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Time Course Studies toward Oxidation of Different Peroxidase Substrates in the Presence of Silver Nanozymes

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Abstract: In this contribution, time course studies were performed toward oxidation of different peroxidase substrates in the presence of silver nanozymes. In this regard, two common peroxidase substrates, TMB and DAB, were selected as model chromogens. The process of the oxidation reaction was probed by recording the absorbance of the colored products using UV-Vis spectrophotometer. Thereafter, the nanozymatic activity was calculated and used as an index for comparing the performance of the silver nanozymes against different substrates in a certain reaction time. The time course curves, revealed that the maximal activity of the silver nanozymes were achieved after a very short time of 3.0 min toward TMB oxidation while regarding DAB, the maximal activity was observed after 25.0 min which is 6.5-fold slower than that of the TMB oxidation. The difference between the DAB and TMB can be assigned to their different reaction pathways and different reactivity. In fact, the oxidation of DAB has occurred via n-electron irreversible pathway while the TMB oxidation has occurred through a simple 2-electon reversible pathway. Besides, DAB is a high stable and less relative compound compared of TMB, resulting different oxidation times, different affinity for binding to nanozyme active nodes which lead to different nanozymatic activities.

Keywords: time-course studies; silver nanozymes; different nanozymatic activities

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, time course studies were performed toward oxidation of different peroxidase substrates in the presence of silver nanozymes. In this regard, two common peroxidase substrates, TMB and DAB, were selected as model chromogens. The process of the oxidation reaction was probed by recording the absorbance of the colored products using UV-Vis spectrophotometer. Thereafter, the nanozymatic activity was calculated and used as an index for comparing the performance of the silver nanozymes against different substrates in a certain reaction time.

2. Experimental section

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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 DAB or 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 30 min in the case of DAB and 5.0 min for TMB oxidation. There after the colored products were analyzed by UV-Vis spectrophotometer at 460.0 nm for DAB and 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.

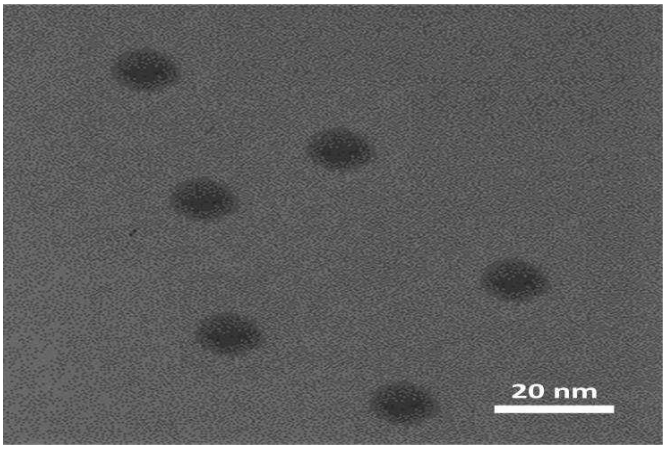

Figure 1. TEM image of as-prepared silver nanoparticles.

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 2. As can be seen from this 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

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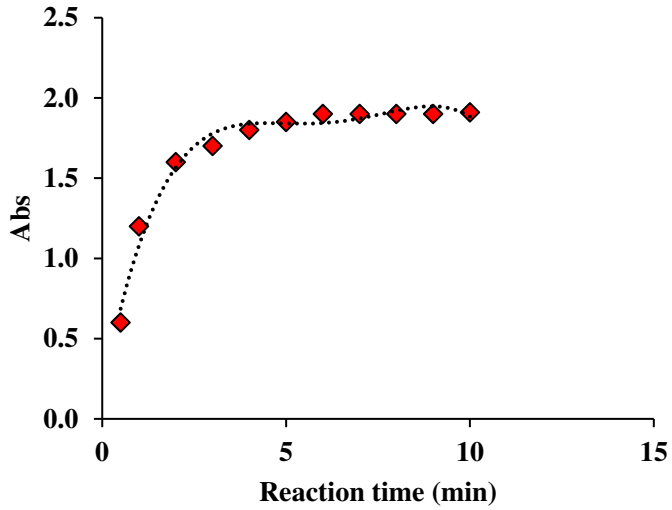

Figure 2. Oxidation of TMB in the presence of silver nanozymes as a function of time.

3.3. Time-course studies toward DAB oxidation

Besides, the peroxidase-like activity of silver nanoparticles toward DAB oxidation was also evaluated. To evaluate the peroxidase-like activity of the as-prepared AgNPs against DAB, the oxidation of DAB 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 brown-colored product via spectrophotometric detection at 460.0 nm. Afterward, the plot of oxidation of DAB in the presence of AgNPs as a function of time was constructed by plotting the absorbance at 460.0 nm as a function of reaction time. The results are shown in Figure 3. As can be seen from this figure, the AgNPs can catalyze the oxidation of DAB to form a brown-colored product with a maximum absorbance at 460.0 nm. As can be seen from Figure 3, the oxidation of DAB was found to be slower in rate than the TMB, reaching an absorbance of 1.5 after 20.0 min. Considering these fact, the as-prepared silver nanoparticles can be utilized as the peroxidase mimics for oxidation of chromophores.

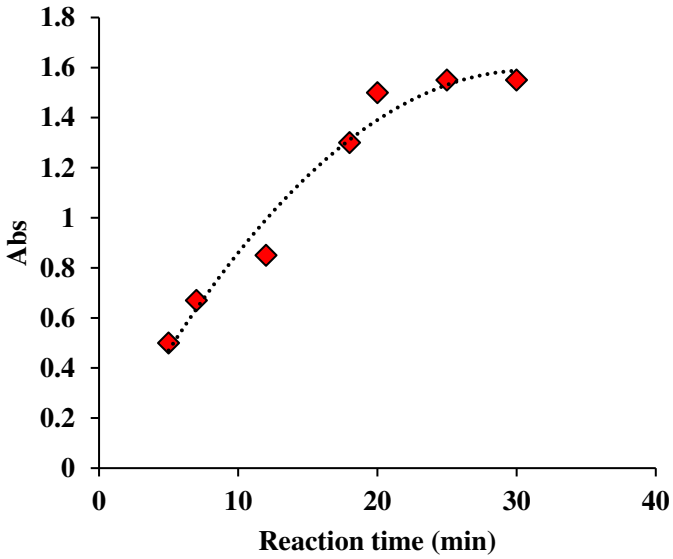

Figure 3. Oxidation of DAB in the presence of silver nanozymes as a function of time.

3.4. Comparing the results of DAB and TMB oxidation over silver nanozymes

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The time course curves, revealed that the maximal activity of the silver nanozymes were achieved after a very short time of 3.0 min toward TMB oxidation while regarding DAB, the maximal activity was observed after 25.0 min which is 6.5-fold solwer than that of the TMB oxidation. The difference between the DAB and TMB can be assigned to their different reaction pathways and different reactivity. In fact, the oxidation of DAB has occurred via n-electron irreversible pathway while the TMB oxidation has occurred through a simple 2-electon reversible pathway. Besides, DAB is a high stable and less relative compound compared of TMB, resulting different oxidation times, different affinity for binding to nanozyme active nodes which lead to different nanozymatic activities.

4. Conclusions

In this contribution, time course studies were performed toward oxidation of different peroxidase substrates in the presence of silver nanozymes. In this regard, two common peroxidase substrates, TMB and DAB, were selected as model chromogens. The process of the oxidation reaction was probed by recording the absorbance of the colored products using UV-Vis spectrophotometer. Thereafter, the nanozymatic activity was calculated and used as an index for comparing the performance of the silver nanozymes against different substrates in a certain reaction time. The time course curves, revealed that the maximal activity of the silver nanozymes were achieved after a very short time of 3.0 min toward TMB oxidation while regarding DAB, the maximal activity was observed after 25.0 min which is 6.5-fold solwer than that of the TMB oxidation. The difference between the DAB and TMB can be assigned to their different reaction pathways and different reactivity. In fact, the oxidation of DAB has occurred via n-electron irreversible pathway while the TMB oxidation has occurred through a simple 2-electon reversible pathway. Besides, DAB is a high stable and less relative compound compared of TMB, resulting different oxidation times, different affinity for binding to nanozyme active nodes which lead to different nanozymatic activities.

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Conflict of interest: None.

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