Evaluation of the effect of glutathione on nanozymatic activity of enzyme-like carbon dots

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December 9, 2023

Abstract

The effect of the glutathione molecules as a possible inhibitor was investigated on the nanozymatic activity of the enzyme-like carbon dots. The peroxidase-like activity of carbon dots was proved via catalysis of peroxidase-mediated oxidation of TMB as a standard peroxidase substrate. The experimental investigations exhibited that the peroxidase-like activity of the carbon dots was significantly inhibited by glutathione molecules. Hence, the inhibitory effect of glutathione molecules on their nanozymatic activity was evaluated, revealing that the relative activity of nanozymes was inhibited by increasing the glutathione concentration in the reaction media, revealing a strong inhibitory effect of glutathione molecules on the nanozymatic activity of the enzyme-like carbon dots.

1. Introduction

Different nanomaterials for instance, metal oxides, metal-based nanoparticles, metal-organic frameworks, and metallic nanoclusters exhibit intrinsic enzyme-like activity [1-12]. 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 produced radicals can then react with chromogenic substrates and oxidize them to their corresponding colored products. The spectrophotometric assay and recording of the absorbance of these products can be used as an index for calculating the nanozymatic activity of the nanoscale peroxidase-like materials [19-25]. Moreover, it is proved by several researches that the enzyme-like activity of the nanozymes can be inhibited by some inhibitors as same as the native enzymes. It is inhibitory effect can be used for several aims especially for sensing and detection toward developing both clinical and analytical protocols [26-30]. It is well known that among different identified enzymes, peroxidase enzymes, especially horseradish peroxidase (HRP), are attractive enzymes from both industrial and clinical points of view [28]. Regarding the peroxidase enzymes, hydrogen peroxide is the initiator of the peroxidase-mediated reactions and the oxidation of a wide range of organic compounds (substrates) including aromatic amines, phenols, and their mixtures can be initiated in the presence of hydrogen peroxide and peroxidase enzyme [28]. However the peroxidase as same as other natural enzymes shows some of the following serious disadvantages such as pH and temperature instability, difficult recovery protocol, short storage time, no reusability, and highly expensive production methods. Hence, to fix these drawbacks, the immobilization of enzymes was proposed [31-33]. However, during most immobilization protocols, the enzyme’s initial activity is reduced and some of them are expensive. Hence, a better solution is needed to overcome these difficulties, the new field of nanozymes is the right solution [28]. In fact, 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 [34, 35], unique optical properties [36-38], high active area [39], antibacterial properties [40], and high biocompatibility [41]. Among different nanomaterials, nanozymes as nanomaterials with high enzyme-like activity can be used to
simulate enzymatic reactions in harsh environmental conditions (for example, higher temperature or wider pH range) [1-28]. Hence, due to their high stability and intrinsic enzyme-like properties, the nanozymes were used for different applications, especially for constructing sensing assays for a wide variety of analytes, e.g., amino acids, glutathione (GSH), tetracycline, metal cations, glucose, H₂O₂, 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. Hence, herein, the effect of the glutathione molecules as a possible inhibitor was investigated on the nanozymatic activity of the enzyme-like carbon dots. The peroxidase-like activity of carbon dots was proved via catalysis of peroxidase-mediated oxidation of TMB as a standard peroxidase substrate. The experimental investigations exhibited that the peroxidase-like activity of the carbon dots was significantly inhibited by glutathione molecules. Hence, the inhibitory effect of glutathione molecules on their nanozymatic activity was evaluated, revealing that the relative activity of nanozymes was inhibited by increasing the glutathione concentration in the reaction media, revealing a strong inhibitory effect of glutathione molecules on the nanozymatic activity of the enzyme-like carbon dots.

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

The effect of the glutathione molecules as a possible inhibitor was investigated on the nanozymatic activity of the enzyme-like carbon dots. The peroxidase-like activity of carbon dots was proved via catalysis of peroxidase-mediated oxidation of TMB as a standard peroxidase substrate. The experimental investigations exhibited that the peroxidase-like activity of the carbon dots was significantly inhibited by glutathione molecules. Hence, the inhibitory effect of glutathione molecules on their nanozymatic activity was evaluated, revealing that the relative activity of nanozymes was inhibited by increasing the glutathione concentration in the reaction media, revealing a strong inhibitory effect of glutathione molecules on the nanozymatic activity of the enzyme-like carbon dots.

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


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