Jangi, S. R. H. (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.
29. Xu, S., Zhang, S., Li, Y., & Liu, J. (2023). Facile Synthesis of Iron and Nitrogen Co-Doped Carbon Dot Nanozyme as Highly Efficient Peroxidase Mimics for Visualized Detection of Metabolites. Molecules, 28(16), 6064.

30. Ray, S., Biswas, R., Banerjee, R., & Biswas, P. (2020). A gold nanoparticle-intercalated mesoporous silica-based nanozyme for the selective colorimetric detection of dopamine. Nanoscale Advances, 2(2), 734-745.
31. Jangi, S. R. H., & 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.
32. Jangi, S. R. H., & Akhond, M. (2022). Introducing a covalent thiol-based protected immobilized acetylcholinesterase with enhanced enzymatic performances for biosynthesis of esters. Process Biochemistry, 120, 138-155.
33. Jangi, S. R. H., 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.
34. 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.

35. 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.

36. Dehghani, Z., Akhond, M., Jangi, S. R. H., & Absalan, G. (2024). Highly sensitive enantioselective spectrofluorimetric determination of R-/S-mandelic acid using l-tryptophan-modified amino-functional silica-coated N-doped carbon dots as novel high-throughput chiral nanoprobes. Talanta, 266, 124977.

37. Hormozi Jangi, S. R., & Gholamhosseinzadeh, E. (2023). Developing an ultra-reproducible and ultrasensitive label-free nanoassay for L-methionine quantification in biological samples toward application in homocystinuria diagnosis. Chemical Papers, 1-13.

38. Jangi, S. R. H., & 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.

39. Thakkar, K. N., Mhatre, S. S., & Parikh, R. Y. (2010). Biological synthesis of metallic nanoparticles. Nanomedicine: nanotechnology, biology and medicine, 6(2), 257-262.

40. Hajipour, M. J., Fromm, K. M., Ashkarran, A. A., de Aberasturi, D. J., de Larramendi, I. R., Rojo, T., ... & Mahmoudi, M. (2012). Antibacterial properties of nanoparticles. Trends in biotechnology, 30(10), 499-511.

41. 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.

42. Jangi, S. R. H., 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.

43. Jangi, S. R. H., Davoudli, H. K., Delshad, Y., Jangi, M. R. H., & 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.

44. 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.

45. Chen, J., Wu, W., Huang, L., Ma, Q., & Dong, S. (2019). Self-indicative gold nanozyme for H2O2 and glucose sensing. Chemistry–A European Journal, 25(51), 11940-11944.

46. 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, 1-10.

47. Singh, M., Weerathunge, P., Liyanage, P. D., Mayes, E., Ramanathan, R., & Bansal, V. (2017). Competitive inhibition of the enzyme-mimic activity of Gd-based nanorods toward highly specific colorimetric sensing of l-cysteine. Langmuir, 33(38), 10006-10015.

48. Singh, S., Tripathi, P., Kumar, N., & Nara, S. (2017). Colorimetric sensing of malathion using palladium-gold bimetallic nanozyme. Biosensors and bioelectronics, 92, 280-286.

49. Liang, C., Liu, B., Li, J., Lu, J., Zhang, E., Deng, Q., ... & Li, T. (2021). A nanoenzyme linked immunochromatographic sensor for rapid and quantitative detection of SARS-CoV-2 nucleocapsid protein in human blood. Sensors and Actuators B: Chemical, 349, 130718.

50. Jangi, S. R. H. (2023). Natural Polyphenols of Pomegranate and Black Tea Juices can Combat COVID-19 through their SARS-CoV-2 3C-like Protease-inhibitory Activity. Qeios.

51. Hormozi Jangi, S. R. (2023). A Brief Overview on Clinical and Epidemiological Features, Mechanism of Action, and Diagnosis of Novel Global Pandemic

Infectious Disease, Covid-19, And its Comparison with Sars, Mers, And H1n1. World J Clin Med Img, 2(1), 45-52.