Effect of Organic Solvent on the Enzyme Bleaching Agent System

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The Orange II decoloration reaction in the presence of various organic solvents with the HRP-H_2O_2 system was examined. In 5% organic solvent mixing aqueous solutions, the decoloration rates of Orange II were about 0.9-0.5 times of those in the aqueous solutions. Decoloration rate of Orange II decreased, as the concentration of organic solvent increases. The reaction of Orange II decoloration stopped at the 60% dimethyl sulfoxide concentration.

Key words : organic solvent
peroxidase
Orange II
decoloration
enzyme

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(2001年12月10日受稿、2002年1月30日審査終了受理)

1. Introduction

The horseradish peroxidase (HRP) system bleaching agent is a new bleaching agent to prevent dye transfer during the wash. The HRP system bleaching agent is developed from the viewpoint of the practical advantage[1-4], that is more effective in preventing the dye transfer than sodium percarbonate system bleaching agent.

The large number of researches of enzyme reaction in organic solvent has been reported[5-7]. To determine cysteine in Orange II decoloration reaction solution at the glucose oxidase (GOD)-HRP system, we applied the determination method of cysteine using 90% dimethyl sulfoxide (DMSO) solution[8] to our enzyme reaction system. As the result, we found that the HRP reaction was stopped in the 90% DMSO solution. However, the detailed examination has not been made on this system of reaction in organic solvent.

In this study, the effect of organic solvent on Orange II decoloration reaction by the HRP-H_2O_2 system was examined in order to clarify the effectiveness of organic solvent to the HRP reaction.

2. Experimental

2.1 Reagent and Apparatus

Chemical formulas of organic solvents used in this work were shown in Table 1. HRP came from Sigma Chemical Co. Molar concentration (mol dm^{-3}=M) of HRP was determined spectrophotometrically, for which the following extinction value was used[9]: $\varepsilon_{403}=1.02\times10^5\ M^{-1}$. Orange II (C.I. Acid Orange 7) was purchased commercially and purified with paper chromatography. All other chemicals were guaranteed reagents and used without further purification. Water was purified using a Millipore. Using a spectrophotometer MPS-2000 (Shimazu) made all of the absorbance measurements.

2.2 Method

Standard procedure of Orange II decoloration reaction in 5% organic solution with HRP system was as followed; Tris-HCl buffer solution (pH9.0) 3.3ml containing 5.6×10^{-5} M of Orange II and 3.8×10^{-7} M of HRP was put in the vial and stirring. The solution of decoloration reaction was prepared by added 0.2ml of organic solvent to it. Next, 2.7ml of this solution were placed in the cell. The reaction was started when 0.3ml of aqueous solution containing 7.5×10^{-4} M H_2O_2 was injected to the cell. The variation of absorbance of Orange II was monitoring at λ_{max} in the presence of solvent.

Table 1. Chemical formulas of organic solvents

Organic solvent	Chemical formula	Molecular weight
methanol	CH_3OH	32.04
2-propanol	$(CH_3)_2CHOH$	60.10
ethanol	C_2H_5OH	46.07
1-butanol	$CH_3(CH_2)_2CH_2OH$	74.13
2-methoxyethanol	$CH_3OCH_2CH_2OH$	76.10
acetonitrile	CH_3CN	41.05
acetone	CH_3COCH_3	58.08
DMSO	$(CH_3)_2SO$	78.14
1, 4-dioxane	$OCH_2CH_2OCH_2CH_2$	88.11

3. Result and Discussion

3.1 pH dependence of the HRP reaction in organic/water mixed solution (organic aqueous solution)

Decoloration curve of Orange II, in 5% (v/v) DMSO aqueous solution at pH9.0, was shown in Fig.1. At first, in the presence of DMSO (line 1), Orange II decoloration was more slowly than that in 100% aqueous solution (line 2). Two curves were coincidence after 4 minutes. The pH dependence of DMSO mixed solution was compared with that of

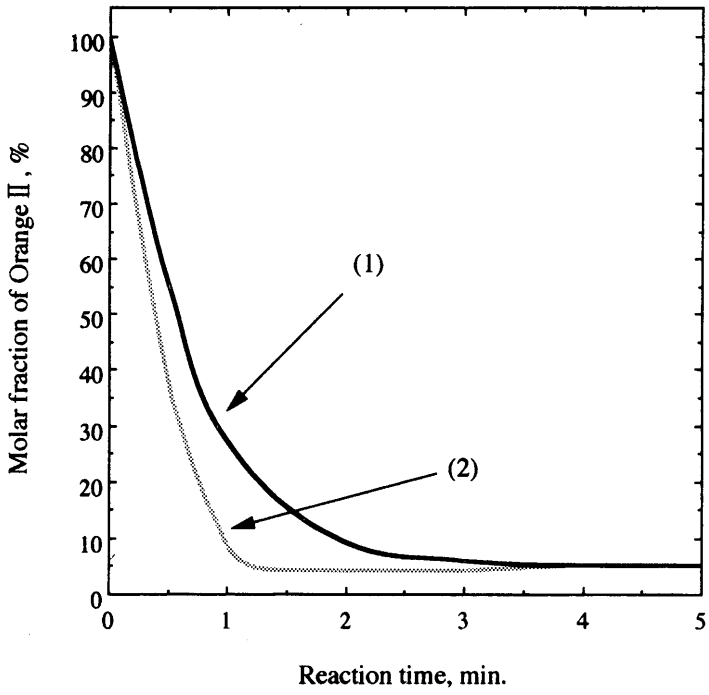

Figure 1. Decoloration curve of Orange II in the presence of 5% DMSO with the HRP-H_2O_2 system.

(1) 5% DMSO solution, (2) water, [HRP]=3.4×10^{-7} M, [Orange II]=5.0×10^{-5} M, [H_2O_2]=7.5×10^{-4} M, Carmody buffer (pH9.0), 20℃.

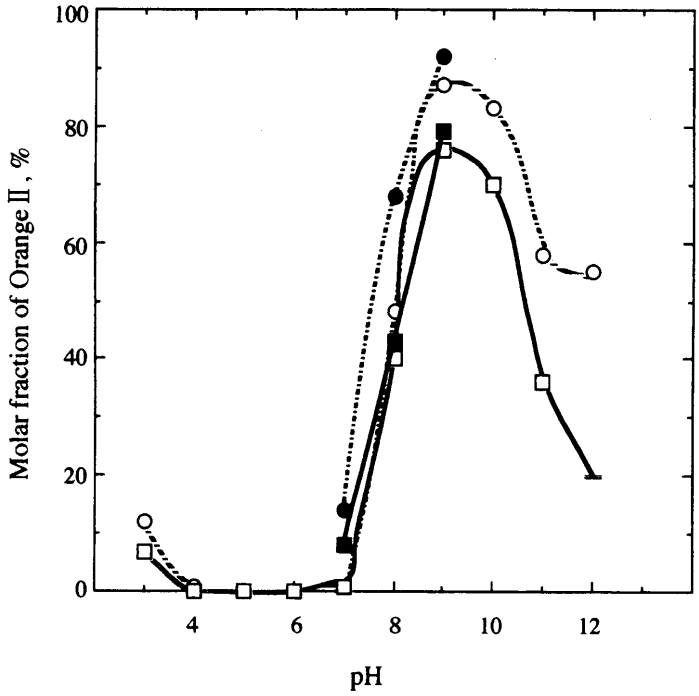

Figure 2. Effect of DMSO to pH dependence of Orange II decoloration (after 1min)

□: 5% DMSO (Carmody buffer), ■: 5% DMSO (Tris-HCl buffer), ○: water (Carmody buffer), ●: water (Tris-HCl buffer), [HRP]=3.4×10^{-7} M, [Orange II]=5.0×10^{-5} M, [H_2O_2]=7.5×10^{-4} M, 20℃.

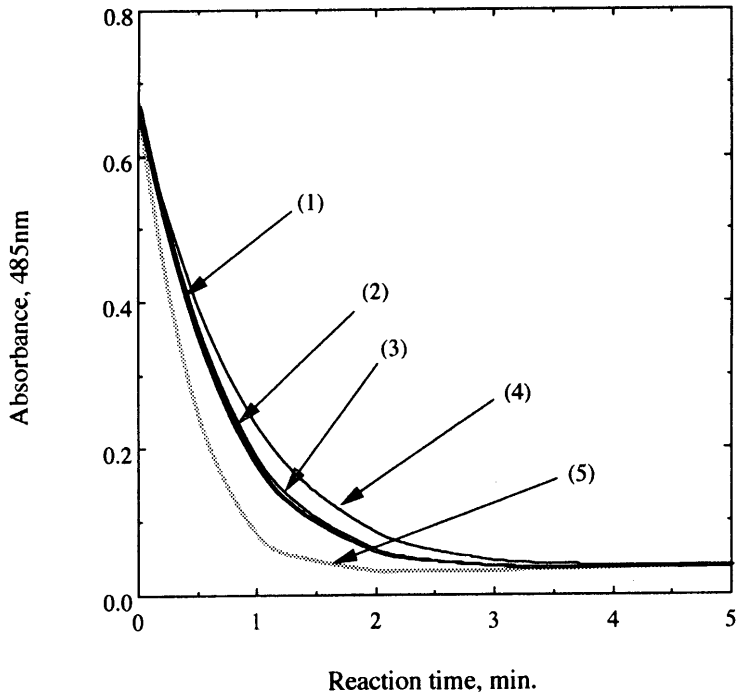

Figure 3. Absorbance variation of Orange II with the HRP-H_2O_2 reaction in aqueous solution containing 5% organic solvent.

(1) 5% DMSO, (2) 5% acetonitrile, (3) 5% acetone, (4) 5% dioxane, (5) water, [HRP]=3.4×10^{-7} M, [Orange II]=5.0×10^{-5} M, [H_2O_2]=7.5×10^{-4} M, Tris-HCl buffer (pH9.0), 20℃.

100% aqueous solution, by the Orange II decoloration ratio at reacted after 1 minute (Fig.2). In 5% DMSO aqueous solution (solid line), the Orange II decoloration ratio decreases a little, but the pH dependence was similar to that in 100% aqueous solution (broken line). Orange II did not decolorate in acid condition, it suggested that the enzyme activity was lost. The Orange II decoloration ratio was maximum at pH9.0, while it was reduced at pH10-12.

Effect of Carmody buffer solution was compared with that of Tris-HCl buffer solution. Tris-HCl buffer solution showed higher Orange II decoloration ratio than Carmody, both of 5% DMSO aqueous solution and 100% aqueous solution. In these results, pH condition of Orange II decoloration reaction in presence of organic solvent was determined to use Tris-HCl buffer, pH9.0.

3.2 Orange II decoloration reaction in nine kinds of organic solvent

Nine kinds of organic solvents (Table 1) were selected for this work. Aqueous solution containing 5% organic solvent was prepared respectively, and used to the HRP-H_2O_2 reaction. Orange II decoloration curves of DMSO, dioxane, acetone, acetonitrile were shown in Fig.3. In these 5% organic aqueous solutions, the Orange II decoloration was more slowly than that in 100% aqueous solution, but there were observed little differences each of them. The rate constants of Orange II were calculated by these decoloration curves. All of 5% organic aqueous solutions were lower rate constant than 100% aqueous solution (Table 2). Methanol, propanol, ethanol, acetonitrile, DMSO showed high rate constants, 0.9-0.7 times 100% aqueous solution. While butanol, acetone, and dioxane were low values such as 0.5 times.

Table 2. Rate constants of Orange II decoloration in 5% organic water mixed solution with the HRP-H_2O_2 system.

Organic solvent	Rate constant min^{-1}	Relative ratio
water	2.81	1.00
methanol	2.56	0.91
2-propanol	1.91	0.68
ethanol	2.52	0.90
1-butanol	1.32	0.47
2-methoxyethanol	1.69	0.60
acetonitrile	1.91	0.68
acetone	1.44	0.51
DMSO	1.97	0.70
1, 4-dioxane	1.21	0.43

3.3 Effect of organic solvent concentration

Methanol, propanol and DMSO, which had high rate constants, were investigated to clear the effect of high organic solvent concentration. Decoloration curves of Orange II in various DMSO concentrations were shown in Fig.4. Decoloration rate of Orange II was inhibited when the concentration of DMSO increased. At the condition of 60% DMSO, Orange II decoloration did not occur and absorbance of reaction solution stabilized for 60 minutes. Reacted after 60 minutes, 5% methanol aqueous solution and 5% propanol aqueous solution showed similar behavior to 5% DMSO aqueous solution (Fig.5). Orange II decoloration made little progress when concentration of 50% methanol and 30% propanol.

Ryu and Dordick[5] reported the following factors of the loss of peroxidase activity by organic solvent: (1) the loss in the catalytic specificity of peroxidase by a penetration of organic solvent. (2) the bulk tertiary structural change of enzyme. Those results we got at high organic solvent concentration suggested that HRP activity must be lost because of the factors indicated by them. Since 60% DMSO aqueous solution stopped Orange II decoloration with the HRP-H_2O_2 system completely, this condition was useful method to stop the HRP-H_2O_2 reaction on the way.

4. Conclusion

Effect of organic solvents on Orange II decoloration reaction with the HRP-H_2O_2 system were examined. The pH dependence of the Orange II decoloration rate in 5% DMSO aqueous solution was similar to that in 100% aqueous solution. Decoloration rate of Orange II decreased when the concentration of organic solvent increased. In the case of DMSO, Orange II decoloration was stopped at 60% concentration. This result of DMSO was useful to stop the HRP-H_2O_2 reaction on the way.

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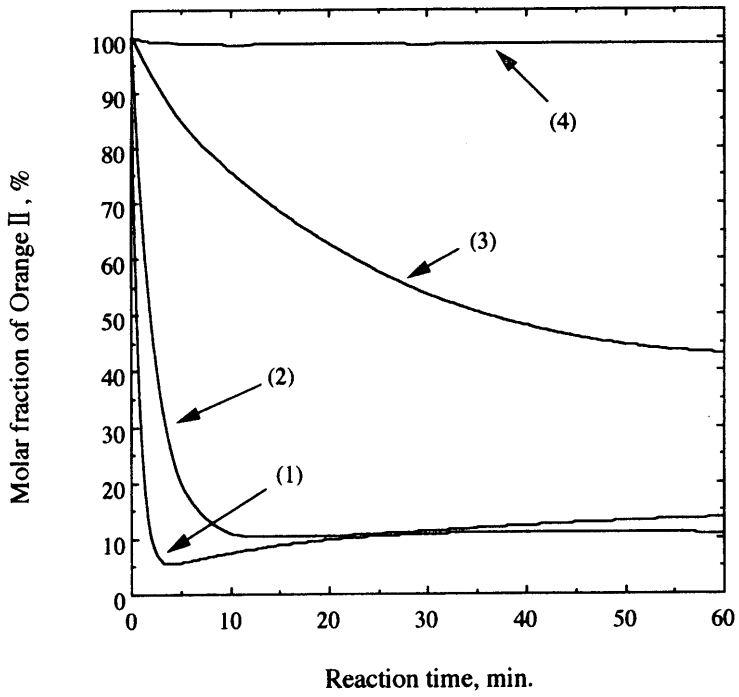

Figure 4. Influence of DMSO concentration to decoloration curve of Orange II with the HRP-H_2O_2 system.

(1) 5% DMSO, (2) 20% DMSO, (3) 40% DMSO, (4) 60% DMSO, [HRP]=3.4×10^{-7} M, [Orange II]=5.0×10^{-5} M, [H_2O_2]=7.5×10^{-4} M, Tris-HCl buffer (pH9.0), 20℃.

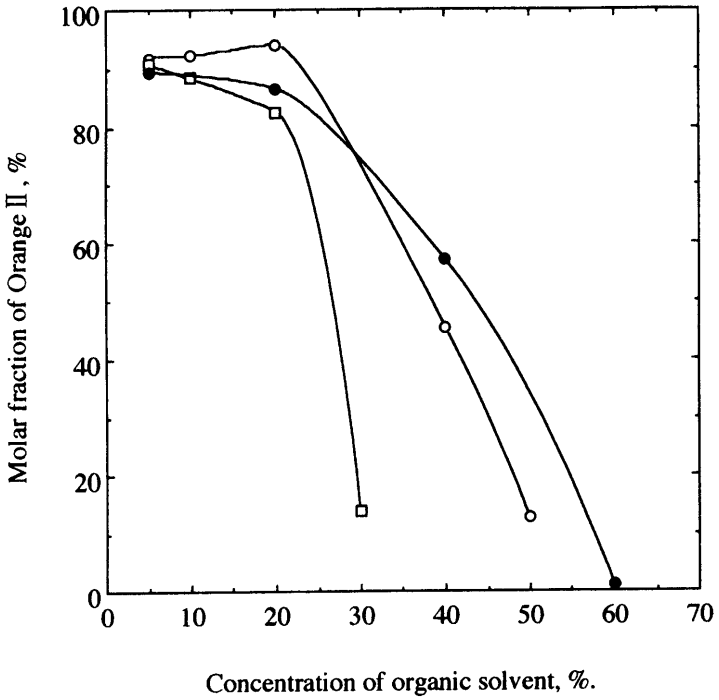

Figure 5. Orange II decoloration ratio with the HRP-H_2O_2 reaction in various concentration of organic solvent.

●: DMSO, ○: methanol, □: 2-propanol, [HRP]=3.4×10^{-7} M, [Orange II]=5.0×10^{-5} M, [H_2O_2]=7.5×10^{-4} M, Tris-HCl buffer (pH9.0), 20℃.

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