Influence of Reaction Time on Silver Nanoparticles-Catalyzed Oxidation of 3,3',5,5'-Tetramethylbenzidine

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Article

Influence of Reaction Time on Silver Nanoparticles-Catalyzed Oxidation of 3,3′,5,5′-Tetramethylbenzidine

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Abstract: Influence of reaction time on silver nanoparticles-catalyzed oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) was evaluated via probing the oxidation process by recording the UV-Visible absorbance of the resulting colored products of TMB oxidation by hydrogen peroxide in the presence of silver nanoparticles as peroxides mimics. The time curve of the oxidation process was constructed, revealed that the concentration of the oxidation product was increased by increasing the reaction time and then levled off because the nanozyme active nodes were saturated after a certain reaction time and consequently the steady-state condition can be observed. The maximal activity of the silver nanozymes was achieved after a very short reaction time as short as 3.0 min and after this time, the color intensity of the oxidation products remained constant, revealing saturation of active nodes of nanoparticles with nanozyme-substrate.

Keywords: silver nanoparticles; peroxidase mimics; time influence on oxidation; nanozyme active nodes

1. Introduction

Nanozymes or nanoparticles with excellent enzyme-like activity are attracted many attention due to their stability and higher efficiency compared to natural enzymes [1–7]. In fact, Natural enzymes show several disadvantages such as low stability (thermal and narrow pH range) [8]. For overcoming these drawbacks, the enzyme immobilization process has been developed [9–13]. The recent progresses on nanochemistry and material science open a new door for developing high performance nano-supports such as MOFs, catalytic materials, and nanoparticles with enzyme-like activity [14–20]. Several of the above-mentioned nanoparticles reveal high peroxidase-like activity which can be used instead of enzymes in the reactions. Recently, the nanozymes had been used for different applications for instance, analytical sensing of species, biocatalysis of reactions instead of natural enzymes, water treatment, dye degradation, sensing and detection [21–24]. Since nanozymes are able to catalyze the oxidation of peroxidase substrates to their corresponding colored products, they have been used for the analytical purposes [1–7]. 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 [1–7]. Silver nanoparticles are well-known as the nanomaterials with -peroxidase-like materials [25–30]. In this contribution, the effect of incubation time on nanozyme-catalyzed oxidation of 3,3′,5,5′-tetramethylbenzidine over silver nanoparticles due to importance of the reaction time on producing the corresponding radicals. The process of the oxidation reaction was probed by recording the absorbance of the colored products using UV-Vis spectrophotometer. Herein, the influence of reaction time on silver nanoparticles-catalyzed oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) was evaluated via probing the oxidation process by recording the UV-Visible absorbance of the resulting colored products of TMB oxidation by hydrogen peroxide in the presence of silver nanoparticles as peroxides mimics. The results of this work revealed that the concentration of the oxidation product was increased by increasing the reaction time and then levled off because the nanozyme active nodes were saturated after a certain reaction time and consequently the steady-state condition can be observed.

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2. Experimental section

2.1. Synthesis of AgNPs

The synthesis was performed based on the process reported by Hormozi Jangi et al [27]. To do this, silver ions were reduced by $NaBH_4$ in the presence of sodium citrate as stabilizer within 3 hours. After this time, the AgNPs were collected and sored at 4 ℃.

2.2. Oxidation reactions

To do the oxidation reactions, the suitable amount of TMB were introduced into the buffer solutions containing silver nanoparticles and hydrogen peroxide with a fixed pH of 7.0 or 4.0, respectively. The reaction was proceeded for about 5.0 min for TMB oxidation. There after the colored products were analyzed by UV-Vis spectrophotometer at 650 nm for TMB.

3. Results and discussion

3.1. Characterization of silver nanozymes

Unmodified silver nanoparticles were synthesized and characterized for their size and morphological properties. In this regard, the TEM image of the as-prepared nanozyme was recorded and the results are shown in Figure 1, as shown in this figure, the as-prepared silver nanoparticles showed uniform morphology with spherical particles. In addition, the as-prepared nanozymes showed a narrow size distribution over 10.3-12.6 nm with an average size of 11.0 nm.

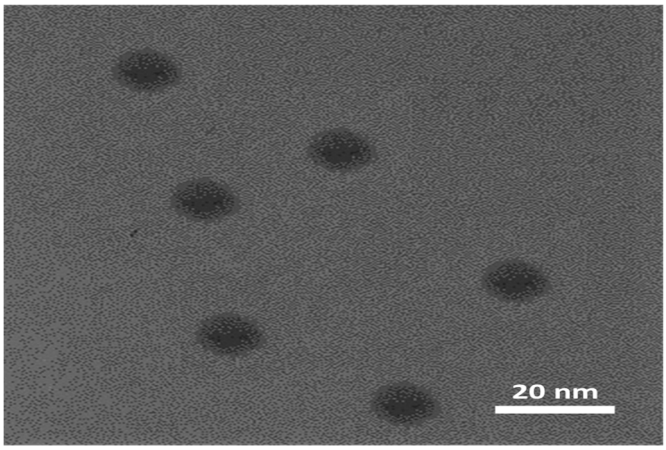

Figure 1. TEM image of as-prepared silver nanoparticles.

3.2. Time-course studies toward TMB oxidation

To evaluate the peroxidase-like activity of the as-prepared AgNPs, the oxidation of TMB was performed by hydrogen peroxide in the presence of AgNPs as the peroxidase mimics. In this regard, the time course studies were performed by probing the blue-colored product via spectrophotometric detection at 650.0 nm. Afterward, the plot of oxidation of TMB in the presence of AgNPs as a function of time was constructed by plotting the absorbance at 650.0 nm as a function of reaction time. The results are shown in Figure 2A-B. As can be seen from these figure, the AgNPs can catalyze the oxidation of TMB to form a blue-colored product with a maximum absorbance at 650.0 nm. Based on the time-course studies, the oxidation of TMB was quickly proceed by AgNPs and the absorbance at 650 nm was reached to 1.9 after a short reaction time of 3.0 min. After this time, the color intensity of the oxidation products remained constant, revealing saturation of active nodes of nanoparticles with nanozyme-substrate.

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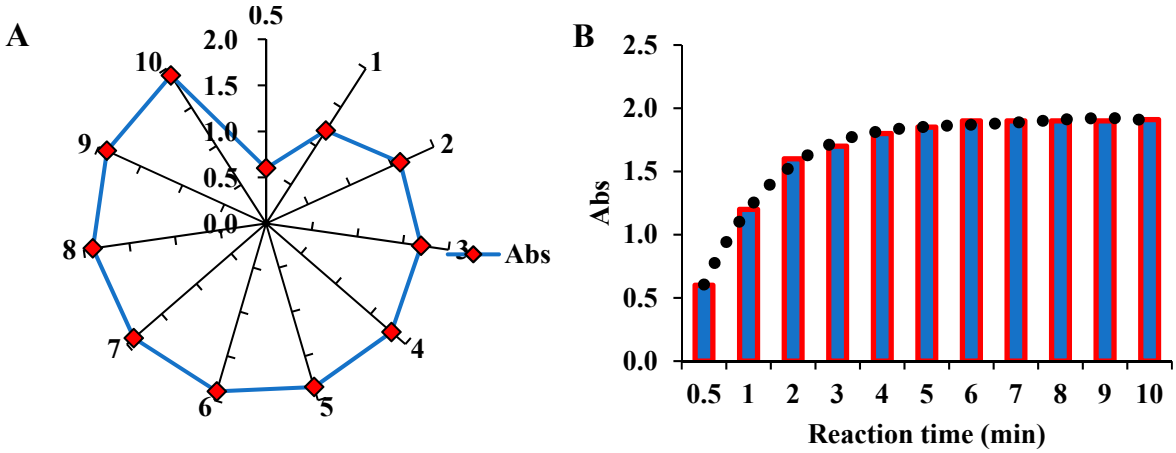

Figure 2. Oxidation of TMB in the presence of silver nanozymes as a function of time, (A) time course radar plot and (B) corresponding histogram.

4. Conclusions

Influence of reaction time on silver nanoparticles-catalyzed oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) was evaluated via probing the oxidation process by recording the UV-Visible absorbance of the resulting colored products of TMB oxidation by hydrogen peroxide in the presence of silver nanoparticles as peroxides mimics. The time curve of the oxidation process was constructed, revealed that the concentration of the oxidation product was increased by increasing the reaction time and then levled off because the nanozyme active nodes were saturated after a certain reaction time and consequently the steady-state condition can be observed. The maximal activity of the silver nanozymes was achieved after a very short reaction time as short as 3.0 min and after this time, the color intensity of the oxidation products remained constant, revealing saturation of active nodes of nanoparticles with nanozyme-substrate.

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Conflict of interest: None.

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