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Kinetic study of the oxidative dehalogenation of 2,4,6-trichlorophenol catalyzed by chloroperoxidase

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ABSTRACT

A sigmoidal behaviour of chloroperoxidase for the oxidative dehalogenation of 2,4,6-trichlorophenol is reported for the first time. Kinetic data were adjusted to the Hill equation and the kinetic parameters were obtained: $n = 1.7 \pm 0.2$, $v_{max} = (8.8 \pm 0.3) \times 10^{-5}$ M min^{-1}, the pseudo-Michaelis constant $K_s^* = (8.6 \pm 0.5) \times 10^{-5}$ M, $k_{cat} = 677 \pm 84$ min^{-1} and the catalytic efficiency $= (8.9 \pm 0.6) \times 10^6$ M^{-1} min^{-1}. The sigmoidal curve could be related to the cooperative binding of the substrate to the enzyme, so that the binding of the first substrate molecule may help the binding of the second one. Further, both substrate molecules could establish Π–Π interactions between them, which would confer more stability to the system.

1. Introduction

Chloroperoxidase (CPO, E.C. 1.11.1.10) is a glycosylated hemoprotein secreted by the fungus *Caldariomyces fumago*. CPO contains an iron protoporphyrin IX (heme) as its prosthetic group and shares structural features with both P450s and peroxidases. Like P450, the axial ligand in CPO is a cysteine (Cys29) sulfur atom rather than a histidine nitrogen atom usually found in peroxidases, but contrary to P450s, polar aminoacids have been found in CPO distal binding site. However, CPO lacks the two distal residues His and Arg, which are normally found in peroxidases [1,2]. Instead, a glutamate residue (Glu183) is found 5 Å away from the Fe atom and a histidine residue (His105) is located near Glu183 fixing it and modulating the acidity of this residue [3].

These structural features make CPO one of the most versatile heme enzymes. After the activation of the ferric heme centre, CPO carries out a wide variety of oxidative reactions [4]. Thus, CPO catalyzes many P450 reactions: heteroatom dealkylation [5], benzylic [6] and propargylic [7] hydroxylation, enantioselective epoxidation of olefins [8], sulfoxidation [9], oxidation of indoles [10] and N-oxidation of arylamines [11]. In addition, CPO also undergoes peroxidations and two-electron oxidation typical of classical peroxidases and catalases, respectively. With regard to oxygen transfer reactions, CPO requires the activation of the ferric centre by a suitable hydroperoxide, yielding the active oxo–iron intermediate, named Compound I (Cpd I), which has been thoroughly studied [3,12,13]. Although the primary function of CPO is halogenation [14], its ability to catalyze dehalogenation reactions has been recently reported [15,16], resulting in reaction products that are less hazardous than the starting halogenated reactives, with the environmental implications that this entails.

Chlorophenols constitute a group of priority pollutants listed by the US Environmental Protection Agency and the European Union (Decission 2455/2001/EC). They may be also generated as by-products during waste incineration, the bleaching of pulp with chlorine and the dechlorination of drinking water. Chlorophenols have been widely employed as pesticides, insecticides, fungicides and dyes. Their toxicity and persistence in the environment increase with the degree of chlorine substitution. In the last years CPO has been used in the degradation of various chlorophenols and even 4-fluorophenol, as Table 1 shows.

In the majority of the enzymatic methods in Table 1, a dark precipitate is formed and the corresponding substrate has been totally degraded. In addition, TCP is often used to test the efficiency of oxidative degradation methods [22]. The ability of CPO to catalyze the oxidative dehalogenation of halophenols has been recently reported and its mechanism elucidated towards TCP [15,16]. However, to the best of our knowledge, there are not thorough kinetic studies of the respective processes. So, in this work we have studied the kinetics of 2,4,6-trichlorophenol oxidative dehalogenation catalyzed by CPO.

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Table 1
Substrates involve in oxidative degradation catalyzed by CPO.

Enzyme	Source of supply	Substrate	References
Chloroperoxidase (CPO)	*Caldariomyces fumago*	Phenol, TCP	[17]
		4-CP	[17,18]
		2,4-DCP	[19]
		2,3,5,6-TeCP, PCP	[20]
		4-FP	[21]

4-CP (4-chlorophenol), 2,4-DCP (2,4-dichlorophenol), 2,3,5,6-TeCP (2,3,5,6-tetrachlorophenol), PCP (pentachlorophenol), and 4-FP (4-fluorophenol).

2. Materials and methods

2.1. Reagents

Chloroperoxidase (CPO, E.C. 1.11.1.10) was purchased from Fluka as suspension (22371 U/mL) in 0.1 M sodium phosphate pH 4.0. The Reizenstahl number (R_z) expresses the purity degree of the CPO preparation, and it was obtained from the A_{403}/A_{280} ratio. CPO used in this study is almost pure, with R_z value of 1.5 [23].

Sodium acetate and acetic acid were obtained from Merck. Hydrogen peroxide was purchased from Prolabo, 2,6-dichloro-1,4-benzoquinone (DCQ) was obtained from Aldrich. Stock standard solutions of TCP and DCQ were prepared in acetonitrile and stored in the refrigerator, and they were used to daily prepare working standard solutions by suitable dilution in a 0.01 M acetate buffer.

Acetonitrile was purchased from J.T. Baker. All other chemicals were analytical-reagent grade. All solutions were prepared with high purity water produced by a Milli-Q purification system (Millipore).

2.2. Apparatus

High performance liquid chromatography (HPLC) was used to analyze the main product of the homogeneous oxidation of TCP catalyzed by CPO. The chromatograph (HPLC 1100 Series, Agilent Technologies, Tokyo, Japan) was coupled to an ion trap MS detector (LC/MSD Trap XCT Plus, Agilent Technologies, Tokyo, Japan), and was used to identify the main reaction product. A precolumn (TR-C-160 with a ODS cartridge) was coupled to the analytical column (150 mm Pinnacle C18 column, 5 μm particle diameter and 4.6 mm I.D.) and both of them were supplied by Teknokroma (Spain). The chromatographic separation was performed with a mobile phase consisting of 0.05 M acetate buffer pH 3.5 (solvent A) and acetonitrile (solvent B). The gradient elution program was as follows: keeping constant 50% B for 6 min, then increasing to 90% B in 1 min and keeping constant until 9 min. Then restored to 50% B and followed by a 5 min equilibration time. The flow rate was set at 1 mL min^{-1} (DCQ retention time: 3.7 min).

For kinetic evaluation, an HPLC instrument made up of a gradient Shimadzu 20A system fit with a SPD-20MA diode array detector and a Rheodyne 7725i rotating valve with a 20 μL loop was used. The chromatographic separation was performed with the same columns and time programme as HPLC-MS measurements. Data analysis was carried out with Shimadzu LC Solution software. The detector was set at 280 nm to detect both TCP (λ_{max} = 290 nm) and DCQ (λ_{max} = 272 nm) in a single chromatogram (retention times: 3.7 min for DCQ and 7.2 min for TCP).

The absorption spectra were performed in a Lambda 20 spectrometer from PerkinElmer (USA).

2.3. CPO characterization

The enzyme concentration given as mol L^{-1} was obtained from UV-absorption measurements of the enzyme solution (sodium acetate 0.1 M, pH 6.0) at 403 nm (extinction coefficient = 75.3 mM^{-1} cm^{-1}) [19]. The resulting data were represented versus the enzyme concentration expressed as U mL^{-1}, and adjusted to straight line. The relationship between both concentration units was provided by the slope as 1 U L^{-1} = $(2.5 \pm 0.3) \times 10^{-5}$ mol L^{-1}.

The iron present in the enzyme was determined by attacking CPO with hydrogen peroxide in acidic medium, which results in the iron release, and it is quantified by ICP-MS. 100 μL of the commercial CPO solution were diluted in 1 mL of $HClO_4$ 2 M and incubated with H_2O_2 15% (w/v) for 14 h under stirring. The mixture was centrifuged and the iron content in the supernatant solution was determined with a 7500ce model ICP-MS (Agilent Technologies, Tokyo, Japan) by measuring m/z 56. The iron content in CPO was estimated as (3 ± 1) % (w/v).

2.4. Oxidative dehalogenation of TCP catalyzed by CPO

The oxidative dehalogenation of TCP was carried out at 25 °C in small flasks (4 mL) with CPO (5×10^3 U L^{-1}) as catalyst. Hydrogen peroxide concentration was fixed at 10^{-3} M for all the experiments to guarantee it is in excess over the other reactants. The oxidative dehalogenation of TCP was determined at different pH values and then, kinetic measurements were carried out at the optimum pH value. No reaction was found when H_2O_2 or CPO alone was added to a TCP solution.

Steady-state kinetic data (reaction rate versus substrate concentration between 5×10^{-6} and 10^{-3} M) were fit to the sigmoidal plot developed as the Hill plot (Eq. (1)). The fitting was done by an iterative procedure following Marquardt–Levenberg non-linear least squares algorithm using Origin 8.1 software.

Additionally, kinetic measurements were also carried out at pH 5.0 varying H_2O_2 concentration at two fixed TCP concentrations (10^{-5} and 10^{-4} M), and the kinetic parameters were estimated.

$$v = \frac{v_{max}[S]^n}{K_S^n + [S]^n} \quad (1)$$

3. Results and discussion

3.1. Identification of the oxidation products of TCP

When TCP (10^{-4} M) reacts with H_2O_2 (10^{-3} M) with the native CPO (5×10^3 U L^{-1}) as catalyst in 0.1 M acetate buffer pH 5.0 (we fixed this pH value to guarantee that TCP is protonated, pK_a = 6.59), the main reaction product was detected by HPLC–UV/VIS (280 nm) at 3.7 min, as shown in Fig. 1A. In order to identify this product, a mass spectrum of this reaction medium (10^{-3} M TCP, 10^{-3} M H_2O_2 and 5×10^5 U L^{-1} CPO) was acquired at 3.7 min by HPLC–MS and m/z 177.0 was observed (Fig. 1B), which matches up with a DCQ standard solution. Therefore we can conclude that DCQ is the main reaction product of the degradation of TCP catalyzed by CPO in the reaction conditions mentioned above. This observation is in accordance with the studies of Osborne et al. [15,16], and the oxidative dehalogenation of TCP catalyzed by CPO is illustrated in Scheme 1.

3.2. Effect of the pH of the reaction medium on the CPO dehalogenating activity

The pH of the reaction medium was evaluated in the range 3.0–7.0. Fig. 2 displays the amount of DCQ generated in Scheme 1 as a function of pH. CPO exhibits a bell-shaped pH profile with an optimum pH range between 4.5 and 5.5. At pH > 4.2 Glu183 [16] is predominantly ionized and facilitates the abstraction of a proton from hydrogen peroxide and the subsequent heterolytic cleavage of O–O bond to produce an oxo–iron (IV) reactive species (Compound I). Laurenti et al. [24] proposed that the oxidative dehalogenation

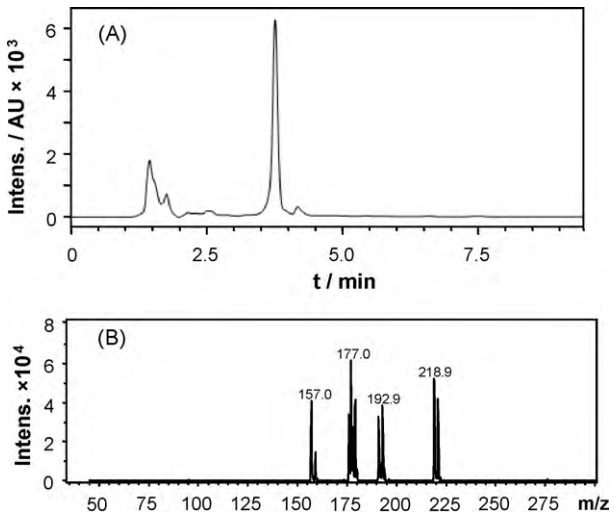
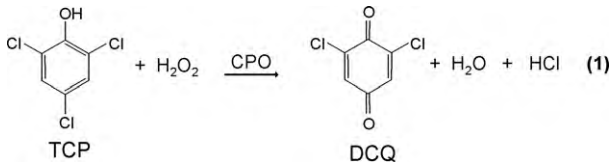


Fig. 1. (A) HPLC–VIS/UV (280 nm) chromatogram of the reaction medium and (B) mass spectra of the reaction medium acquired at 3.7 min (DCQ, $m/z = 177$). Reaction conditions: 5×10^5 U L^{-1} CPO, 10^{-3} M H_2O_2, 10^{-3} M TCP in 0.01 M acetate buffer pH 5.0 with 10% DMSO, reaction time: 2 min.



Scheme 1.

of halogenated phenols catalyzed by HRP only takes place with the neutral form of the substrate, so low amounts of DCQ would be formed at pH 6.5. This is the trend observed in Fig. 2, and it agrees with the pK_a value of TCP (6.59). However, there are some evidences that dehaloperoxidase from *Amphitrite ornata* (DHP) [25,26] could catalyze the oxidative dehalogeantion of various halogenated substrates with both the neutral and the anionic form of the substrate. In our case, it is possible to explain the shape of the full curve pH versus the amount of DCQ generated. The maximum of the bell-shaped curve in Fig. 2 corresponds to the pH interval where Glu183 is predominantly ionized (pH > 4.2) while TCP remains neutral (pH < 6.59). Therefore we can confirm the importance of the acid–base character of the CPO aminoacidic residue Glu183, and conclude that reaction from Scheme (1) occurs from the neutral form of TCP.

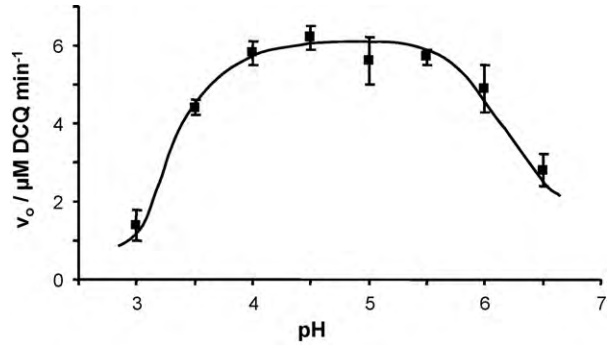


Fig. 2. DCQ concentration measured in the reaction medium at various pH values. Reaction conditions: 5×10^3 U L^{-1} CPO, 10^{-3} M H_2O_2, 10^{-5} M TCP in 0.01 M acetate or phosphate buffer, reaction time: 1 min.

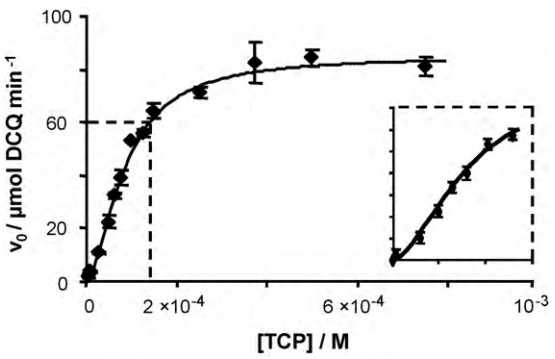


Fig. 3. Initial reaction rate at different TCP concentrations. Reaction conditions: 5×10^3 U L^{-1} CPO, 10^{-3} M H_2O_2 in 0.01 M acetate buffer pH 5.0, reaction time: 1 min.

Furthermore, La Rotta et al. [19] observed two active pH values (pH 3.0 and 6.0) for 2,4-DCP peroxidation with CPO as catalyst and Manoj and Hager [4] have related the presence of more than one optimal pH range to substrate binding to different sites and they have reported pH 3.0 for chloride-assisted peroxidations and pH 5.0 for peroxidations carried out in absence of chloride ion. These two optimal pH values were not observed in Fig. 2, although chloride is a by-product.

3.3. Kinetics of the oxidative dehalogenation of TCP catalyzed by CPO

The time-course of DCQ formation from a 10^{-5} M TCP solution was monitored for 25 min and it increases reaching a plateau after 2 min (data not shown). Steady-state rate of the reaction was estimated by measuring the concentration of the generated DCQ at a fixed reaction time of 1 min. A plot of the rate of oxidation versus TCP concentration yielded an atypical sigmoidal profile (Fig. 3).

These data properly fit the Hill equation (1), as shows the r^2 value of 0.995. The Hill parameter ($n = 1.7 \pm 0.2$) indicates that cooperative effects may take place in the oxidative dehalogenation of TCP [27]. Sigmoidal kinetics have been also observed for the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) [4], 4,6-dimethyldibenzothiophene [28] and several polycyclic aromatic hydrocarbons (anthracene, naphthalene, pyrene) [29] with CPO as catalyst.

Moreover, $v_{max} = (8.8 \pm 0.3) \times 10^{-5}$ M min^{-1} and the pseudo-Michaelis constant $K_s^* = (8.6 \pm 0.5) \times 10^{-5}$ M were obtained for the oxidative dehalogenation of TCO catalyzed by CPO in the present conditions. However, the cooperative effects could be related to the degree of substation of the chlorophenol, since no sigmoidal curves had been obtained when this enzyme catalyzed the oxidative dehalogenation of pentachlorophenol or 2,3,5,6-tetrachlorophenol [20].

k_{cat}, the turnover number of the enzyme, cannot be determined without knowing the concentration of the enzyme. So the native CPO concentration was estimated by UV-absorption measurements as $(1.3 \pm 0.2) \times 10^{-7}$ mol L^{-1}. Moreover, the iron concentration in this native CPO solution was also estimated by ICP-MS as $(1.2 \pm 0.4) \times 10^{-7}$ mol L^{-1}. These data indicate that the whole iron in CPO is catalytically active and a k_{cat} value of 677 ± 84 min^{-1} was obtained. This value can be compared with the ones obtained for TCP with a wide variety of enzymes (see Table 2), which shows that native CPO is one of the most efficient catalyst for the oxidative dehalogenation of TCP.

The catalytic efficiency (k_{cat}/K_s^*) balances the catalytic activity of the enzyme against its affinity. This parameter takes the value $(8.9 \pm 0.6) \times 10^6$ M^{-1} min^{-1} for Scheme 1, which is slightly lower than the one reported for 2,3,5,6-tetrachlorophenol

Table 2
Turnover numbers (min^{-1}) for catalytic degradation of TCP.

Enzyme/catalyst	Turnover (min^{-1})	Reaction conditions	References
CPO	1200	Potassium phosphate pH 3[a]	[15]
DHP	198	Sodium acetate pH 5.4[a]	[30]
	6120	Sodium acetate pH 5.4[a]	[30]
HRP	3300	Sodium citrate pH 3.0[a]	[31]
	4080	Sodium acetate pH 4.0[a]	[31]
Mb	0.74	Potassium phosphate pH 7.0[b]	[32]
LiP	300	Sodium acetate pH 2.5–3.0[a]	[33]
FeTPPS	1200	Sodium citrate/sodium phosphate pH 3.0[c]	[34]

CPO: chloroperoxidase from *Caldariomyces fumago*, DHP: dehaloperoxidase from *Amphitrite ornata*, HRP: horseradish peroxidase, Mb: myoglobin from horse heart, LiP: lignin peroxidase from *Phanerochaete chrysosporium*, TPPS: *meso*-tetrakis(4-sulfonatophenyl)porphyrinato.

[a] 100 μM TCP, 400 μM H_2O_2, 0.1 μM enzyme, and 100 mM buffer.
[b] 150 μM TCP, 65 μM H_2O_2, 60 μM enzyme, and 100 mM buffer.
[c] 10 mM TCP, 50 mM H_2O_2, 0.03 mM FeTPPS, and 100 mM buffer.

($6 \times 10^8\ M^{-1}\ min^{-1}$) and pentachlorophenol ($2.3 \times 10^7\ M^{-1}\ min^{-1}$) where no cooperative effects were observed [20]. Moreover, similar catalytic efficiencies were observed for 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($1.2 \times 10^7\ M^{-1}\ min^{-1}$) [4], anthracene ($5.4 \times 10^7\ M^{-1}\ min^{-1}$), naphthalene ($6.8 \times 10^7\ M^{-1}\ min^{-1}$) and pyrene ($4.5 \times 10^8\ M^{-1}\ min^{-1}$) from sigmoidal kinetics [29].

The kinetic characterization was completed with the constants obtained as a function of varying H_2O_2 at pH 5.0 and a fixed TCP concentration (10^{-5} or 10^{-4} M), and the kinetic parameters were recorded in Table 3.

The cooperativity in P450s has been observed in the 1980s [35] and it has been related to the existence of kinetically distinct isoforms and distinct ligand binding sites [36–38]. However, evidence of simultaneous binding of multiple ligands to a single active site has been demonstrated [39–41] and this last hypothesis is widely accepted [35]. Since CPO shares structural features with P450 [1,2], previous works [28,29] associate the sigmoidal kinetics of CPO to the existence of two substrate molecules at the CPO active site, and a theoretical study of substrate docking into the enzyme active site [29,42] demonstrates that a Π–Π dimer is energetically favored against the binding of monomeric substrate. Furthermore, Manoj and Hager [4] corroborate the existence of at least two different sites in CPO for carrying out oxidative reactions. Since the CPO preparation used in this work is almost pure ($R_z = 1.5$), the sigmoidal kinetics cannot be explained in terms of different isoforms of the enzyme. Therefore, these sigmoidal effects could be related to the multiplicity of TCP binding within a large CPO active site, in such a way that the binding of one TCP molecule facilitates the successive binding of one more TCP molecule ($n = 1.7 \pm 0.2$). Assuming that substrates that posses aromatic rings in their structure are able to form Π–Π dimers [29,42], two TCP molecules could interact in the reaction medium or in the active centre of CPO, which is also in accordance with the cooperativity index value. So we can conclude that homotropic cooperativity takes place in the oxidative dehalogenation of TCP catalyzed by CPO in the reaction conditions described in this work.

Table 3
Kinetic parameters obtained by varying H_2O_2 concentration at pH 5.0 and a fixed concentration of TCP (10^{-5} or 10^{-4} M).

Kinetic parameters	[TCP] = 10^{-5} M	[TCP] = 10^{-4} M
n	2.2 ± 0.4	1.3 ± 0.1
v_{max} ($M\ min^{-1}$)	$(4.9 \pm 0.1) \times 10^{-6}$	$(1.8 \pm 0.2) \times 10^{-5}$
k_{cat} (min^{-1})	38 ± 6	139 ± 26
K_s^* (M)	$(6.2 \pm 0.4) \times 10^{-6}$	$(2.3 \pm 0.4) \times 10^{-3}$
k_{cat}/K_s^* ($M^{-1}\ min^{-1}$)	$(6 \pm 1) \times 10^6$	$(6 \pm 2) \times 10^4$
R^2	0.991	0.999

4. Conclusion

The kinetics of the oxidative dehalogenation of TCP catalyzed by CPO was studied. A sigmoidal profile was observed for the first time when the effect of the substrate concentration on the initial reaction rate was studied. A cooperative index of 1.7 ± 0.2 was obtained, which is more likely to be due to the formation of Π–Π dimers at the binding site that to the presence of enzyme isoforms, since the enzyme used had a high degree of purity. The kinetic parameters n, v_{max}, k_{cat}, the pseudo-Michaelis constant and the catalytic efficiency were estimated, and we can conclude that CPO is one of the most efficient catalysts for TCP dehalogenation following HRP, even when sigmoidal kinetics are observed.

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References

[1] M. Sundaramoorthy, J. Terner, T.L. Poulos, Structure 3 (1995) 1367–1377.
[2] M. Sundaramoorthy, J. Terner, T.L. Poulos, Chem. Biol. 5 (1998) 461–473.
[3] H. Chen, H. Hirao, E. Derat, I. Schlichting, S. Shaik, J. Phys. Chem. B 112 (2008) 9490–9500.
[4] K.M. Manoj, L.P. Hager, Biochemistry 47 (2008) 2997–3003.
[5] G.L. Ketters, P.F. Hollenberg, Arch. Biochem. Biophys. 233 (1984) 315–321.
[6] A. Zaks, D.R. Dodds, J. Am. Chem. Soc. 117 (1995) 10419–10424.
[7] S. Hu, L.P. Hager, J. Am. Chem. Soc. 121 (1999) 872–873.
[8] F.J. Lakner, K.P. Cain, L.P. Hager, J. Am. Chem. Soc. 119 (1997) 443–444.
[9] S. Colonna, N. Gaggero, G. Carrera, P. Pasta, Chem. Commun. 5 (1997) 439–440.
[10] M.P.J. van Deurzen, F. van Rantwijk, R.A. Sheldon, J. Mol. Catal. B 2 (1996) 33–42.
[11] D.R. Doerge, M.D. Corbett, Chem. Res. Toxicol. 4 (1991) 556–560.
[12] M. Filizola, G.H. Loew, J. Am. Chem. Soc. 122 (2000) 3599–3605.
[13] W.D. Woggon, H.A. Wagenknecht, C. Claude, J. Inorg. Biochem. 83 (2001) 289–300.
[14] L.P. Hager, D.R. Morris, F.S. Brown, H.J. Eberwein, J. Biol. Chem. 241 (1996) 769.
[15] R.L. Osborne, G.M. Raner, L.P. Hager, J.H. Dawson, J. Am. Chem. Soc. 128 (2006) 1036–1037.
[16] R.L. Osborne, M.K. Coggins, J. Terner, J.H. Dawson, J. Am. Chem. Soc. 129 (2007) 14838–14839.
[17] C.E. La Rotta, E. D'Elia, E.P.S. Bon, Electron. J. Biotechnol. 10 (2007) 24–30.
[18] C.E. La Rotta, E.P.S. Bon, Appl. Biochem. Biotechnol. 98–100 (2002) 191–203.
[19] C.E. La Rotta, S. Lütz, A. Liesse, E.S. Bon, Enzyme Microb. Technol. 37 (2005) 582–588.
[20] A. Longoria, R. Tinoco, R. Vázquez-Duhalt, Chemosphere 72 (2008) 485–490.
[21] C.D. Murphy, Biotechnol. Lett. 29 (2007) 45–49.
[22] G. Lente, J.H. Espenson, Chem. Commun. 10 (2003) 1162–1163.
[23] K.M. Manoj, Biochim. Biophys. Acta 1764 (2006) 1325–1339.
[24] E. Laurenti, E. Ghibaudi, S. Ardissone, R.P. Ferrari, J. Inorg. Biochem. 95 (2003) 171–176.
[25] R.L. Osborne, M.K. Coggins, G.M. Raner, M. Walla, J.H. Dawson, Biochemistry 48 (2009) 4231–4328.
[26] S. Franzen, L.B. Gilvey, J.L. Belyea, Biochim. Biophys. Acta 1774 (2007) 121–130.
[27] I.H. Segel, Enzyme Kinetics, Behaviour and Analysis of Rapid Equilibrium and Steady-State Systems, John Wiley & Sons, USA, 1993, pp. 346–375.
[28] E. Torres, J. Aburto, Arch. Biochem. Biophys. 437 (2005) 224–232.
[29] J. Aburto, J. Correa-Basurto, E. Torres, Arch. Biochem. Biophys. 480 (2008) 33–40.
[30] R.L. Osborne, L.D. Taylor, K.P. Han, J.H. Dawson, Biochem. Biophys. Res. Commun. 324 (2004) 1194–1198.
[31] R.P. Ferrari, E. Laurenti, F. Trotta, J. Biol. Inorg. Chem. 4 (1999) 232–237.
[32] R.L. Osborne, M.K. Coggins, M. Walla, J.H. Dawson, Biochemistry 46 (2007) 9823–9829.
[33] K.H. Hammel, P.J. Tardone, Biochemistry 27 (1988) 6563–6568.
[34] G. Labat, J.L. Seris, B. Meunier, Angew. Chem. Int. Ed. Engl. 29 (1990) 1471–1473.
[35] W.M. Atkins, Annu. Rev. Pharmacol. Toxicol. 45 (2005) 291–310.

[36] A.P. Koley, J.T. Buters, R.C. Robinson, A. Markowitz, F.K. Friedman, J. Biol. Chem. 272 (1997) 3149–3152.
[37] N.A. Hosea, G.P. Miller, F.P. Guengerich, Biochemistry 39 (2000) 5929–5939.
[38] H. Fernando, J.R. Halpert, D.R. Davydov, Biochemistry 45 (2006) 4199–4209.
[39] M. Shou, J. Grogan, J.A. Mancewicz, K.W. Krausz, F.J. González, H.V. Gelboin, K.R. Korzekwa, Biochemistry 33 (1994) 6450–6455.
[40] M.J. Dabrowski, M.L. Schrag, L.C. Wienkers, W.M. Atkins, J. Am. Chem. Soc. 124 (2002) 11866–11867.
[41] I.G. Denisov, B.J. Baas, Y.V. Grinkova, S.G. Sligar, J. Biol. Chem. 282 (2007) 7066–7076.
[42] J.C. Basurto, J. Aburto, J.T. Ferrara, E. Torres, Mol. Simul. 33 (2007) 649–654.