ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 4 (1998) 155–159

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Thermodynamic hydrophobicity of aqueous mixtures of water-miscible organic solvents predicts peroxidase activity

Eduardo Torres [a], Barbara Siminovich [a], Eduardo Barzana [b], Rafael Vazquez-Duhalt [a,*]

[a] *Biotechnology Institute U.N.A.M., Apartado Postal 510-3, Cuernavaca, Morelos 62250, Mexico*
[b] *Faculty of Chemistry U.N.A.M., Mexico D.F., Mexico*

Received 7 July 1997; accepted 13 August 1997

Abstract

The effect of different water-miscible organic solvents on biocatalytic activities of chloroperoxidase from *Caldariomyces fumago* and horseradish peroxidase was determined. A new hydrophobicity parameter for water–organic solvent mixtures was used to predict the enzyme behavior. This thermodynamic concept of hydrophobicity also describes the catalytic behavior of three other biocatalysts with peroxidase activity. So far, all reported data of peroxidase activity in increasing concentrations of water-miscible organic solvents are effectively predicted by the thermodynamic model presented in this work. © 1998 Elsevier Science B.V.

1. Introduction

Enzymatic catalysis in organic solvents has opened a new field in the biotechnological applications of proteins [1–3]. Four main advantages could be obtained by using organic solvents in catalytic systems: (a) Hydrophobic substrates that have a serious mass transfer limitation in aqueous systems could be biotransformed, such as polycyclic aromatic hydrocarbons [4] and asphaltenes [5]. (b) Reactions which are thermodynamically impossible in aqueous systems could be performed in organic solvents, such as peptide synthesis by proteases [6], transesterification by lipases [7], and condensation of glucose by glucosidase to obtain disaccharides [8]. (c) In a low water media several enzymes are more thermostable than in aqueous systems, allowing the performance of biocatalytic reactions at high temperature [9]. (d) Finally, from an industrial point of view, products and enzyme recovery after reaction could be easier from organic solvents than from water, because of the high vapor-pressure of organic solvents.

During the last decade several studies have been focused on the use of physical properties of solvents to predict the catalytic behavior of enzymes in organic solvents [10–14]. The most common being the logarithm of the partition coefficient octanol/water ($\log P$) of pure solvent. Nevertheless, this parameter is far from being general [8], and it cannot be applied for

* Corresponding author. Biotechnology Institute U.N.A.M., Apartado Postal 510-3, Cuernavaca, Morelos 62271, Mexico. Fax: +52-73-172388.

PII S1381-1177(97)00030-1

aqueous mixtures containing water-miscible organic solvents. In this work, the biocatalytic behavior of four peroxidases and cytochrome *c* is correlated with a new hydrophobicity parameter.

2. Experimental

2.1. Chemicals

Purified chloroperoxidase ($R_z = 1.41$) from *Caldariomyces fumago* was a gift from Professor M.A. Pickard (University of Alberta, Canada). Horseradish peroxidase, pyrene, hydrogen peroxide and guaiacol were obtained from Sigma Chemical (St. Louis, MO). HPLC-grade solvents, tetrahydrofuran, acetonitrile, isopropanol, ethanol, and methanol, were purchased from Fisher Scientific (Springfield, NJ). Buffer salts were obtained from J.T. Baker (Phillipsburg, NJ).

2.2. Reaction conditions

Chloroperoxidase activity was estimated in 1 ml reaction mixture containing 20 μM pyrene, 20 mM KCl and different proportions of water-miscible organic solvents in 60 mM acetate buffer pH 3.0. The reaction was started by adding 0.25 mM hydrogen peroxide, and monitored at 335 nm. The specific activity was determined by using an extinction coefficient of 32.6 mM^{-1} cm^{-1} for pyrene [4]. Activity of horseradish peroxidase was estimated as the absorbance increase at 470 nm in 1 ml reaction mixture containing 16 mM guaiacol, 1 mM hydrogen peroxide, and different proportions of water-miscible organic solvents in a 60 mM phosphate buffer pH 6.1 [15].

3. Results and discussion

Organic solvent concentrations at which half of the maximum activity is obtained (C_{50}) were determined for chloroperoxidase and horseradish peroxidase in different aqueous mixtures of water-miscible organic solvents (Table 1). In addition, literature data of four other enzymes (lignin peroxidase, horseradish peroxidase, lactoperoxidase, and cytochrome c) have been used.

In a recent study we have proposed a new hydrophobicity parameter for water–organic solvent mixtures [16]. This hydrophobicity concept (H) was obtained considering the substrate partitioning between the active site of the enzyme and the bulk solvent mixture (Fig. 1). Increasing the solvent hydrophobicity (H), the substrate is partitioned preferably from the active site toward the solvent, reducing the biocatalytic activity. Substrate interaction with active site of the enzyme decreases when hydrophobicity of the solvent is increased [16]. This new hydrophobicity parameter (H) is directly correlated with the thermodynamic activity of the organic solvent (a_s) in the mixture, and inversely correlated with the solvent polarity [$E_T(30)$]:

$$H = \frac{a_s}{E_T(30)}$$

We have used thermodynamic activities, for different organic solvent concentrations (a_s), determined from isothermal data for vapor–liquid equilibrium at 25°C, and calculated by the NRTL equations [17]. In contrast to concentrations, thermodynamic activities represent the available amount of organic solvent that participates in the total hydrophobicity of the system. Thermodynamic activity of solvent ($a_s = \gamma_s[X_s]$) in an aqueous solution of a water-miscible organic solvent at any concentration can be determined by the NRTL equation:

$$\ln \gamma_s = X_w^2 \left[\frac{A_2}{RT} \left(\frac{e^{-\alpha(A_2/RT)}}{X_s + X_w e^{-\alpha(A_2/RT)}} \right)^2 + \frac{(A_1/RT) e^{-\alpha(A_1/RT)}}{\left(X_w + X_s e^{-\alpha(A_1/RT)}\right)^2} \right]$$

Table 1
Threshold concentration (C_{50}), organic solvent activity and hydrophobicity of the solvent system at C_{50} concentrations (H_{50}) for the peroxidase activity in water–organic solvent media

Enzyme	Organic solvent	C_{50} (%v/v)	Ref.	a_s	H_{50} (μmol/cal)
Chloroperoxidase	tetrahydrofuran	17.0	this work	0.52	13.9
	acetonitrile	18.5	this work	0.42	9.1
	isopropanol	32.5	this work	0.43	8.8
	ethanol	22.0	this work	0.25	4.8
	methanol	26.0	this work	0.20	3.6
Horseradish peroxidase	tetrahydrofuran	18.0	this work	0.55	14.7
	acetone	35.0	[19]	0.48	11.4
	acetonitrile	20.0	this work	0.44	9.6
	methanol	31.0	[19]	0.15	2.7
	ethylene glycol	34	[19]	0.13	2.3
Lignin peroxidase	tetrahydrofuran	14.7	[16]	0.48	12.8
	acetonitrile	19.1	[16]	0.43	9.2
	isopropanol	18.8	[16]	0.31	6.3
	ethanol	27.9	[16]	0.31	6.0
	methanol	21.7	[16]	0.17	2.9
Lactoperoxidase	acetone	35.0	[19]	0.48	11.4
	methanol	35.0	[19]	0.17	3.1
	ethylene glycol	36.0	[19]	0.14	2.5
Cytochrome *c*	tetrahydrofuran	19.1	[16]	0.57	15.1
	acetonitrile	28.0	[16]	0.54	11.7
	isopropanol	27.6	[16]	0.41	8.4
	ethanol	32.9	[16]	0.36	6.8
	methanol	27.8	[16]	0.21	3.8

in which γ_s is the activity coefficient of the solvent, X_w and X_s are the molar fractions of water and organic solvent, respectively, R is the gas constant in cal/mol · K, T is the temperature in K, α is the nonrandomness parameter, and A_1 and A_2 are parameters for the interaction between components (water and organic solvent). The parameters for the NRTL equation; A_1, A_2, and α are available in the literature for several aqueous–organic systems [17].

Organic solvent polarity was estimated by the Dimroth–Reichardt parameter [$E_T(30)$], which is an empirical parameter for solvent polarity and is directly related to the free energy (ΔG) of the solvation process [18]. This solvatochromic parameter includes the total sum of the interactions between the solute and the solvent molecules by measuring the change in the molecular structure of a dye in different solvents. The solvent polarity parameter is ob-

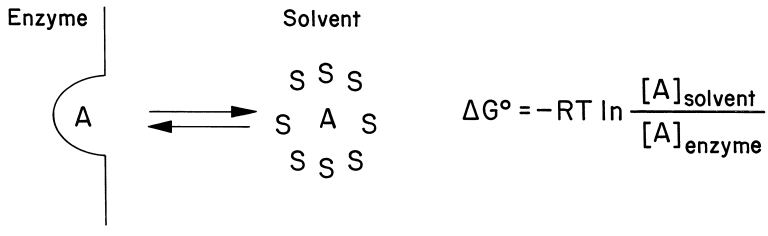
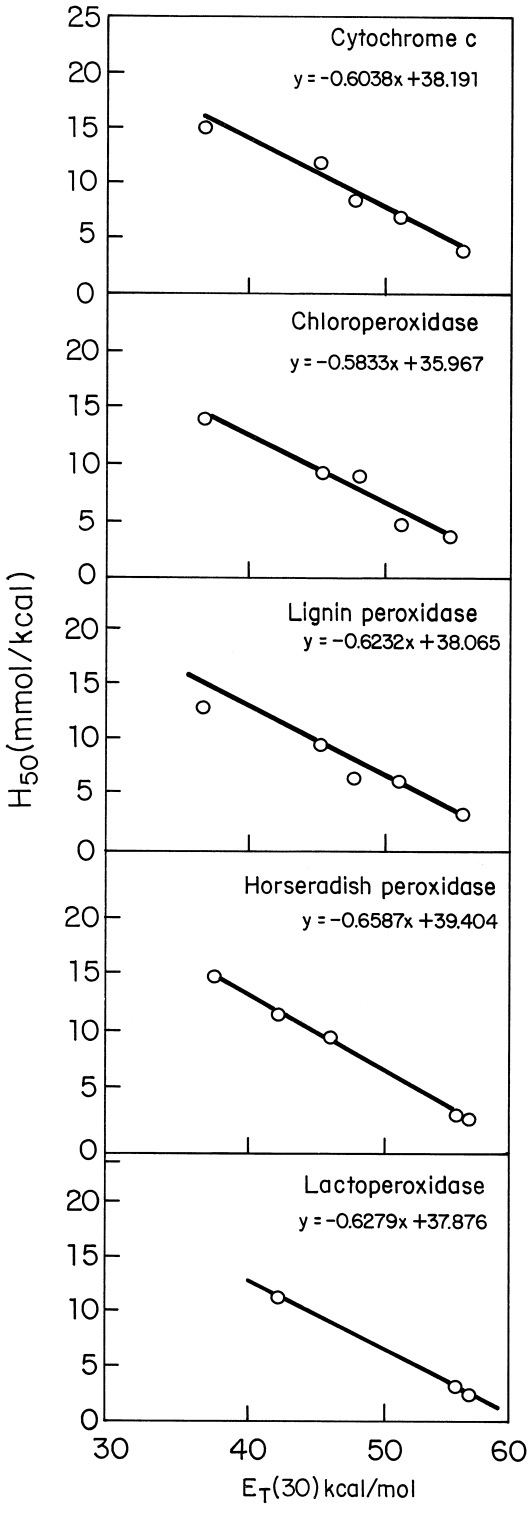


Fig. 1. Substrate partitioning between the active site of the enzyme and bulk solvent.



tained from the change in the electron transition energy of a negatively solvatochromic dye (Reichard's dye), which is a pyridiniophenolate. The transition energies [$E_T(30)$] can be used directly as empirical parameters of the solvent polarity [18] and were calculated with the following equation:

$$E_T(30)\ [\text{kcal/mol}] = h\upsilon N = h\left(\frac{c}{\lambda}\right)N$$

in which h is Planck's constant, υ is the light frequency, c is the velocity of light, λ is the wavelength, and N is Avogadro's number.

Table 1 shows the values of H_{50}, which is the hydrophobicity of the solvent system in which half of the maximum peroxidase activity is found. In other words, it is the hydrophobicity of a solvent system at the C_{50} concentration. Fig. 2 shows that a linear correlation has been found for all peroxidases and for cytochrome c between the value of H_{50} and the solvent polarity [$E_T(30)$]. This linear correlation has also been obtained for different substrates, including pyrene, in the cases of lignin peroxidase and cytochrome *c* [16]. Our results show that threshold hydrophobicities H_{50} of horseradish peroxidase, chloroperoxidase, and lactoperoxidase can also be predicted for all solvents and substrates, as follows:

$$H_{50} = b - a\left[E_T(30)\right]$$

Thus, this new hydrophobicity parameter, which is defined by both the thermodynamic activity of the organic solvent and the solvent polarity, predicts the biocatalytic behavior of four peroxidases and cytochrome *c* in systems containing water-miscible organic solvents. So far, all

Fig. 2. Linear correlation between the hydrophobicity of solvent mixture at C_{50} concentration (H_{50}) and the polarity parameter of the organic solvent [$E_T(30)$]. Pure solvent polarities, $E_T(30)$, in kcal/mol are: Tetrahydrofuran, 37.4; acetone, 42.2; acetonitrile, 46.0; isopropanol, 48.6; ethanol, 51.9; methanol, 55.5; and ethylene glycol, 56.3 (from Reichardt [18]).

available data of peroxidase reactions in water-miscible organic solvents fit the proposed mathematical model showing excellent correlation coefficients.

Acknowledgements

This work was funded by DGAPA-UNAM Grant 21459 and Conacyt Grant 4217A.

References

[1] A. Zaks, A.M. Klibanov, J. Biol. Chem. 263 (1988) 3194.
[2] Y.L. Khmelnitsky, A.V. Levashov, N.L. Klyachko, K. Martinek, Enzyme Microb. Technol. 10 (1988) 710.
[3] J.S. Dordick, Enzyme Microb. Technol. 11 (1989) 194.
[4] R. Vazquez-Duhalt, D.W.S. Westlake, P.M. Fedorak, Appl. Environ. Microbiol. 60 (1994) 459.
[5] P.M. Fedorak, K.M. Semple, R. Vazquez-Duhalt, D.W.S. Westlake, Enzyme Microb. Technol. 15 (1993) 429.
[6] H. Kise, A. Hayakawa, Enzyme Microb. Technol. 13 (1991) 584.
[7] W. Chulalaksananukul, J.S. Condoret, D. Combes, Enzyme Microb. Technol. 14 (1992) 293.
[8] V. Laroute, R.M. Willemont, Enzyme Microb. Technol. 14 (1992) 528.
[9] A. Zaks, A.M. Klibanov, Science 224 (1984) 1249.
[10] C. Laane, S. Boeren, K. Vos, C. Veeger, Biotechnol. Bioeng. 30 (1987) 81.
[11] P.J. Halling, Biotechnol. Bioeng. 35 (1990) 691.
[12] Y.L. Khmelnitsky, V.V. Mozhaev, A.B. Belova, M.V. Sergeeva, K. Martinek, Eur. J. Biochem. 198 (1991) 31.
[13] A. Manjon, J.M. Obon, M. Canovas, J.L. Iborra, in: J. Tramper, M.H. Vermue, H.H. Beeftink, U. von Stockar (Eds.), Biocatalysis in Non-Conventional Media, Elsevier Science Publishers, Holland, p. 121.
[14] P.J. Halling, Enzyme Microb. Technol. 16 (1994) 178.
[15] R. Vazquez-Duhalt, P.M. Fedorak, D.W.S. Westlake, Enzyme Microb. Technol. 14 (1992) 837.
[16] E. Torres, R. Tinoco, R. Vazquez-Duhalt, J. Biotechnol. 49 (1996) 59.
[17] J. Gmehling, U. Onken, W. Arlt, Vapor–Liquid Equilibrium Data Collection. 1a. DECHEMA Deutsche Gesellschaft fur Chemisches Apparatewesen, Frankfurt, Germany, 1981.
[18] C. Reichardt, Angew. Chem. Int. Ed. Engl. 18 (1979) 98.
[19] K. Sato, H. Hasumi, A. Tsukidate, J. Sakuruda, S. Nakamura, T. Hosoya, Biochim. Biophys. Acta 1253 (1995) 94.