Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 44 (2007) 53–59
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Catalytic activity of *Cerrena unicolor* laccase in aqueous solutions of water-miscible organic solvents—Experimental and numerical description

J. Rodakiewicz-Nowak [a], A. Jarosz-Wilkołazka [b,*]

[a] *Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Kraków, Poland*
[b] *Department of Biochemistry, M. Curie-Skłodowska University, Lublin, Poland*

Received 22 March 2006; received in revised form 25 July 2006; accepted 19 August 2006
Available online 5 October 2006

Abstract

The stability and catalytic activity during oxidation of syringaldazine (SGZ) by laccase from *Cerrena unicolor* (CUL) was studied in aqueous solutions of ethanol, acetone and dimethyl sulfoxide (DMSO). Additional, the oxidation of other two substrates, catechol (CAT) and 2,6-dimethoxyphenol (DMOP), was studied in ethanol and in DMSO solutions, respectively. It was observed that in 3 M solutions of the studied solvents CUL was practically stable for 30 min of the experiments, which show its good applicability in solutions of water-miscible organic solvents. Moreover, it was observed that the effect of the tested organic solvents on the maximum rate of oxidation values (V_{max}) of different substrates was not dependent on the substrate. Within the studied range of concentrations (up to 8 M) ethanol did not affect V_{max} values and the presence of DMSO reduced it at roughly the same rate for the studied substrates. In the case of SGZ as a substrate, the effect of the solvent on V_{max} values increased in the order ethanol < acetone < DMSO and in the case of K_M in the order DMSO < acetone < ethanol. The experimental relationships between the content of organic solvents and the observed kinetic parameters were numerically described using the exponential relationships on the solvent concentrations, in the first approximations replacing the variability of the activity coefficients of the reactants. The fitted exponential coefficients were observed with the values observed for other fungal laccases and discussed.

Keywords: Laccase; Catalytic activity; Ethanol; Dimethyl sulfoxide; Acetone

1. Introduction

Laccases (benzenediol: oxygen oxidoreductases, E.C. 1.10.3.2) are copper enzymes (four copper atoms per subunit), catalysing the oxidation of a wide range of phenolic compounds, arylamine compounds and some inorganics, simultaneously reducing oxygen to water [1,2]. Laccases and laccase-expressing white rot fungi are intensively studied as promising agents in environmental remediation (degradation of halogen substituted phenols and anilines, pesticides, dioxins, chlorolignins, and azo dyes), in the biobleaching of a wood pulp, and in degradation of polyaromatic hydrocarbons [3]. Laccases (but also lignin peroxidases and Mn-dependent peroxidases) catalyse these processes, either alone or in the presence of appropriate mediators. Many of laccase-catalysed processes are carried out in the presence of organic solvents [4–7]. The effect of organic solvents on the stability and catalytic activity of laccases depends on the source of the enzyme, cultivation conditions, and purification procedure [8–11]. Therefore, a method of comparison the influence of different organic solvents on various samples of laccases has the practical importance.

The addition of water-miscible organic solvents to the aqueous medium of enzymatic reactions usually reduces the observed reaction rates. The molecules of solvent may directly interact with the enzymes, thus changing their structure, exchanging water molecules in the active centre of proteins, causing irreversible inactivation of the enzymes [12–14], but it is not always the case. However, in all cases the addition of organic solvents affects various physico-chemical properties of the medium of enzymatic reaction, such as its hydrophobicity, dielectric constant, pH, varying the chemical potentials of all reactants present in the solutions, and the free energy of substrate binding by the enzyme, even when the observed maximum reaction rates remain practically not affected. It was also observed for laccase from *Phlebia radiata* in solutions of short chain alkanols [11].

* Corresponding author. Tel.: +48 81 537 56 65; fax: +48 81 537 51 02.
E-mail addresses: ncrodaki@cyf-kr.edu.pl (J. Rodakiewicz-Nowak), ajarosz@biotop.umcs.lublin.pl (A. Jarosz-Wilkołazka).

1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.08.005

Catalytic activity of the enzymes strongly depends on their ability to bind the substrates, thus on the energy difference between binding the substrate by the enzyme and substrate–solvent interactions [15,16]. Thermodynamic description of the influence of organic solvents on the enzymes activity has already a long history. Dordick [16] was the first who tried to separate the effect of the solvent addition on the enzyme from that exerted on substrate. He found the correlation between the hydrophobicity, Hammett constant and Taft constant of the substrate and the observed catalytic activity of the enzyme during the oxidation of several *m*- and *p*-substituted phenols by horseradish peroxidase in various mixtures of organic solvents and water [16,17]. The observed relationships seem to indicate moreover that even 80% (v/v) dioxane, methanol and acetonitrile did not destabilize the peroxidase–substrate complex. Wescott and Klibanov described the substrate specificity of subtilisin Carlsberg in water and anhydrous organic solvents and compared the partitioning of two substrates between water and the solvent [18].

The possible effect of organic solvents on the enzyme reaction rate through changes of the thermodynamic activity of water and the activity coefficients of the substrate and enzyme species was widely discussed by Lee and co-workers [19,20]. Smith and Canady [21] showed that the effect of the solvent on the activity coefficient of diluted solutions of an organic substrate may be approximated with the ratio of its solubility in water and in the respective solution of the organic solvent, varying exponentially with the solvent concentration over a considerable range of the solvent contents. The authors described the variation of the kinetic parameters of the hydrolysis of methylhydrocinnamate catalysed by α-chymotrypsin in aqueous methanol solutions using the experimentally determined the relationship of solubility [21].

The kinetics of laccase-catalysed reactions is a complex, two-site ping-pong bi-bi type of reaction [22], intensively studied in many laboratories [23]. Nevertheless, the simple Michaelis–Menten equation is commonly used to describe the kinetics of laccase-catalysed reaction, as the enzyme is always oversaturated with oxygen as the second substrate. The fulfilment of the Michaelis–Menten equation for complex enzymatic reactions means that both k_{cat} and K_M are the functions of the kinetic constants of the steps of the catalytic process, and the apparent K_M contains the true substrate binding constant. For laccases the exact relationships are not known yet.

We may expect that at least for some enzymes the solvent effect on the substrate will predominate in the variability of the apparent K_M with the type and concentration of the solvent. Our recent results on the solvent effects on the apparent K_M values for the oxidation of Mn^{2+} ions and DMOP catalysed by the versatile peroxidase from *Bjerkandera fumosa* confirm this hypothesis [24]. The observed K_M values increase with the solvents concentrations for DMOP, and decrease for Mn^{2+}, along with the change of their solubility. Moreover, studying various phenols oxidizing enzymes, such as bacterial tyrosinase [25], *P. radiata* laccase [26] and *B. fumosa* peroxidase [24], we have found that the observed K_M values, V_{max} and V_{max}/K_M values varied exponentially with the solvent concentrations, while studied over the range of its low and moderate contents. This prompted us to use the exponential relationships in describing and comparing the non-specific influence of various solvents on fungal laccases of various sources and to check how much the observed parameters of the solvent influence on V_{max} and K_M depend on the laccase sample, for the oxidation of selected substrates under the same experimental conditions.

2. Experimental

2.1. Materials

The white rot fungus *Cerrena unicolor* was cultivated in liquid, submerged cultures in 5 L bottles using mineral medium according to Lindeberg and Holm [27]. The culture medium was filtered and concentrated by ultrafiltration using a 10 kDa cut-off membrane (Amicon). The concentrated fluid was applied to a Sephadex G-25 column and the elution was performed with 1 mM Tris–HCl buffer, pH 7. The laccase-active fractions were collected, and lyophilised. Then, they were purified by the anion exchange chromatography on DEAE Toyopearl 650 M and dialysed [28].

DMSO of spectroscopic grade, KH_2PO_4, Na_2HPO_4, p.a. were from Fluka (Switzerland), ethanol and acetone p.a. were from POCh (Poland), 4-hydroxy-3,5-dimethoxybenzaldehyde azine (syringaldazine, SGZ), 2,6-dimethoxyphenol (DMOP) and catechol (CAT) were from Sigma/Aldrich (Germany).

2.2. Methods

Kinetic measurements of enzymatic activity were carried out using UV/Vis spectrophotometer Shimadzu PC2101 in 100 mM sodium acetate buffer pH 5.52, at 30 °C for SGZ and DMOP and in 100 mM Na_2HPO_4/KH_2PO_4 buffer pH 5.7 for CAT, at 30 °C. The rate of SGZ oxidation was studied at 525 nm ($\varepsilon = 63\,000\ M^{-1}\ cm^{-1}$ [29]), the rate of CAT oxidation was studied at 410 nm ($\varepsilon = 2211\ M^{-1}\ cm^{-1}$ [30]), and the rate of DMOP oxidation was measured at 468 nm ($\varepsilon = 14\,800\ M^{-1}\ cm^{-1}$ [31]). It is worthy to note that in the last case many authors used different values of absorption coefficient: $49\,600\ M^{-1}\ cm^{-1}$ [32], $35\,645\ M^{-1}\ cm^{-1}$ [30] or $27\,500\ M^{-1}\ cm^{-1}$ [33]. This absorption coefficient is difficult to measure, due to the subsequent polymerization of the oxidation product what happens both in aqueous and mixed aqueous–organic solvent solutions of high water content. The studied range of substrate concentrations exceeded at least three times the fitted K_m values for each studied Michaelis–Menten relationship.

The experimental data were first analysed graphically and the linearity of the C_S/V_{in} versus C_S plot, where C_S represents the substrate concentration and V_{in} denotes the initial reaction rate, was checked. No systematic deviations from linearity were observed. Afterwards, they were fitted to the non-linear Michaelis–Menten equation. All numerical fittings were done using Origin 5.0.

CUL stability in aqueous buffer solutions in 3 M DMSO, acetone or ethanol was studied in triplicate, using the initial rate of 70 μM DMOP oxidation as a marker of the laccase catalytic

Table 1
Models of the effect of organic solvents on the kinetic parameters of the Michaelis–Menten equation

Model	V_{max}	K_M	lim V_{max}	lim K_M
I	V^0_{max}	$K^0_M \exp(\varepsilon C_{sol})$	V^0_{max}	$K^0_M(1+\varepsilon C_{sol})$
II	$V^0_{max} \exp(-\beta C_{sol})$	$K^0_M \exp(\varepsilon C_{sol})$	$V^0_{max}/(1+\beta C_{sol})$	$K^0_M(1+\varepsilon C_{sol})$
III	$V^0_{max} - \delta C_{sol}$	$K^0_M \exp(\varepsilon C_{sol})$	$V^0_{max} - \delta C_{sol}$	$K^0_M(1+\varepsilon C_{sol})$
IV	$V^0_{max} \exp(-\beta C_{sol})$	$K^0_M \exp(\varepsilon C_{sol})(1+C_{sol}/K_I)$	$V^0_{max}/(1+\beta C_{sol})$	$K^0_M(1+\varepsilon C_{sol})(1+C_{sol}/K_I)$
V	$V^0_{max} \exp(-\beta C_{sol})/(1+C_{sol}/K_{IS})$	$K^0_M \exp(\varepsilon C_{sol})(1+C_{sol}/K_I)/(1+C_{sol}/K_{IS})$	$V^0_{max}/[(1+\beta C_{sol})(1+C_{sol}/K_{IS})]$	$K^0_M(1+\varepsilon C_{sol})(1+C_{sol}/K_I)/(1+C_{sol}/K_{IS})$
VI	$V^0_{max} - \delta C_{sol}$	$K^0_M \exp(\varepsilon C_{sol})(1+C_{sol}/K_I)$	$V^0_{max} - \delta C_{sol}$	$K^0_M(1+\varepsilon C_{sol})(1+C_{sol}/K_I)$

where V_{in} is the initial reaction rate; V_{max} the maximum reaction rate; K_M the Michaelis constant; C_S the substrate analytical concentration; a_s the thermodynamic activity of the substrate; C_{sol} the concentration of the organic solvent; ε the substrate activity coefficient, leading to the apparent inhibition constant; β and δ the parameters of the variability of V_{max} with the solvent concentration, as explained in the text above the table; K_I the competitive inhibition constant; K_{IS} the mixed inhibition constant; the superscript zero denotes the solution that does not contain an organic solvent. Limiting conditions for lim V_{max} and lim K_M are as follows: low values of εC_{sol} or low values of βC_{sol}, respectively.

activity after the incubation in the respective reaction medium for a desired time.

Protein concentrations were determined using Bradford reagent and bovine serum albumin as a standard [34].

3. Results and discussion

3.1. Stability of laccase from C. unicolor (CUL) in solutions of the studied organic solvents

CUL stability in the aqueous buffer solutions and in 3 M solutions of DMSO, acetone and ethanol was studied for 30 min (Fig. 1), by comparing the initial rate of DMOP oxidation after the desired time of the enzyme incubation in a thermostatted cuvette (30 °C).

It may be seen that the presence of organic solvents immediately decreases the observed reaction rate, but the observed catalytic activity of CUL does not reduce during the first 30 min of the enzyme incubation. The enzyme does not denaturate under these conditions and may be practically applied for performing various reactions requiring the presence of organic solvents in the system.

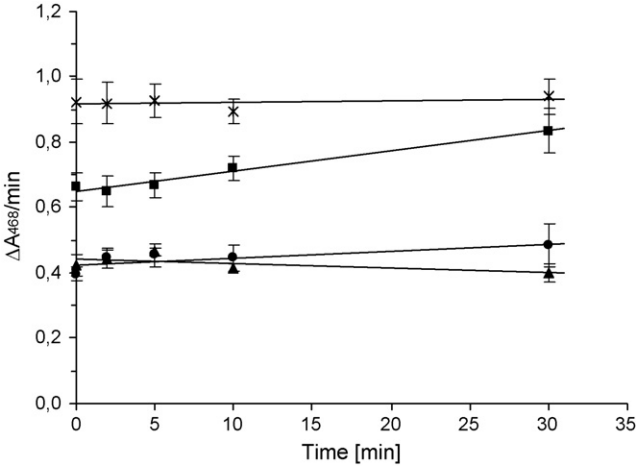

Fig. 1. The effect of organic solvents on the initial rate of DMOP oxidation (ΔA_{468}/min) catalysed by CUL in the buffer (×) or in the presence of ethanol (■), acetone (●), and DMSO (▲).

3.2. Numerical description of the non-specific influence of organic solvents on the kinetic parameters of the Michaelis–Menten equation

Organic solvents affect the whole system, changing the solvation of molecules and thermodynamic activities of all its components, including proteins. Earlier studies of the effect of low amounts of water-miscible organic solvents (up to 2.5 M) on the activity of many enzymes, including laccases, have shown that this effect may be apparently described using the formal equation of the competitive or mixed inhibition [11]. Smith and Canady [21] showed that these equations might be also obtained assuming that the effect of the organic solvent on the activity coefficients of the substrates and most possibly of enzyme and enzyme–substrate complexes take an exponential form. In such a case, the observed Michaelis constants are exponentially or linearly dependent on the concentration of the organic solvent [21]. The verification of the Smith and Canady's assumptions requires the comparison of several enzymes in oxidation of the same substrates, not performed yet.

Enzymatic reactions catalysed by laccases in aqueous solutions may be formally described by the Michaelis–Menten equations (1, 2) in the absence or presence of organic solvents (abbreviations are explained under Table 1):

$$V_{in} = \frac{V^0_{max} C_s}{K^0_M + C_s} \quad (1)$$

$$V_{in} = \frac{V_{max} a_s}{K_M + a_s} \quad (2)$$

We may assume after Smith and Canady [21] that in the solutions of the solvent the thermodynamic activity of substrate (a_s) is depend on its concentration (C_s) and on the concentration of solvent (C_{sol}), with exponential term replacing the activity coefficient of the substrate: $a_s = C_s \exp(-\varepsilon C_{sol})$. Smith and Canady observed that for the particular case of the studied reaction also V_{max} was exponentially dependent on C_{sol} with practically

the same exponential parameter [21]. We assume that for simple enzymatic reactions, truly following the Michaelis–Menten kinetics the exponential term $\exp(-\beta C_{sol})$ gives the ratio of the activity coefficients of the transition state and of the enzyme. For more complex enzymatic reactions, this term may be a function of more than one activity coefficient of various transition forms of the enzyme. Very often both activity coefficients vary similarly with the solvent concentration, thus giving the V_{max} equal to V^0_{max}. If the solvent does not affect specifically the active centre of the enzyme and k_{cat} does not change with solvent concentration and its presence than we may observe V_{max} equal to V^0_{max}. At sufficiently low products of εC_{sol} and βC_{sol} the respective exponents tend to the forms: $1+\varepsilon C_{sol}$ and $1+\beta C_{sol}$. In some cases, the observed values of V_{max} linearly decrease with the solvent concentration beyond the limit of extrapolation of the exponential term to the linearity. It is taken into account in the Model III (Table 1), with the linearity coefficient δ.

Inserting the appropriate forms of V_{max} and a_s into Eq. (2) we obtain the general equation of the enzymatic reaction in the solvent system:

$$V_{in} = \frac{V^0_{max}\exp(-\beta C_{sol})C_s}{K^0_M \exp(\varepsilon C_{sol}) + C_s} \quad (3)$$

Table 1 summarizes various possible equations describing the effect of organic solvent on the enzyme catalytic activity resulting from Eq. (3).

The limiting terms of the values V_{max} and K_M presented for the Models I and II resemble the equations describing the competitive and mixed inhibition of the enzymatic reactions. In general, organic solvents are neither competitive nor mixed inhibitors, because they do not replace substrate molecules from the active centre of the enzyme, and their inhibition concentrations are much higher than for the true inhibitors. To show the equations describing the double role of the solvents as non-specific inhibitors and the competitive or mixed inhibitors of the enzymes the Models IV–VI have been also introduced, with Eq. (4) for the competitive inhibition:

$$K_M = K^0_M \exp(\varepsilon C_{sol})\frac{1+C_{sol}}{K_I} \quad (4)$$

and Eqs. (5a) and (5b) for the mixed inhibition:

$$K_M = K^0_M \exp(\varepsilon C_{sol})\frac{1+C_{sol}/K_I}{1+C_{sol}/K_{IS}} \quad (5a)$$

and

$$V_{max} = \frac{V^0_{max}}{1+C_{sol}/K_{IS}} \quad (5b)$$

3.3. Effect of water-miscible organic solvents on the observed catalytic activity of CUL

Three laccase substrates were studied in this work. The maximum oxidization rates in buffer did not vary much for them. They reached 30.8 μmol/min^{-1} mg^{-1} of CUL for DMOP, 28.3 μmol/min^{-1} mg^{-1} of CUL for CAT and about 22 μmol/min^{-1} mg^{-1} of CUL for SGZ (extrapolated value). K^0_M values were 0.6 mM for CAT, 10 μM for DMOP and less than 10 μM for SGZ. The range of solvent concentrations for SGZ starts from 1 M or more solutions because of the poor solubility of SGZ in water, limiting the possibility of determining the Michaelis–Menten kinetic parameters.

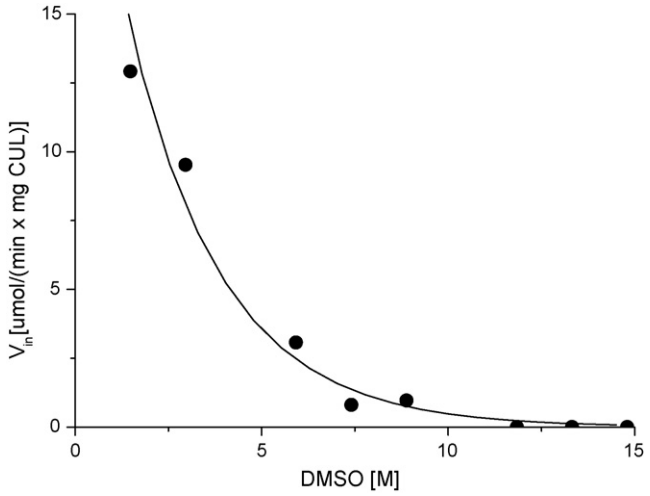

Fig. 2. The effect of DMSO on the oxidation of 0.02 M DMOP catalysed by CUL.

Fig. 2 shows that at the beginning the reaction rate of DMOP oxidation catalysed by CUL drops down relatively rapidly, even at high substrate and DMSO concentrations, at which a rise of the catalytic activity was observed for laccases from *P. radiata* (PRL) and *Pyricularia oryzae* (POL) [26]. All mentioned laccases (CUL, POL and PRL) show the reaction rate declining exponentially to about 10 M DMSO solutions and this decrease is much intensive for CUL than for the POL and PRL.

Figs. 3 and 4 show the effect of DMSO on the V_{max} and K_M values for the oxidation reaction of DMOP and SGZ by laccase from *C. unicolor* (CUL).

The values of V_{max} decrease almost linearly with DMSO concentration at roughly the same rate in the case of both studied substrates (Fig. 3). K_M values in the case of DMOP oxidation increase linearly with the DMSO concentration for both substrates, but with different rate (Fig. 4).

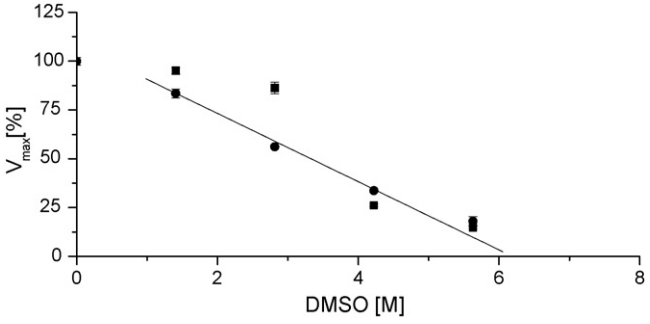

Fig. 3. The effect of DMSO on V_{max} values of the oxidation of SGZ (■) and DMOP (●), catalysed by CUL (points determined experimental, lines fitted with the parameters given in Table 2).

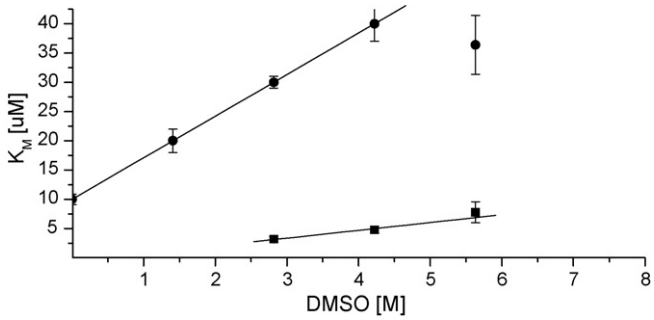

Fig. 4. The effect of DMSO on K_M values of the oxidation of SGZ (■) and DMOP (●), catalysed by CUL (points determined experimental, lines fitted with the parameters given in Table 2).

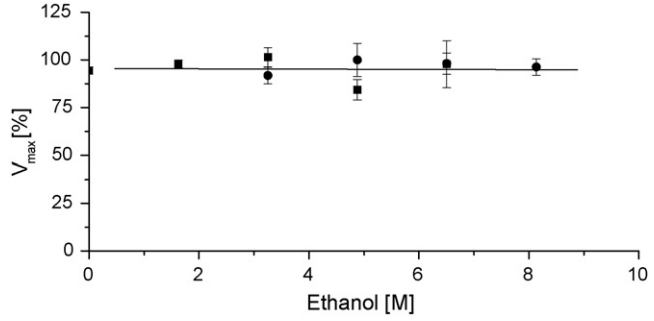

Fig. 5. The effect of ethanol on V_{max} of the oxidation of SGZ (●) and CAT (■), catalysed by CUL (points determined experimental, lines fitted with the parameters given in Table 2).

The values of V_{max} for the oxidation of SGZ and CAT (Fig. 5) do not depend upon addition of ethanol over a wide range of its concentrations, similarly as it was observed for laccase from *P. radiata* [11], while K_M values (Fig. 6) increase exponentially with the addition of ethanol, similarly as it was observed for laccases from *Panus tigrinus* and *T. versicolor* [35]. Both in the case of DMSO and ethanol, the effect of the solvent on V_{max} is not dependent on the tested substrate, and the influence on K_M values is again substrate-dependent (cp. Fig. 4).

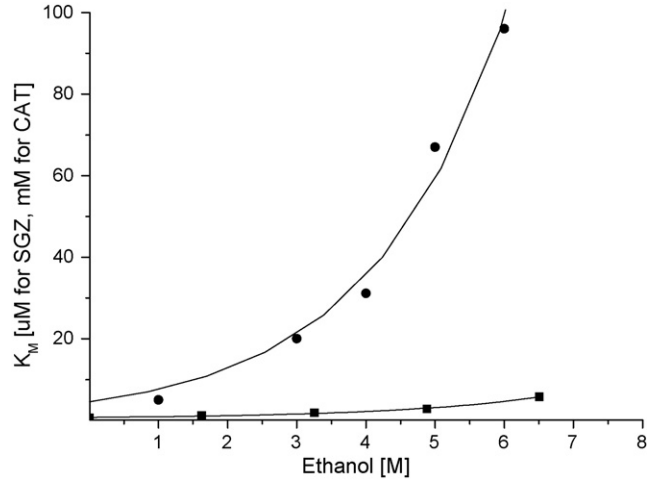

Fig. 6. The effect of ethanol on K_M values of the oxidation of SGZ (●) and CAT (■), catalysed by CUL (points determined experimental, lines fitted with the parameters given in Table 2).

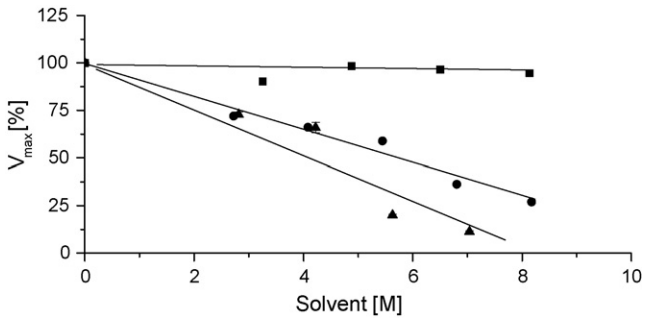

Fig. 7. The effect of organic solvents on V_{max} values of the oxidation of SGZ catalysed by CUL in the presence of ethanol (■), acetone (●), and DMSO (▲); points determined experimental, lines fitted with the parameters given in Table 2.

Figs. 7 and 8 compare the effects of ethanol, acetone, and DMSO on the values of V_{max} and K_M for syringaldazine oxidation. The effect of the studied organic solvents on V_{max} values (ethanol < acetone < DMSO) is different from the results obtained by Khmelnitsky et al. [36] for laccase from *Coriolus versicolor*, where the following series was observed under the enzyme denaturating conditions: DMSO < ethanol < acetone. In the case of *P. radiata* laccase (PRL), DMSO was the only one strongly inactivating solvent over a studied range of low solvent concentrations [11]. The effect of the solvents on K_M values for CUL and SGZ (DMSO < acetone < ethanol) is reversed in comparison to that observed for PRL and DMOP (ethanol < acetone < DMSO) [11].

3.4. Application of the theoretical models to the effect of water-miscible organic solvents on the catalytic activity of CUL

Figs. 2–7 show that the effect of various water-miscible solvents on CUL catalytic activity, within the studied range of the solvent concentrations may be described using the following models: ethanol, Model I; acetone and DMSO, Model III.

Table 2 collects the values of the adjusted model coefficients (δ, ε, β). In Table 3, the numerical data for other laccases are shown, based on the earlier experimental data [11]. For comparison of the data in Tables 2 and 3, Table 2 contains also a column showing the values of β (Model II) for the same experimental data.

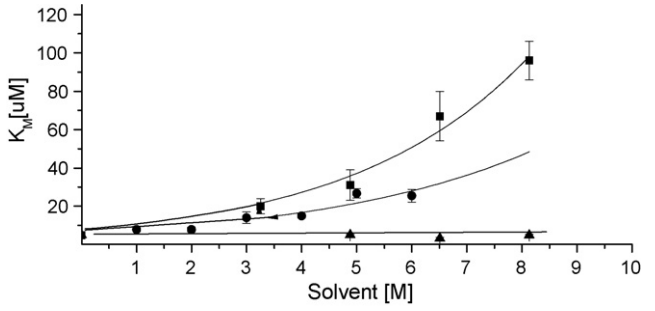

Fig. 8. The effect of organic solvents on K_M values of the oxidation of SGZ catalysed by CUL in the presence of ethanol (■), acetone (●), and DMSO (▲); points determined experimental, lines fitted with the parameters given in Table 2.

Table 2
Inhibition parameters of organic solvents on CUL catalytic activity (Models I–III)

Solvent	Substrate	δ (M^{-1})	ε (M^{-1})	β (M^{-1})
Ethanol	SGZ	0	0.51 ± 0.06	0
Ethanol	CAT	0	0.36 ± 0.03	0
Acetone	SGZ	1.80 ± 0.25	0.26 ± 0.05	0.16 ± 0.09
DMSO	SGZ	5.50 ± 1.30	0.10 ± 0.04	0.36 ± 0.14
DMSO	DMOP	4.70 ± 0.20	0.73 ± 0.07	0.25 ± 0.04

The comparison of the values of ε (the effect of moderate concentrations of organic solvents on K_M) for various laccases shows the following:

- K_M values are not solely dependent on the variations of the substrate activity in solutions of organic solvents, but the exponential relationship is very useful in comparison the influence of organic solvents on various laccases.
- The exponential relationships cover a relatively wide range of solvent concentrations, even the solvent-sensitive laccases.
- In the case of CUL, PTL, and CVL the effect is similar for the studied phenolic substrates oxidized by an individual enzyme in the presence of a selected solvent, except for SGZ, oxidized by CUL in DMSO. On contrary, the earlier studied PRL has showed the stronger effects for SGZ than for DMOP.
- The described effect depends not only on the solvent properties (mainly its hydrophobicity) but also on laccase source and preparation.
- The following order of the ε values may be observed for SGZ oxidation: **CUL**–DMSO < **CUL**–acetone < **CUL**–ethanol ≈ **CVL**–ethanol < **PTL**–ethanol.
- The following order of the ε values may be observed for DMOP oxidation: **CVL**–ethanol < **PRL**–ethanol < **PRL**–DMSO ≈ **PTL**–ethanol ≈ **CUL**–DMSO.
- $\varepsilon = 0.7\ M^{-1}$ may be used for predicting the solvent effect on substrate K_M values for many typical solvents, simple phenolic compounds and fungal laccases. In many cases, it will overestimate the effect.

The comparison of the β values (the effect of the moderate concentrations of organic solvents on V_{max} values) shows the following:

- Ethanol does not affect much any of the studied laccases;
- DMSO strongly affects CUL and PRL and this effect depends on the tested laccase and independent on the investigated substrate.

Table 3
Inhibition parameters of organic solvents on the catalytic activity of other laccases (Models I and II)[a]

Enzyme–solvent	Substrate	ε (M^{-1})	β (M^{-1})
PRL–DMSO (0–3.5 M)	DMOP	0.70 ± 0.08	0.57 ± 0.04
CBL–ethanol (0–5 M)	DMOP	0.44 ± 0.03	0.07 ± 0.01
	SGZ	0.37 ± 0.01	0.12 ± 0.01
PBL–ethanol (0–3 M)	DMOP	0.71 ± 0.06	0.06 ± 0.01
	SGZ	0.78 ± 0.10	0.31 ± 0.05
PRL–ethanol (0–2.5 M)	DMOP	nd	0
	SGZ	nd	0

[a] Model parameters calculated for the data presented earlier: PRL, *P. radiata* laccase; CVL, *C. versicolor* laccase; PTL, blue laccase from *P. tigrinus* [11]; nd, not determined beyond the linear range.

The data collected in Tables 2 and 3 show also the variation of $\ln V_{max}/K_M$ for various studied systems (given by the difference between the values of β and ε). Except for SGZ in DMSO oxidized in the presence of CUL, the values are negative and vary between −0.1 and −0.6.

4. Conclusions

The experimental and numerical results show the regions of organic solvent concentration, in which laccase from *C. unicolor* and other laccases may be used for conversion of substrates that are poorly soluble in water. CUL is more sensitive to acetone than laccase from *P. radiata*, comparably sensitive but more effective in DMSO than laccase from *P. radiata* and less sensitive to the presence of ethanol than the studied samples of laccases from *C. versicolor* and *P. tigrinus.*

The observed K_M values of the investigated phenolic substrates of various studied laccases vary in a similar way for the individual enzymes and solvents, they increase with the solvent hydrophobicity and to some extent they are dependent on the origin of laccase. Short chain alkanols exert little influence on many fungal laccases over a long range of their concentrations. For relatively little sensitive laccases, for example, that from *C. versicolor* [36] and from *P. radiata* [11] the denaturating effect of the solvents (*C. versicolor* laccase) and variations of K_M values (*P. radiata* laccase) were parallel. It is not the case for the investigated here, more sensitive highly purified laccase from *C. unicolor*.

Acknowledgment

This work was partially supported by the State Committee for Scientific Research (139/E-339/SPB/6, PR UE/DIE 450/2004-2007).

References

[1] C.F. Thurston, Microbiology 140 (1994) 19–26.
[2] A.I. Yaropolov, O.V. Skorobogatko, S.S. Vartanov, S.D. Varfomeleev, Appl. Biochem. Biotechnol. 49 (1994) 257–280.
[3] A. Leonowicz, A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wojtaś-Wasilewska, N.-S. Cho, M. Hofrichter, J. Rogalski, Fungal. Genet. Biol. 27 (1999) 175–185.
[4] O. Milstein, A. Hüttermann, A. Majcherczyk, K. Schulze, R. Fründ, H.-D. Lüdemann, J. Biotechnol. 30 (1993) 37–47.
[5] O. Milstein, A. Hüttermann, R. Fründ, H.-D. Lüdemann, Appl. Microb. Biotechnol. 40 (1994) 760–767.
[6] C. Mai, O. Milstein, A. Hüttermann, Appl. Microb. Biotechnol. 51 (1999) 527–531.
[7] C. Mai, W. Schormann, A. Hüttermann, Enzyme Microb. Technol. 28 (2001) 460–466.

[8] J. Rogalski, E. Jóźwik, A. Hatakka, A. Leonowicz, J. Mol. Catal. B 95 (1995) 99–108.
[9] J. Rogalski, A. Dawidowicz, E. Jóźwik, A. Leonowicz, J. Mol. Catal. B 6 (1999) 29–39.
[10] J. Rodakiewicz-Nowak, Top. Catal. 11/12 (2000) 419–434.
[11] J. Rodakiewicz-Nowak, B. Dudek, S.M. Kasture, J. Haber, J. Mol. Catal. B 11 (2000) 1–11.
[12] J.S. Dordick, Enzyme Microb. Technol. 11 (1989) 194–211.
[13] Y.-J. Zheng, R.L. Ornstein, J. Am. Chem. Soc. 118 (1996) 4175–4180.
[14] Q.X. Chen, X.D. Liu, H. Huang, Biochemistry (Moscow) 68 (2003) 644–649.
[15] A. Fersht, Enzyme Structure and Mechanism, W.H. Freeman and Co., New York, 1985.
[16] J.S. Dordick, Biotechnol. Progr. 8 (1992) 259–267.
[17] K. Ryu, J.S. Dordick, Biotechnol. Tech. 6 (1992) 272–282.
[18] Ch.R. Wescott, A.M. Klibanov, J. Am. Chem. Soc. 115 (1993) 1629–1631.
[19] S.B. Lee, K.-J. Kim, J. Ferment. Bioeng. 79 (1995) 473–478.
[20] S.B. Lee, J. Ferment. Bioeng. 79 (1995) 479–484.
[21] R.R. Smith, W.J. Canady, Biophys. Chem. 43 (1992) 173–187.
[22] S. Garavaglia, M.T. Cambria, M. Miglio, S. Ragusa, V. Iacobazzi, F. Palmieri, C. D'Ambrosio, A. Scaloni, M. Rizzi, J. Mol. Biol. 342 (2004) 1519–1531.
[23] L. Quintanar, J. Yoon, C.P. Aznar, A.E. Palmer, K.K. Andersson, R.D. Britt, E.I. Solomon, J. Am. Chem. Soc. 127 (2005) 13832–13845.
[24] J. Rodakiewicz-Nowak, A. Jarosz-Wilkołazka, J. Luterek, Appl. Catal. A: Gen. 308 (2006) 56–61.
[25] J. Rodakiewicz-Nowak, M. Ito, J. Chem. Technol. Biotechnol. 78 (2003) 809–816.
[26] J. Rodakiewicz-Nowak, S.M. Kasture, J. Haber, Proceedings of the 36th Colloquium on Catalysis, Kraków, March 17–19, 2004.
[27] G. Lindeberg, G. Holm, Physiol. Plant. 5 (1952) 100–114.
[28] J. Luterek, L. Gianfreda, M. Wojtaś-Wasilewska, J. Rogalski, M. Jaszek, E. Malarczyk, A. Dawidowicz, M. Fink-Boots, G. Ginalska, A. Leonowicz, Acta Microbiol. Polon. 46 (1997) 297–311.
[29] A. Leonowicz, K. Grzywnowicz, Enzyme Microb. Technol. 3 (1981) 55–58.
[30] H. Jung, F. Xu, K. Li, Enzyme Microb. Technol. 30 (2002) 161–168.
[31] D. Slomczyński, J.P. Nakas, S.W. Tannenbaum, Appl. Environ. Microb. 611 (1995) 907–911.
[32] H. Wariishi, K. Valli, M.H. Gold, J. Biol. Chem. 267 (1992) 23688–23695.
[33] E. Rodriguez, O. Nuero, F. Guillen, A.T. Martinez, M.J. Martinez, Soil Biol. Biochem. 36 (2004) 909–916.
[34] M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[35] J. Rodakiewicz-Nowak, J. Haber, N.N. Pozdnyakova, A.A. Leontievsky, L.A. Golovleva, Biosci. Rep. 19 (1999) 589–600.
[36] Y.L. Khmelnitsky, V.V. Mozhaev, A.B. Belova, M.V. Sergeeva, K. Martinek, Eur. J. Biochem. 198 (1991) 31–41.