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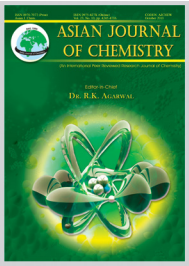

Investigation on the Potential use of the Mimetic Peroxidase-Catalyzed Reaction of Hydrogen Peroxide and Catechol in Aqueous-Organic Mixtures

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The reaction between hydrogen peroxide and catechol catalyzed by mimetic peroxidase hemin has been adapted to determination of trace amounts of H_2O_2. The present work employs hemin as mimetic peroxidase to catalyze the oxidation of catechol by H_2O_2. Its intermediate is obtained in aqueous-organic two phases and the absorption peaks at 260 nm. The catalytic optimum conditions for hemin-H_2O_2-catechol two phases system were studied. As low as 2×10^{-6} M H_2O_2 could be detected with a linear range from 3×10^{-6} M to 1×10^{-4} M *via* spectrophotometry.

Key Words: Hemin, Mimetic enzyme, Aqueous-organic mixtures.

INTRODUCTION

Over the past few years, the development of the natural peroxidases has gained much attention. They were applied for determination of H_2O_2 or phenolic substances. However, their industrial applications have been limited by the expensive cost and the poor stability[1]. Accordingly, the substituted substances of peroxidases that remain the catalytic activity with similar structure of enzyme have been attracting more interest[2]. The peroxidase mimics as the substituted substances of the natural peroxidase were considerable important in the catalytic application[3]. Hemin as mimetic enzyme with a good stability and relative low cost has been shown to possess excellent catalytic activities in aqueous solution[4,5]. Shen *et al.*[6] applied hemin as the peroxidase substitute in the catalytic oxidation of Bromopyrogallol red by H_2O_2 through spectrophotometric method.

As it is known that H_2O_2 is a significant substance in the catalytic and analytical chemistry[7]. To study the catalytic behaviour of hemin toward the oxidative reaction of phenolic substrate is important to determine H_2O_2[8,9]. Meanwhile, catechol is a basilic intermediate chemical and environmental pollutant and its oxidate decrease by H_2O_2 has aroused great attention, so it was used in the present work[10].

In recent years, biocatalysts have been extensively investigated in organic media[11,12]. The range of biocatalysis has considerably extended with the use of organic phase for enzyme and mimic enzyme catalysis[13]. Many investigations indicate that enzymes are able to maintain their catalytic activity in organic solvents[14,15]. It is known that organic phase possess some advantages, such as improved thermostability and sensitivity of enzymes, reduction of interference reaction, but the toxicity, volatility and other subsidiary effects of organic solvents cannot be removed, which limits the widely application of them[16,17]. Therefore, in more recent years, aqueous-organic cosolvent mixtures, namely, previously mix certain amounts of water with the hydrophilic organic phase has gained considerably research. Guo and Mabrouk[18] reported that the stability research of enzyme in aqueous-organic mixtures.

In this paper, we proposed on the application of aqueous-organic cosolvent mixtures aimed at determining H_2O_2 using hemin as mimetic enzyme through spectrophotometry. We found that hemin can maintain their catalytic activity in the mixed solution of isopropanol and water. At the same time, the effects of temperature, reaction time and amount of water to the catalytic activity of hemin were also discussed.

EXPERIMENTAL

Hydrogen peroxide (30 % w/v solution) and isopropanol were obtained from Shanghai Chemical Reagent Company. Catechol and hemin were also obtained from Shanghai Chemical Reagent Company. All chemicals were of analytical-reagent grade. Deionized water was used in all experiments. H_2O_2 stock standard solution was prepared by diluting 1 mL of 30 % H_2O_2

to 50 mL water and standardized by titration with $KMnO_4$, which was 2.0×10^{-2} mol/L in H_2O_2. Catechol stock standard solution of 2.0×10^{-3} mol/L was obtained by dissolving the appropriate weight of catechol in water and further diluted with deionized water. Hemin stock standard solution was prepared by ultrasonicating 3.3 mg hemin in NaOH solution (pH = 10) for 2 min, then diluted with 0.05 M Na_2HPO_4 solution, the concentration of hemin was 1.1×10^{-4} mol/L. All stock standard solution was stored at 4 ºC.

Aqueous-organic mixtures were prepared with different volume ratios of deionized water to isopropanol. They were mixed in a reactor with homogenizer for 1 min. 200 mL H_2O_2 stock solution and 200 mL of 2.0×10^{-3} mol/L catechol stock solution were added into the reactor, respectively. Then the appropriate volume of hemin solution was added into the mixtures to react at 35 ºC about 5 min. At last, the absorbance of oxidation product was measured at 260 nm.

Absorption: $\Delta A = A$ (hemin) - A(blank)

RESULTS AND DISCUSSION

Fig. 1 shows the UV-VIS absorption spectra of catechol-H_2O_2-hemin catalytic system in aqueous-organic mixtures. Curve a and b show the absorption peak of a single catechol solution and the mixed solution of catechol and H_2O_2, respectively. The characteristic peak of catechol was observed at about 271 nm. Curve c indicates that the absorption peak of free hemin is located at 400 nm. Curve d shows an outstanding sharp peak appears at 260 nm after adding the appropriate amount of hemin solution into the mixed solution of catechol and H_2O_2 and reacted at 35 ºC for 5 min, which indicating hemin as mimetic enzyme has a similar catalytic property for the reaction of the oxidation of catechol by H_2O_2 in aqueous-organic media. *Ortho*-quinone is the oxidation product of this reaction system according to the response equation, therefore, the absorption peak at 260 nm is considered to be the peak of oxidation product *o*-quinone.

Catechol is an excellent spectrophotometric substrate in H_2O_2-hemin catalytic system, so it was chosen as the substrate in this research. The effect of substrate concentration on this reaction system was investigated over the range from 0.2×10^{-4} to 1.4×10^{-4} M and the results are shown in Fig. 2. Both in the absence and presence of hemin, the whole trend goes up with the increase of catechol concentration under the same experimental conditions (Fig. 2a). On the other hand, it can be clearly seen from figure 2b that ΔA get the highest point when the catechol concentration was 1.0×10^{-4} M (Fig. 2b). Therefore, the opportune concentration of substrate at 1.0×10^{-4} M was recommended in this work.

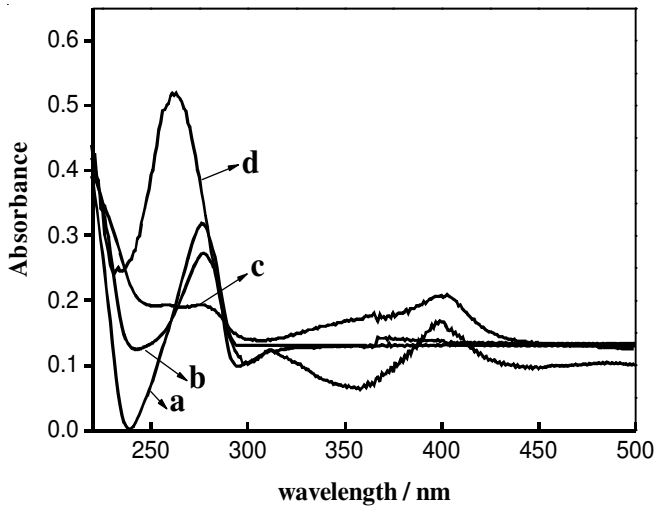

Fig. 1. UV-VIS absorption spectra of (a) catechol solution system (b) catechol and H_2O_2 solution system (c) free hemin solution system (d) catechol-H_2O_2-hemin system in aqueous-organic mixtures

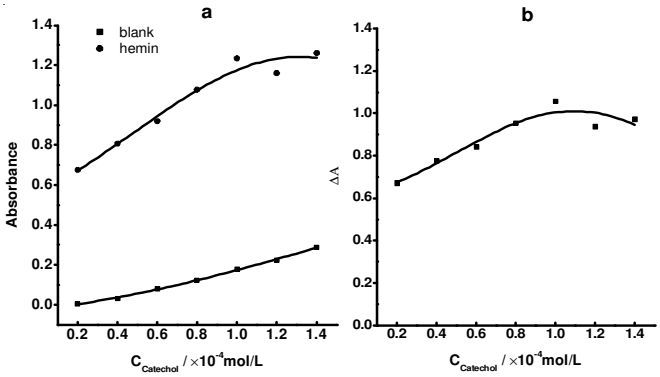

Fig. 2. (a) Concentration of catechol-response curves in the absence and presence of hemin; (b) The different concentration of catechol-A curve at 260 nm; H_2O_2: 1.0×10^{-3} M, hemin: 2.8×10^{-5} M, temperature: 35 ºC, reaction time: 5min

Fig. 3 shows the temperature effect on the catalytic activity of hemin in aqueous-organic mixtures. It can be seen that the absorption peak gradually increased from 25 to 35 ºC and improved rapidly to reach the maximum until 50 ºC. Then, the absorption peak decreases correspondingly when the temperature exceeds 50 ºC. The results indicate that hemin still maintained catalytic property and thermal stability at high temperatures. However, the volatile speed of organic phases accelerating with the increase of temperature. Thus, in order to maintain the accuracy of the experiment results, the temperature was controlled in the range of 25 to 35 ºC.

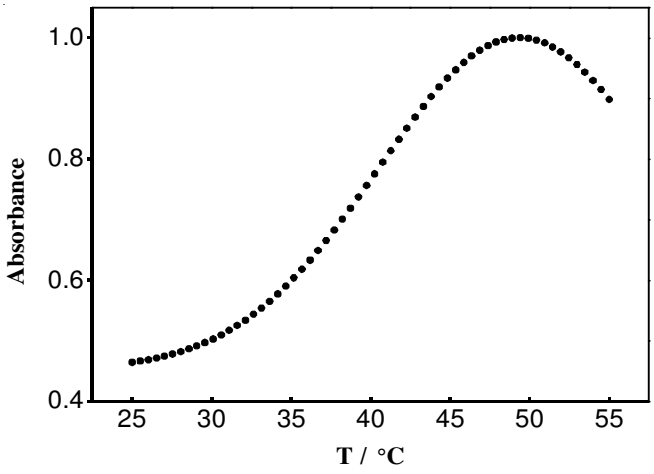

Fig. 3. Temperature-response curve on the catalytic activity of hemin in aqueous-organic medium. H_2O_2: 1.0×10^{-3} M, hemin: 2.8×10^{-5} M, Catechol: 1.0×10^{-4} M, reaction time: 5min

After adding the different amount of hemin solution, the effectiveness of the catalytic activity of hemin was studied by

the oxidation of the substrate in aqueous-organic medium and the results are shown in Fig. 4. It can be seen that the absorption value of the oxidation product at 260 nm grows with the increase of the amount of hemin. The result also suggests that the concentration of hemin has relevant effect on the reaction time. With the increase of the hemin concentration, the absorption value reached an approximately constant and maximum value after reacting for 14, 12 and 8 min, respectively.

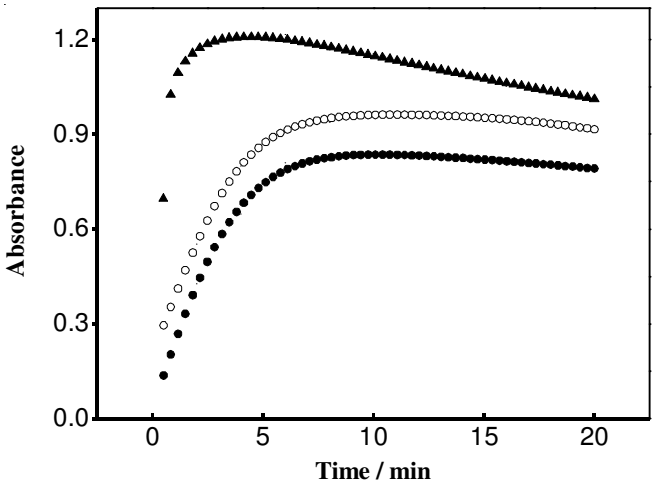

Fig. 4. Concentration of hemin and the relatively reaction time-response curves for H_2O_2 detection. Experiment condition as follow, H_2O_2: 1.0×10^{-3} M, catechol: 1.0×10^{-4} M, hemin (-●-) 1.5×10^{-5} M, hemin (-o-) 2.0×10^{-5} M, hemin (-▲-) 2.8×10^{-5} M, temperature: 35 ºC

The concentration of H_2O_2 greatly influenced the catalytic activity in this system, the absorption value increased after adding more H_2O_2. Fig. 5a shows that the absorption value sharply grows with the increase of the concentration of H_2O_2 up to 0.5×10^{-3} M, when the concentration exceeds it, the absorption value increases slowly. Under the optimal conditions, the corresponding calibration plot is shown in Fig. 5b. It gave a linear response to H_2O_2 in a range of 3.0×10^{-6} M to 1.0×10^{-4} M, with the detection limit of 2.0×10^{-6} M.

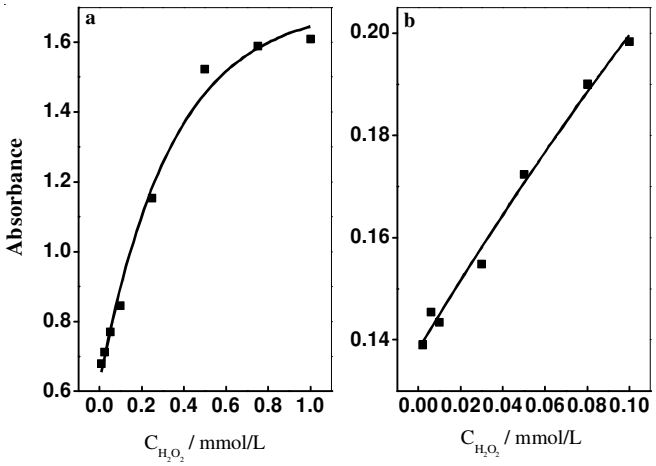

Fig. 5. (a) Concentration of H_2O_2-response curve for $H2O_2$ detection in aqueous-organic medium and (b) the calibration graph for the determination of H_2O_2. Hemin: 1.9×10^{-5} M, catechol: 1.0×10^{-4} M, temperature: 35 ºC, reaction time: 6 min

Conclusion

In this paper, hemin was used to catalyze the reduction of hydrogen peroxide, with the subsequent oxidation of catechol in aqueous-organic medium. The results obtained in this work suggested hemin still has catalytic activity with adding organic phase, the relative longer response time may be contributed to its catalytic property. Future study will aim on the improvement of its catalytic activity in aqueous-organic and non-aqueous medium.

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