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Effects of water-miscible organic solvents on the reaction of lignin peroxidase of *Phanerochaete chrysosporium*

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Abstract

The oxidation of lignin peroxidase (LiP) of *Phanerochaete chrysosporium* was examined using various phenolics and aromatic amines as substrates in organic solvents. LiP oxidized 3,3′-dimethoxybenzidine more effectively in several 70% aqueous water-miscible organic solvents including ethylene glycol and methylcellosolve than in water. LiP activity in water-miscible organic solvents was correlated with the Dimroth–Reichardt parameter ($E_T(30)$) of the solvents, which is directly related to free energy of the solvation process. In addition, absorption spectra of LiP in several glycols were similar to those in succinate buffer. These results suggest that these glycols do not disturb the conformation around the active center, heme, of LiP. Furthermore, LiP catalyzed the oxidation of several phenolics and aromatic amines including 3,3′-dimethoxybenzidine, *o*-, and *p*-phenylenediamines and *o*-aminophenol in 70% aqueous ethylene glycol solution. These substrates were shown to have low ionization potential and high hydrophobicity, and thus to be suitable substrates for the reaction of LiP in water-miscible organic solvents.

Keywords: *Phanerochaete chrysosporium*; Lignin peroxidase; Organic solvents; $E_T(30)$; Substrate specificity

1. Introduction

A ligninolytic enzyme, lignin peroxidase (LiP), has been found in culture filtrates of various white-rot basidiomycetes including *Phanerochaete crysosporium* [1], *Coriolus versicolor* [2], *Phlebia radiata* [3] and *Bjerkandera adusta* [4]. This enzyme catalyzes the H_2O_2-dependent oxidation of lignin and related compounds, halogenated phenolic compounds, polycyclic aromatic hydrocarbonds and other aromatic compounds by a one-electron oxidation mechanism followed by a series of non-enzymatic reactions finally yielding various degradation products [5,6]. Most of these compounds, however, are insoluble in water. Hence, development of a reaction system using organic solvents as the reaction media is necessary for the degradation and transformation of these compounds. It has become clear that many enzymes can function in organic solvents as well as in water [7–9]. In organic solvents, proteases and lipases catalyze reactions which are thermodynamically impossible in water such as peptide syntheses [10] and transesterification [11]. In addition, horseradish peroxidase (HRP) has been found to oxidize phenolics to give the poly-

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merization products [12]. Furthermore, laccase, a ligninolytic enzyme, has been reported to catalyze the oxidation of lignin and substituted phenols in organic solvents [13]. In addition to these observations indicating that enzymes can function in organic solvents, the effects of the properties of organic solvents on enzyme function were also investigated to predict optimal solvent and substrate choices for these reactions [14,15].

Recently, LiP was also shown to be able to catalyze the oxidation of 3,3′-dimethoxybenzidine [16] and polycyclic aromatic hydrocarbons [17]. Moreover, LiP was chemically modified to increase its activity in reaction mixtures containing organic solvents [18,19]. However, the effects of organic solvents on the catalytic activity and substrate specificity of LiP are not well understood. Particularly, no systematic evaluation has yet been performed on the effects of the physical and chemical characteristics of organic solvents and substrates including propensity for hydrogen bond formation, hydrophobicity and ionization potential on the reaction of LiP in organic solvents. Elucidation of the effects of these parameters on the reaction will enable optimal choices of substrates and solvents to be made for the reaction of LiP in organic solvents.

In this study, the reaction of LiP of *P. chrysosporium* was examined using phenolics and aromatic amines in several organic solvents. Furthermore, the effects of various physicochemical parameters of organic solvents on the reaction of LiP are discussed.

2. Materials and methods

2.1. Purification of LiP

LiP was purified from cultures of *P. chrysosporium* strain ATCC 34541 as previously described [16]. The purified LiP had a p*I* of 4.5 and a molecular weight of 40,000. LiP activity was measured spectrophotometrically as described by Tien and Kirk [20] using veratryl alcohol as a substrate. One unit of LiP oxidizes 1 nmol of veratryl alcohol to veratryl aldehyde per minute. Protein was measured by the method of Bradford [21].

2.2. LiP activity assay in organic solvents

In water-miscible organic solvents, the activity was measured spectrophotometrically using a reaction mixture containing 14 mM substrate, 0.25 mM H_2O_2, 70% (v/v) organic solvent and the enzyme solution in a final volume of 1 ml. For measurement of substrate specificity in 70% ethylene glycol medium, 0.1 M Na–tartrate buffer (pH 3.0) was added. On the other hand, in water-immiscible organic solvents, the activity was measured by the method of Vanzquez–Duhalt et al. [22] using 14 mM substrates. One unit of LiP activity in an organic solvent was defined as the amount of enzyme that increased absorbance by 1 per minute.

2.3. Hydrophobicity of substrates

Hydrophobicity of the substrates was estimated as the partition coefficient of substrate between water and *n*-octanol [23]. *n*-Octanol solutions of substrate and water were placed into test tubes and shaken for 30 min at 28°C. After separation into two phases by centrifugation at 11,000 rpm for 5 min, the absorbance of each phase was measured at 280 nm. The partition coefficient ($K_{o/w}$) was defined by

$$K_{o/w} = C_o/C_w$$

where C_w and C_o were the molar concentrations of solute in water and *n*-octanol, respectively.

2.4. Ionization potential of substrates

Ionization potential of each substrate was measured by the absorption maximum of the charge-transfer complex of the substrate and chloranil [24]. A saturated solution of chloranil in chloroform (2 ml) was mixed with the sub-

strate dissolved in a few drops of chloroform. A colored complex was immediately obtained and its absorption spectrum was recorded on a Shimadzu UV-160A Spectrophotometer. Ionization potential values were calculated from the equation IP = $1.228 E_{CT} + 5.038$ (eV), in which the E_{CT} energy corresponds to the absorption maximum of the charge-transfer band of the complex between substrate and chloranil.

2.5. Critical micelle concentration (CMC) of organic solvents

Critical concentration for micelle formation in organic solvents was determined by a modification of the method of Muto et al. [25] Spectral changes of 7,7′,8,8′-tetracyanoquinodimethane (TCNQ) due to the formation of TCNQ-surfactant micelle complex were measured using Nonidet P-40 as a surfactant. TCNQ (5 mg) was added to 10 ml of a mixture containing various concentrations of Nonidet P-40 and organic solvents in L-type tubes. The mixtures were shaken for 72 h at 40°C. After shaking, excess TCNQ was filtered off through glass filters. The absorption spectra of the samples were measured. The absorption maxima obtained were plotted against the logarithm of Nonidet P-40 concentration, and the inflection point coincided with the CMC value.

3. Results

3.1. Activity of LiP in organic solvents

LiP activity in organic solvents was investigated. The best catalytic activity was obtained in a reaction mixture containing 70% ethylene glycol [16]. Therefore, the reaction was conducted in reaction mixtures containing various organic solvents at 70%.

As summarized in Table 1, LiP oxidized 3,3′-dimethoxybenzidine in reaction mixtures containing 70% water-miscible organic solvents including ethylene glycol, diethylene glycol, methylcellosolve and acetone. The LiP activity in 70% aqueous ethylene glycol was 3.7-fold higher than that observed in water. In contrast, the activities in aqueous acetone, methylcellosolve and diethylene glycol media were lower than that in water. However, methanol, tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), dioxane and water-immiscible organic solvents resulted in loss of the activity. These findings indicate that some glycols and acetone can be used as solvents for the oxidation of 3,3′-dimethoxybenzidine by LiP.

To facilitate selection of the best solvents for optimum activity, the relationships between the activity of LiP and physicochemical parameters

Table 1
Activity of LiP in organic solvents

Solvents	Specific activity (U/mg)	$E_T(30)$ [a] (kcal/mol)	CMC (mM)
Water	159	63.1	0.2
70% Acetone	37	42.2	— [c]
70% Dioxane	0	36.0	— [c]
70% DMF	0	43.8	n.d. [d]
70% Ethylene glycol	591	56.3	142.8
70% Methylcellosolve	52	52.3	112.2
70% 1,2-Dimethoxyethane	12	38.2	n.d. [d]
70% THF	0	37.4	n.d. [d]
70% Diethylene glycol	118	53.8	52.8
70% DEGME	21	n.l. [b]	n.d. [d]
70% DEGDE	5	38.6	119.1
70% Pyridine	3	40.2	n.d. [d]
70% Methanol	0	55.5	— [c]
70% 2-Propanol	7	48.6	— [c]
Chloroform	1	39.1	
Ethyl acetate	0	38.1	
Benzene	1	34.5	
Toluene	0	33.9	

LiP activity was measured as described in Section 2 using 3,3′-dimethoxybenzidine dihydrochloride and 3.3′-dimethoxybenzidine as substrates in water and in organic solvents, respectively. One unit of enzyme produced an absorbance of 1.0 after 1 min. Critical micelle concentration (CMC) was measured as described in Section 2.

DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; DEGME, diethylene glycol monomethyl ether; DEGDE, diethylene glycol dimethyl ether.

[a] Data were taken from Ref. [25].

[b] Values not listed in Ref. [25].

[c] These solvents had no obvious CMC value.

[d] Could not be determined.

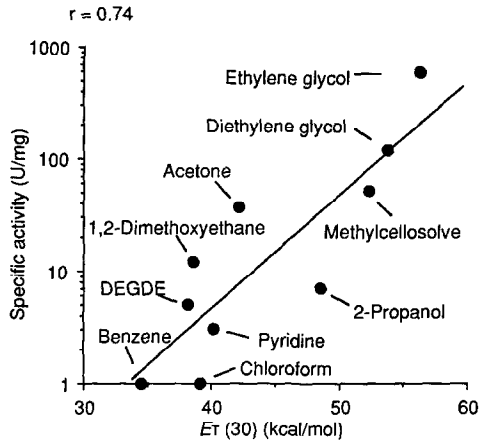

Fig. 1. Relationship between the specific activity of LiP and solvent polarity, $E_T(30)$. The values of $E_T(30)$ were taken from Ref. [25] and are listed in Table 1.

of organic solvents were investigated. As shown in Fig. 1, the specific activity of LiP in different organic solvent systems correlated well with the Dimroth–Reichardt parameter $E_T(30)$.

The activity of LiP in organic solvents and the micelle formation abilities of these solvents were also investigated. To examine the micelle formation abilities of organic solvents, critical micelle concentration (CMC) of the surfactant was measured in various organic solvents. The obtained CMC values of organic solvents are also shown in Table 1. Organic solvents which have low CMC values easily formed micelles, whereas micelles were not formed in organic solvents with no CMC value. Ethylene glycol, methylcellosolve, diethylene glycol, and diethylene glycol dimethyl ether (DEGDE) were found to be micelle-forming solvents, and the activity of LiP was retained in reaction mixtures containing these solvents. However, acetone, dioxane, methanol, 2-propanol had no obvious CMC and markedly decreased the activity of LiP. Acetone has no obvious CMC. The CMC values of THF, diethylene glycol monomethyl ether and 1,2-dimethoxyethane could not be determined by the method using TCNQ, because Nonidet P-40 reacted with TCNQ.

3.2. Absorption spectra of LiP in organic solvents

The spectra of LiP in various solutions are shown in Table 2. LiP showed strong absorption at 407 nm with weak maxima at 500 and 630 nm in 20 mM succinate buffer (pH 4.5). Similar spectra were obtained in 70% aqueous ethylene glycol, diethylene glycol and DEGDE medium, indicating that the heme of LiP was little affected by addition of these organic solvents. However, the intensity of the Soret band was

Table 2
Spectral characteristics of LiP in water-miscible organic solvents

Solvents	Absorption maxima (nm) [ε (mM^{-1} cm^{-1})]		
Succinate buffer (pH 4.5)	408 [186]	500 [21]	634 [14]
70% Acetone	408 [169]	492 [24]	648 [obscure]
70% Dioxane	407 [71]	570 [Broad]	
70% DMF	403 [151]	568 [9]	608 [9]
70% Ethylene glycol	409 [183]	500 [24]	636 [4]
70% Methylcellosolve	401 [162]	472 [Sh 25.0]	594 [19]
70% 1,2-Dimethoxyethane	400 [149]	580 [20]	
70% THF	401 [44]	470 [19]	
70% Diethylene glycol	409 [180]	502 [24]	640 [11]
70% DEGME	420 [112]	550 [17]	
70% DEGDE	409 [155]	500 [19]	644 [11]
70% Pyridine	408 [156]	530 [27]	558 [27]
70% Methanol	399 [262]	488 [Sh 46]	
70% 2-Propanol	400 [152]	472 [Sh 26]	588 [20]

Sh: shoulder peak. DMF, *N,N*-dimethylformamide; THF, tetrahydrofuran; DEGME, diethylene glycol monomethyl ether; DEGDE, diethylene glycol dimethyl ether.

reduced in the presence of 70% dioxane and THF, indicating that these solvents affect the structure of the active site. Acetone seemed to have little effect on heme. The other solvents had strong effects on the heme group in LiP. In addition, the absorption maxima of LiP in ethylene glycol and diethylene glycol gradually changed after a few minutes; new peaks in the spectra in ethylene glycol and diethylene glycol appeared at 410, 538 and 578 nm and 412, 542 and 576 nm, respectively.

3.3. Substrate specificity of LiP in ethylene glycol

Various compounds which have been used as the substrates of laccase and peroxidases were screened as substrates in 70% aqueous ethylene glycol medium. As shown in Table 3, all aromatic compounds tested were oxidized by LiP in the presence or absence of 70% ethylene glycol, except for *p*-aminophenol and hydroquinone. Aromatic amines including 3,3′-dimethoxybenzidine, *o*- and *p*-phenylenediamines and *o*-aminophenol were more easily oxidized in 70% aqueous ethylene glycol medium than phenolic compounds. Furthermore, EG/AQ, which indicates the ratio of the increase of the reaction estimated by comparing the activity in the solvents with those in water, of the *o*-substituted phenylenediamine was higher than those of *p*- and *m*-substituted derivatives. Apparent differences in the substrate specificities were found between water and 70% aqueous ethylene

Table 3
Substrate specificities of LiP in aqueous and ethylene glycol solution

Substrates	λ_{max} (nm)	Specific activity (U/mg)		EQ/AQ (%)	IP (eV)	Log (C_o/C_w)
		AQ	EG			
3,3′-dimethoxybenzidine [a]	444	182	979	538	6.72	1.58
o-Phenylenediamine	440	656	291	44	6.95	0.00
p-Phenylenediamine	459	153	142	93	6.89	−0.30
m-Phenylenediamine	400	17	1	6	7.61	−0.40
Aniline	705	72	5	7	7.32	0.77
o-Anisidine	500	34	10	29	7.11	0.78
p-Anisidine	550	122	6	5	7.02	0.45
m-Anisidine	400	23	0	0	7.25	1.44
o-Aminophenol	440	166	245	148	7.45	0.47
p-Aminophenol	440	0	0	0	— [b]	0.07
m-Aminophenol	440	59	1	2	7.27	0.24
Phenol	399	171	4	2	7.77	1.65
Catechol	398	23	8	35	7.52	0.91
Hydroquinone	— [b]	0	0	0	7.23	0.36
Resorcinol	320	5	0	0	7.55	0.85
Pyrogallol	300	29	5	17	7.50	0.12
1,2,4-Benzenetriol	471	0	0	0	— [b]	0.35
Guaiacol	465	169	2	1	7.51	1.01
HQME	371	21	0	0	7.22	1.50
3-Methoxyphenol	320	9	0	0	7.68	1.41
2,6-Dimethoxyphenol	468	607	2	0.3	7.56	0.24
Vanillyl alcohol	310	2	0	0	7.38	0.22
Veratryl alcohol	310	128	0	0	7.35	0.51

The LiP activity was determined using reaction mixtures containing 14 mM substrate, 100 mM sodium tartrate (pH 3.0), enzyme solution, 0.25 mM H_2O_2, and 70% organic solvents. IP and hydrophobicity ($\log(C_o/C_w)$) of the substrates were determined as described in Section 2. The λ_{max} of the reaction products was determined by measurement of absorption spectrum of the mixture reacted for a few minutes. HQME: hydroquinone monomethyl ether; IP: ionization potential.

[a] The activity was measured using 3,3′-dimethoxybenzidine dihydrochloride and 3,3′-dimethoxybenzidine as the substrates in water and 70% ethylene glycol solution, respectively.

[b] Not determined.

glycol; enzyme activities on 3,3′-dimethoxybenzidine and *o*-aminophenol were higher in ethylene glycol than in water. However, addition of other substrates resulted in loss of activity in 70% aqueous ethylene glycol. Oxidation of varatryl alcohol, the most widely used substrate of LiP, was not catalyzed in 70% aqueous ethylene glycol.

3.4. Effects of ethylene glycol concentration on oxidation of the substrates

The effects of ethylene glycol concentration on the oxidation the various phenolics and aromatic amines were investigated to optimize the reaction conditions. Interestingly, at concentrations of ethylene glycol between 40–70%, an increase of LiP activity in 3,3′-dimethoxybenzidine oxidation was observed (Fig. 2A). Similar increases were observed with *o*-aminophenol, catechol and *p*-phenylenediamine as substrates. However, in reactions using guaiacol, 2,6-dimethoxyphenol, veratryl alcohol, and *m*-anisidine as substrates, an increase in the

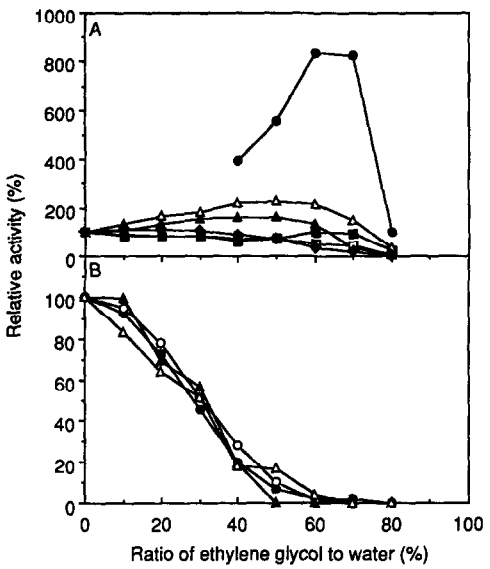

Fig. 2. The activity of LiP was measured using reaction mixtures containing 14 mM substrates, 100 mM sodium tartrate (pH 3.0), 0.25 mM H_2O_2, the enzyme solution, and organic solvent. A: Oxidation rates of 3,3′-dimethoxybenzidine (●), 3,3′-dimethoxybenzidine dihydrochloride (○), *o*-phenylenediamine (□), *p*-phenylenediamine (■), catechol (▲), *o*-aminophenol (△), and pyrogallol (◆) decreased with increasing concentration of ethylene glycol. B: Oxidation rates of veratryl alcohol (▲), guaiacol (●), 2,6-dimethoxyphenol (○), and *m*-anisidine (△) by LiP increased with increasing concentration of ethylene glycol.

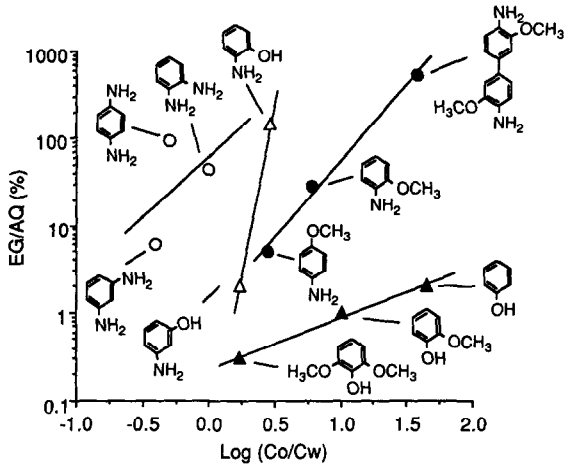

Fig. 3. Hydrophobicity of substrates and LiP activity in 70% aqueous ethylene glycol solution. Hydrophobicity of the substrates was estimated as the partition coefficient of substrate between water and *n*-octanol as described in Section 2. Correlation coefficients for anisidines, phenols, aminophenols and phenylenediamines were 0.99, 0.99, 1.00 and 0.23, respectively.

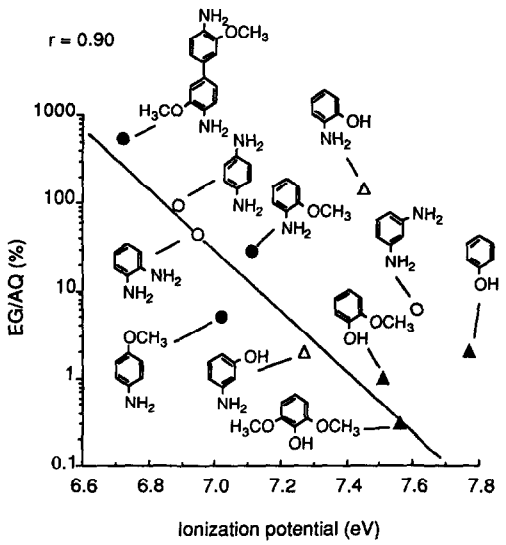

Fig. 4. Ionization potentials of substrates and LiP activity in 70% aqueous ethylene glycol solution. Ionization potentials of the substrates were obtained from the charge-transfer absorption spectra of chloranil and the substrates as described in Section 2. EG/AQ: (activity in 70% aqueous ethylene glycol solution/activity of buffer solution)×100.

ethylene glycol concentration resulted in a marked decrease in the activity (Fig. 2B). Similar results were also obtained with *m*-phenylenediamine, aniline, *o*-, *p*-, and *m*-anisidines, *m*-aminophenol, phenol, resorcinol, hydroquinone monomethyl ether, 3-methoxyphenol, and vanillyl alcohol as the substrates (data not shown). EG/AQ values for the various substrates plotted versus the hydrophobicity of the substrates used are shown in Fig. 3. Substrates were subdivided into four groups: anisidines, phenylenediamines, phenolics, and aminophenols. A simple direct positive correlation was observed for the reaction of LiP in each group. Fig. 4 shows EG/AQ values for the various substrates plotted against the ionization potential. There was a general trend toward higher activity in 70% aqueous ethylene glycol medium with lower ionization potential.

4. Discussion

The specific activity of LiP in different organic solvent systems was found to correlate well with the Dimroth–Reichardt parameter $E_T(30)$ (Table 1, Fig. 1), as was reported in the oxidation of pinacyanol chloride by cytochrome *c* [26]. In addition, the activity of LiP was retained in reaction mixtures containing micelle-forming solvents such as ethylene glycol and methylcellosolve, whereas non-micelle-forming solvents including dioxane, methanol and 2-propanol markedly decreased the activity (Table 1). The values of $E_T(30)$ of organic solvents were measured by the solvatochromism method [27]. The solvatochromism method measures a change in the molecular structure of the dye which is representative of the changes in the three-dimensional structures due to solvent–protein interactions [28]. These values have been used for the determination of the denaturation capacity of solvents in the mathematical model of Khmelnitsky et al. [28]. Therefore, the $E_T(30)$ value is thought to be representative of the conformational changes of enzymes in organic solvents. In other words, LiP is thought to retain its activity in organic solvents which do not cause a significant change in its conformation. In general, the catalytically active conformation of an enzyme is known to be retained by orienting hydrophobic and hydrophilic amino groups to the inside and the surface of the enzyme, respectively. In aqueous solution, surfactant molecules are also known to exist in the form of micelles. Micelles are fairly monodisperse compact aggregates where the hydrophobic groups of the surfactant molecules are sequestered into the center with the hydrophilic groups facing outwards. The structure of micelles is very likely to resemble those of enzymes, suggesting that organic solvents capable of micelle formation are suitable for the reaction of enzymes in organic solvents. In fact, the abilities of organic solvents to maintain the protein structure have been estimated [29], and the relationships between micelle formation ability of organic solvents and enzyme activity in these solvents have been discussed in several reports [30–32] (for review see Ref. [7]). However, good enzyme activity was also found in reaction mixtures containing acetone which had no obvious CMC. The reason for this is not clear at present. Other methods may be available for the measurement of CMC of acetone and organic solvents, the values of which could not be determined here.

LiP contains one iron protoporphyrin IX per molecule as a prosthetic group [33]. Organic solvents are thought to affect not only the conformation of LiP but also the active site, heme. However, spectra of LiP in 70% aqueous ethylene glycol, diethylene glycol, DEGDE and acetone medium were similar to that in 20 mM succinate buffer (pH 4.5), indicating that the heme of LiP was little affected by these organic solvents (Table 2). The other solvents were found to have strong effects on the heme group in LiP, probably due to the rapid complexation of the heme iron with solvent molecules as ligands or alteration of the active site structure by penetration of the solvent into the active site

of LiP. However, penetration of the solvent into the active site was suggested to occur gradually even in ethylene glycol and diethylene glycol, because the absorption maxima of LiP in these solvents gradually changed after a few minutes and new peaks appeared (see Section 3).

Furthermore, the effects of organic solvents on substrate specificity of LiP were investigated using ethylene glycol as the solvent. LiP oxidized the aromatic compounds effectively in the presence of 70% ethylene glycol (Table 3). Especially, aromatic amines were more easily oxidized in 70% aqueous ethylene glycol medium than phenolic compounds. In addition, the *o*-substituted aromatic compounds are favorable for the oxidation in 70% aqueous ethylene glycol medium, and this suggested that the reactivity of *o*- and *p*-substituted aromatic amines was more efficiently enhanced by electron resonance than that of the *m*-substitutive derivative [34]. LiP failed to catalyze the oxidation of veratryl alcohol in the ethylene glycol medium. This was thought to be caused by the decrease in the redox potential of LiP in organic solvents. Interestingly, at concentrations of ethylene glycol between 40–70%, increases of LiP activity in 3,3′-dimethoxybenzidine, *o*-aminophenol, catechol and *p*-phenylenediamine oxidations were observed (Fig. 2). In general, organic solvents are believed to decrease the catalytic activities of enzymes. However, these findings indicate that the activity of LiP in organic solvents depends on not only the nature of solvent but also on that of the substrate. Similar results were obtained in the reaction of HRP in organic solvents. Ryu and Dordick reported enhancement of the catalytic activity of HRP using *p*-cresol as a substrate in reaction mixtures containing 60–80% dioxane [35]. They also discussed the effects of hydrophobicity of substrate on the catalytic turnover of HRP in water-miscible organic solvents including dioxane and methanol. A simple direct positive correlation between hydrophobicity of the substrates and the activity was also observed for the reaction of LiP in 70% ethylene glycol medium (Fig. 3). These results suggested that hydrophobic aromatic compounds are more favorable substrates in 70% ethylene glycol than hydrophilic compounds. Hydrophobic aromatic compounds likely provide increased binding energy for catalysis over hydrophilic aromatics and the activity increased in less polar media, as was reported in the reaction of HRP by Ryu and Dordick [35].

In addition, ionization potentials of substrates were also reported to be important in the reaction of peroxidase [36–38]. The capacities of aromatic compounds to form free radicals are related to their ionization potential. The ionization potential of a molecule is defined as the energy required to completely remove an electron from the neutral particle in its ground state. The removal of one-electron from the compound generates a free radical. The ability of LiP to oxidize the substrates in 70% ethylene glycol medium was also correlated with ionization potential of the substrates (Fig. 4). Therefore, in addition to hydrophobicity, ionization potentials of the substrates is thought to also be important for the increase in activity of LiP in reaction mixtures containing water-miscible organic solvents.

A possible mechanism of the reaction of LiP in organic solvents is shown in Fig. 5 based on the results obtained in this study. In general, enzymes are known to have a hydration shell. In reaction mixtures containing water-miscible or-

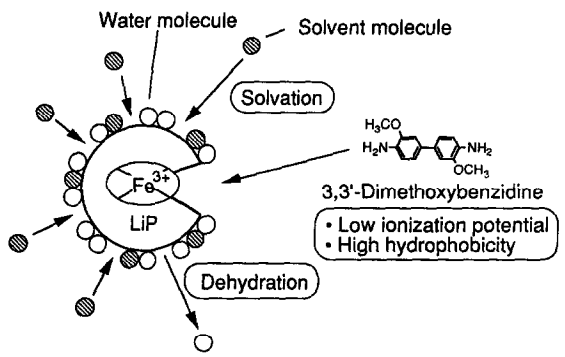

Fig. 5. Reaction of LiP in reaction mixtures containing water-miscible organic solvents.

ganic solvents, distortion of the hydration shell caused by introduction of organic solvent into the enzyme solution upsets the system of interactions supporting the native conformation, which results in the loss of catalytic activity. However, water-miscible organic solvents such as ethylene glycol, diethylene glycol and methylcellosolve, which possess sufficient solvation capabilities, are thought to easily solvate the enzyme in place of water molecules. In addition, these solvents are thought not to disturb the environment of the active center. Moreover, 3,3′-dimethoxybenzidine was the most suitable substrate among those tested, because it has high hydrophobicity and low ionization potential. Such substrates are thought to provide increased binding energy resulting in an increase in the activity in these water-miscible organic solvents [35].

References

[1] M. Tien and T.K. Kirk, Science 221 (1983) 661.
[2] P.J. Dodson, C.S. Evans, P.J. Harvey and J.M. Palmer, FEMS Microbiol. Lett. 42 (1987) 17.
[3] M.-L. Niku-Paavola, E. Karhunen, P. Salola and V. Raunio, Biochem. J. 254 (1988) 877.
[4] Y. Kimura, Y. Asada and M. Kuwahara, Appl. Microbiol. Biotechnol. 32 (1990) 436.
[5] M.H. Gold, H. Wariishi and K. Valli, ASC Symp. Ser. 389 (1989) 127.
[6] K.E. Hammel, Enzyme Microb. Technol. 11 (1989) 776.
[7] Y.L. Khmelnitsky, A.V. Levashov, N.L. Klyachko and K. Martinek, Enzyme Microb. Technol. 10 (1988) 710.
[8] J.S. Dordick, Enzyme Microb. Technol. 11 (1989) 194.
[9] J.S. Dordick, Curr. Opin. Biotechnol. 2 (1991) 401.
[10] H. Kise and A. Hayakawa, Enzyme Microb. Technol. 13 (1991) 548.
[11] W. Chulalaksananukul, J.S. Condoret and D. Combes, Enzyme Microb. Technol. 14 (1992) 293.
[12] J.S. Dordick, M.A. Marletta and A.M. Klibanov, Biotech. Bioeng. 30 (1987) 31.
[13] O. Milstein, A. Huttermann, A. Majcherczyk and K. Schulze, J. Biotechnol. 30 (1993) 37.
[14] K. Ryu and J.S. Dordick, J. Am. Chem. Soc. 111 (1989) 8026.
[15] E. Rubio, A. Fernandez-Mayorales and A.M. Klibanov, J. Am. Chem. Soc. 113 (1991) 695.
[16] S. Yoshida, T. Watanabe, Y. Honda and M. Kuwahara, Biosci. Biotech. Biochem. 60 (1996) 711.
[17] R. Vazquez-Duhalt, D.W.S. Westlake and P.M. Fedorak, Appl. Environ. Microbiol. 60 (1994) 459.
[18] R. Vazquez-Duhalt, D.W.S. Westlake and P.M. Fedorak, Appl. Microbiol. Biotechnol. 42 (1995) 675.
[19] S. Yoshida, T. Watanabe, Y. Honda and M. Kuwahara, Biosci. Biotech. Biochem. 60 (1996) 1805.
[20] M. Tien and T.K. Kirk, Proc. Natl. Acad. Sci. USA 81 (1984) 2280.
[21] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[22] R. Vazquez-Duhalt, P.M. Fedorak and D.W.S. Westlake, Enzyme Microb. Technol. 14 (1992) 837.
[23] M. Shigematsu, H. Sugino, Y. Shinoda and M. Tanahashi, Mokuzai Gakkaishi 41 (1995) 1151.
[24] E.L. Cavalieri, E.G. Rogan, R.W. Roth, R.K. Saugier and A. Hakam, Chem. Biol. Interact. 47 (1983) 87.
[25] S. Muto, Y. Aono and K. Meguro, Bull. Chem. Soc. Jpn. 46 (1973) 2872.
[26] R. Vazquez-Duhalt, K.M. Semple, D.W.S. Westlake and P.M. Fedorak, Enzyme Microb. Technol. 15 (1993) 936.
[27] C. Reichardt, Angew. Chem. Int. Ed. Engl. 18 (1979) 98.
[28] Y.L. Khmelnitsky, V.V. Mozhaev, A.B. Belova, M.V. Segreeva and K. Martinek, Eur. J. Biochem. 198 (1991) 31.
[29] A. Ray, Nature 231 (1974) 313.
[30] A.M. Klibanov, A.N. Semenov and K. Martinek, Bioorgan. Khiniya (Russ.) 4 (1978) 82.
[31] K. Martinek, A.M. Klibanov and I.V. Berezin, J. Solid-phase Biochem. 2 (1977) 343.
[32] K. Martinek and A.N. Semenov, J. Appl. Biochem. 3 (1981) 93.
[33] V. Renganathan and M.H. Gold, Biochemistry 25 (1986) 1626.
[34] S.H. Pine, Organic Chemistry, 5th Ed. (McGraw-Hill Co., Singapore, 1987) p. 909.
[35] K. Ryu and J. Dordick, Resources Conserv. Recycl. 3 (1990) 177.
[36] Ph.-J. Kersten, B. Kalyanaraman, K. Hammel and T.K. Kirk, Lignin Enzymatic and Microbial Degradation, Symposium International, Paris, France, 23–24 avril 1987, p. 75.
[37] K.E. Hammel, B. Kalyanaraman and T.K. Kirk, J. Biol. Chem. 261 (1986) 16948.
[38] E. Cavalieri and E. Rogan, Environ. Health Perspect. 64 (1985) 69.