Journal of Molecular Catalysis A: Chemical 353–354 (2012) 117–121

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Catalysis A: Chemical

journal homepage: www.elsevier.com/locate/molcata

Hemo-acrylic polymers as catalyst for the oxidative dehalogenation of 2,4,6-trichlorophenol. Chloroperoxidase's mimic imprinting effects

Goretti Díaz-Díaz, M. Carmen Blanco-López, M. Jesús Lobo-Castañón, Arturo J. Miranda-Ordieres, Paulino Tuñón-Blanco*

Departamento de Química Física y Analítica, Universidad de Oviedo, Julián Clavería, 8, 33006 Oviedo, Spain

ARTICLE INFO

Article history:
Received 6 July 2011
Received in revised form
30 September 2011
Accepted 13 November 2011
Available online 22 November 2011

Keywords:
Heterogeneous catalyst
Chloroperoxidase mimic
2,4,6-Trichlorophenol dechlorination
Molecularly imprinted polymer

ABSTRACT

Acrylic polymers with catalytic activity for the oxidative degradation of 2,4,6-trichlorophenol (TCP) were developed. In order to mimic the active site of chloroperoxidase (CPO), chloro-iron(III)-protoporphyrin IX was used as the catalytic centre, and methacrylamide (MA) and 4-vinylpyridine (VPY) were used as the monomers that build up the active sites. Taking as basis 3:1 (w/w) acid:basic aminoacidic composition of CPO, three MA:VPY combinations were tested: one keeping the same ratio (3:1) i.e. 25% VPY in the functional monomer mixture, one with lower content of the basic monomer (9:1) i.e. 10% VPY, and one with higher concentration of it (1:1) i.e. 50% VPY. Polymers synthesized with the lowest VPY content exhibited the highest catalytic efficiency, which was improved by the creation of specific TCP binding sites through molecular imprinting technology. In these way, synthetic enzymes with useful properties for analytical and bioremediation applications were obtained.

1. Introduction

Chlorophenols constitute a group of priority pollutants listed by the US Environmental Protection Agency (EPA) and the European Union (EU) in the Clean Water Act and the Decision 2455/2001/EC, respectively. They have been widely employed in different manufacturing processes, and they also result from pulp bleaching, drinking water dechlorination and incineration processes. Chlorophenols can be found in ground and wastewaters [1], and their toxicity and persistence in the environment increase with the degree of chlorine substitution. Therefore, 2,4,6-trichlorophenol (TCP) is one of the most harmful chlorophenols due to the pulmonary lesions that it causes.

When chlorophenols reach soils or natural water reservoirs, they are degraded by microorganisms, which live in muds, sludges and sewages. This natural degradation consists in reductive dehalogenation in anaerobic conditions or in oxidative pathways. However, the majority of chlorophenols are poisonous to microorganisms, and therefore a biological pre-treatment is required. Depending on the enzyme, the reaction conditions and the chlorophenol characteristics, the sole use of enzymes could lead to the complete degradation of the pollutant, and the subsequent treatment with microorganisms could be avoided [2,3]. Peroxidases have been commonly used for this purpose [4,5]. As an alternative solution there is a recent interest in developing artificial catalysts mimicking these natural enzymes. These synthetic receptors can work in harsh conditions (pH, temperature), which provides advantages over natural enzymes. As a first approach to develop catalysts that mimic the degradation ability of natural enzymes over chlorophenols, water soluble iron(III)-porphyrins have been used as homogeneous catalysts in batch assays [6,7]. A step forward is the immobilization of metalloporphyrins on different solid supports that has been found efficient not only to ensure catalyst recovery but also to prevent loss of activity through catalyst aggregation [8–12]. Although these systems allow the catalytic degradation of chlorophenols, they lack of selectivity. In this context, the generation of recognition and catalytic sites in polymeric macromolecular synthetic receptors by molecular imprinting technology have emerged as an attractive approach in order to mimic the catalytic ability of enzymes [13,14]. These artificial receptors consisted on organic polymers synthesized from a suitable combination of monomers and cross-linkers, where the catalytic centre is included. Metalloporphyrins have been used as catalytic centres, and the resulting artificial catalysts exhibit both hydrophobic binding pockets and polar recognition groups, capable of recognizing polar functional groups under aqueous conditions [15–17].

Abbreviations: TCP, 2,4,6-trichlorophenol; CPO, chloroperoxidase; MA, methacrylamide; VPY, 4-vinylpyridine; EPA, US Environmental Protection Agency; EU, European Union; 4-C-3-MP, 4-chloro-3-methylphenol; 2,4-DCP, 2,4-dichlorophenol; 2,4-DNP, 2,4-dinitrophenol; DCQ, 2,6-dichloro-1,4-benzoquinone; AIBN, 2,2′-azo-bis-(isobutyronitrile); DMSO, dimethylsulfoxide; EGDMA, ethileneglycoldimethacrylate; HPLC, High Performance Liquid Chromatography; ODS, octadecylsilane.

* Corresponding author. Tel.: +34 985103487; fax: +34 985103125.
E-mail address: ptb@uniovi.es (P. Tuñón-Blanco).

1381-1169/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcata.2011.11.014

Taking chloroperoxidase as a model enzyme, the objective of this work was the synthesis of a polymer that resembles CPO and mimics its catalytic activity towards the oxidative dehalogenation of TCP, which is one of the most substituted chlorophenols. To achieve this aim, we have reproduced the catalytic centre of CPO with Fe(III)-protoporphyrin IX, which has been covalently included as a co-monomer in the polymer backbone through its two vinyl groups. In our previous works, we have used either a neutral (methacrylamide, MA) [15,18] or a basic (4-vinylpyridine, VPY) [15] functional monomer to prepare the catalysts. But in order to mimic the aminoacidic residues of CPO, both neutral and basic monomers are needed. Therefore, in this work we propose the use of mixtures of the two monomers for preparing the polymers. Different MA:VPY compositions have been tested in order to study the effect of these two monomers in the catalytic behaviour of the synthetic catalyst. In addition, we have tried to improve the properties of the catalysts through an imprinting process, with the ultimate goal of obtaining a catalytic synthetic receptor with analytical and biotechnological applications such as development of sensors for the detection of the pollutant or decontamination applications. Equilibrium experiments and kinetic evaluation were carried out through batch assays in order to assess the catalytic efficiency and selectivity of these polymers towards the oxidative dehalogenation of TCP.

2. Experimental

2.1. Reagents

Chlorohemin (iron(III)-protoporphyrin IX) was purchased from Frontier Scientific (UK). 2,4,6-Trichlorophenol (TCP), 4-chloro-3-methylphenol (4-C-3MP), α,α′-azoisobutyronitrile (AIBN) and dimethylsulfoxide (DMSO) were purchased from Fluka. Ethylene glycol dimethacrylate (EGDMA), acetic acid and perchloric acid were purchased from Merck. Methacrylamide (MA), 4-vinylpiridine (VPY), 2,6-dichloro-1,4-benzoquinone (DCQ) 2-chloro-1,4-benzoquinone and 3-methyl-1,4-benzoquinone were purchased from Aldrich. Hydrogen peroxide was obtained from Prolabo. Methanol and acetonitrile were purchased from J.T. Baker. All chemicals were of analytical grade and used as received except for EGDMA, whose inhibitors were removed by successive liquid–liquid extractions. Buffer solutions were prepared with high purity water produced by a Milli-Q purification system (Millipore), and stock standard solutions of phenols were used to daily prepare working standard solutions by suitable dilution in a 0.01 M acetate buffer.

2.2. Apparatus

An HPLC instrument (Shimadzu 20A) made up of a gradient system fitted with a SPD-20MA diode array detector and a Rheodyne 7725i rotating valve with a 20 μL loop was used. 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.) supplied by Teknokroma. 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: the percentage of solvent B was set at 50% B for the first 6 min, increased to 90% B in 1 min and then kept constant until 9 min. The flow rate was 1 mL min^{-1}. Data analysis was carried out with Shimadzu LC Solution software. The detector was set at 280 nm to detect both TCP and the products of the catalytic reaction in a single chromatogram.

2.3. Procedures

The preparation of the polymers was carried out as follows: chlorohemin (5 μmol), MA and VPY (25 μmol total amount, with different ratios of MA to VPY as explained below), and EGDMA (250 μmol) were placed in a vial, and DMSO (7.5 mL) was employed as porogenic solvent to achieve the solubilization of hemin. The vial was sealed and the mixture was purged with nitrogen for 15 min. Then AIBN (240 mg) was quickly added and the mixture was purged again for 5 min. The polymerization reaction was carried out in an oven at 65 °C for 24 h. As a first approach, we have selected a proportion of MA to VPY in the pre-polymerization mixture of 3:1, which matches the aminoacidic content of the natural CPO, 16% (w/w) of neutral aminoacids and 6% of basic aminoacids over the whole protein [19]. In addition, two more MA:VPY ratios of 1:1 and 9:1 were tested. These proportions correspond to VPY molar content of 25, 50 and 10% VPY with respect to MA. The resultant polymer was subjected to Soxhlet extraction using methanol with 15% (v/v) acetic acid in order to remove the non-polymerized hemin and then washed with methanol to remove the acid. Finally, the polymers were crushed and sieved to obtain particles sized below 25 μm that were used in the batch experiments. Molecularly imprinted polymers (MIPs) were synthesized in the same way, adding TCP (5 μmol) in the pre-polymerization mixture. TCP was subsequently extracted from the polymer by Soxhlet, which was carried out until no TCP was detected by cyclic voltammetry.

The iron present in the polymers was determined by ICP-MS measuring the released iron after lixiviation of the polymers with hydrogen peroxide 15% (w/v) in acidic medium (2 M $HClO_4$). 20 mg of each polymer were suspended in 1 mL of $HClO_4$ 2 M, H_2O_2 15% (w/v) was added, and the mixture was stirred at 600 rpm for 14 h. Before ICP-MS analysis, the mixture was centrifuged at 10,000 rpm, and iron was determined in the supernatant. The lixiviation was carried out again for 15 min in the same conditions, and significant iron quantities were only detected in the first supernatant.

The polymers were morphologically characterized by scanning electron microscopy (SEM) on a MEB JEOL-6100 instrument. SEM specimens were prepared by placing a little amount of the polymer in a support and coating it with gold under vacuum.

Kinetics of the oxidative transformation of TCP with the different polymers as catalyst was studied through batch experiments in acetate 0.01 M pH 5.0, with 10% DMSO in order to improve the wettability of the polymers. The concentration of polymer and H_2O_2 was fixed at 0.5 g L^{-1} and 10^{-3} M, respectively, measuring the initial reaction rate to increasing concentrations of TCP.

The binding of TCP to the polymers was studied by equilibrium experiments, carried out in 0.01 M acetate buffer with 10% of DMSO, and varying the TCP concentrations from 2.5×10^{-5} to 5×10^{-4} M with a fixed concentration of polymer (125 μg mL^{-1}) for 12 h in a tilting mixer. The amount of free TCP was determined by HPLC-UV/VIS.

Selectivity studies were performed in two different ways: firstly, TCP kinetics was carried out at a fixed concentration of the interferent (10^{-4} M), and secondly, the interferent itself was used as substrate of the catalytic reaction, and the resulting product was quantified by HPLC-UV/VIS.

3. Results and discussion

3.1. Morphological characterization of the polymers

The morphology of the polymers was studied by SEM experiments. In all cases, polymer particles exhibited an irregular shape and a dual distribution of sizes. However, the size differences between the two particle populations were more remarkable for

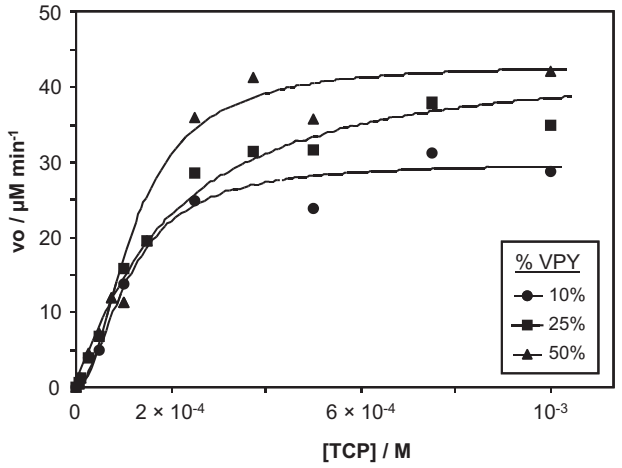

Fig. 1. Initial reaction rate at different TCP concentrations with the polymers as catalysts. Reaction conditions: 0.5 g L^{-1}, polymer, 10^{-3} M H_2O_2 in 0.01 M acetate buffer with 10% DMSO, reaction time: 2 min.

the 10% VPY polymers (5 ± 2 and 26 ± 4 μm) compared to the ones synthesized with 25 and 50% VPY (10 ± 3 and 31 ± 4 μm), which did not exhibit significant differences between them. This fact indicated that the particle size increased with the VPY content. Comparing these results with those of the polymers synthesized with a unique functional monomer [15], 10% VPY polymers are formed by particles with intermediate sizes between the polymers synthesized with MA (10 ± 3 and 20 ± 3 μm) or VPY (10 ± 3 and 31 ± 4 μm), while 25 and 50% VPY ones resembled the ones synthesized with VPY.

3.2. Oxidative dehalogenation of TCP with polymers as catalysts

The oxidative degradation of TCP was carried out with the synthesized polymers as catalysts. The first step was to confirm that they exhibit dehaloperoxidase activity, giving 2,6-dichloro-1,4-benzoquinone (DCQ) as the main reaction product, which matches with that obtained when either CPO [20–22] or polymers synthesized with only MA [15,18] or only VPY [15].

The reaction–progress curve was obtained by mixing each polymer (10, 25 and 50% VPY) as catalysts with the substrate (TCP 10^{-5} M) and the oxidant (H_2O_2 10^{-3} M), and measuring the DCQ generated over a period of 25 min at 30 s intervals. The initial velocity region of the catalytic reaction was below 5 min. Initial rates, obtained by measuring the concentration of the generated DCQ at a fixed reaction time of 2 min, were measured as a function of the TCP concentration to generate the saturation curve (Fig. 1). These data do not obey the Michaleis–Menten equation but yield sigmoidal profiles that were fit to Hill equation by an iterative procedure following Marquardt–Levenberg non-linear least squares algorithm using Origin 8.1 software. A similar behaviour was obtained for the natural enzyme, CPO, catalyzing the same process [18].

The Hill equation provides the values for the parameters n, v_{max} and the pseudo-Michaelis constant (K_s*), which are shown in Table 1. The Hill parameter (n) indicates the presence of cooperative effects ($n > 1$) [23]. We had previously observed this behaviour ($n = 1.7 \pm 0.2$) when we studied the oxidative dehalogenation of TCP catalyzed by CPO [20] and by polymers synthesized with just MA ($n = 1.6 \pm 0.4$) or VPY ($n = 2.7 \pm 0.5$) [15]. The lower n values correspond to the polymers synthesized with 25% VPY while the rest exhibit Hill parameters close to 2, which indicates that there could be up to two TCP molecules bound at the catalytic centre of the polymer. However, the v_{max} values resemble the ones obtained for VPY-based polymers ($1.35 \pm 0.05 \times 10^{-5}$ M min^{-1}), and this parameter takes its maximum values when 25% VPY polymers catalyze the oxidative dehalogenation of TCP. An additional parameter useful to describe the efficiency of a biomimetic catalyst is the catalytic constant (k_{cat}) or turnover number that can be calculated as the ratio between v_{max} and the concentration of catalyst. The turnover number for the different polymers was obtained assuming that the concentration of the active sites is equivalent to the amount of chlorohemin present in the polymers. The catalytic constants displayed similar values to k_{cat} obtained for MA-based polymers (0.45 ± 0.04 min^{-1}), which were slightly higher than the obtained for VPY-based polymers (0.34 ± 0.01 min^{-1}) [15]. The pseudo-Michaelis constant K_s* indicates that these polymers exhibit affinities similar to those of the polymers prepared with the basic functional monomer VPY ($7.8 \pm 0.5 \times 10^{-5}$ M) [15].

Finally, the global effect of k_{cat} and K_s* was evaluated as the ratio k_{cat}/K_s* (catalytic efficiency) [14]. The catalytic efficiency is similar to the VPY-based catalyst and one order of magnitude lower than the MA-based one [15]. k_{cat}/K_s* takes it lowest value for the 50% VPY polymer whereas similar values were shown for the polymers synthesized with 10 or 25% VPY, which indicates that the polymers containing the lower VPY percentages mimicked more efficiently the CPO catalytic dehalogenation activity. It should be taken into account that the aminoacidic composition of these polymers resembled the basic aminoacidic percentage found in chloroperoxidase.

3.3. Oxidative dehalogenation of TCP with molecularly imprinted polymers

In order to improve the catalytic properties of the polymers, specific binding sites for the substrate, TCP, were introduced during their synthesis. Molecular imprinting technology was used for this purpose [14], and TCP was included in the pre-polymerization mixture, giving 10, 25 and 50% VPY-MIPs. These imprinted polymers exhibit similar morphology when compared to their respective non-imprinted counterparts. They were also made up of spherical substructures, whose rapprochement created pores that were larger in the MIP as compared to the NIP, which indicates that the accessibility of TCP to the recognition cavities will be favored. The oxidative dehalogenation of TCP was carried out with MIPs as catalysts, and the parameters n, v_{max} and K_s*, which were compiled in Table 2, follow the same trend as those of the non-imprinted polymers discussed previously. However, the catalytic efficiency reaches its minimum value for 25% VPY-MIP. This fact could indicate the higher ability of this polymer to specifically bind TCP and catalyze its oxidative dehalogenation, due to the presence of specific template binding sites created during the polymerization process. The imprinting factor (IF) also reflects this fact, and it can be estimated as the ratio MIP/NIP of the respective catalytic efficiencies. According to this parameter, polymers synthesized with 10% VPY exhibit a marked imprinting effect. Therefore, based on the

Table 1
Kinetic parameters for the polymers synthesized in this work.

Polymer	n	$v_{max} \times 10^{-5}$ (M min^{-1})	k_{cat} (min^{-1})	K_s* $\times 10^{-4}$ (M)	k_{cat}/K_s* $\times 10^{3}$ (M^{-1} min^{-1})	R^2
10% VPY	1.8 ± 0.6	1.5 ± 0.1	0.42 ± 0.03	1.1 ± 0.2	3.8 ± 0.7	0.973
25% VPY	1.3 ± 0.2	2.1 ± 0.2	0.55 ± 0.05	1.5 ± 0.2	3.6 ± 0.6	0.993
50% VPY	2.0 ± 0.4	2.1 ± 0.2	0.42 ± 0.04	1.4 ± 0.2	3.0 ± 0.5	0.968

Table 2
Kinetic parameters of the molecularly imprinted polymers (MIPs).

Polymer	n	$v_{max} \times 10^{-5}$ (M min^{-1})	k_{cat} (min^{-1})	$K_s^* \times 10^{-4}$ (M)	$k_{cat}/K_s^* \times 10^3$ (M^{-1} min^{-1})	R^2	IF
10% VPY-MIP	1.9 ± 0.2	1.26 ± 0.04	0.54 ± 0.02	1.22 ± 0.08	4.5 ± 0.3	0.998	1.2
25% VPY-MIP	1.2 ± 0.2	2.2 ± 0.2	0.57 ± 0.05	1.8 ± 0.3	3.2 ± 0.6	0.992	0.9
50% VPY-MIP	1.9 ± 0.3	1.78 ± 0.06	0.45 ± 0.02	1.18 ± 0.08	3.8 ± 0.3	0.993	1.3

Table 3
Binding parameters of the polymers synthesized with 10% VPY: $K_{k\,min-k\,max}$ (affinity constant), $N_{k\,min-k\,max}$ (average number of binding sites) and m (heterogeneity index).

Polymer	m	$N_{k\,min-k\,max}$ (μmol g^{-1})	$K_{k\,min-k\,max}$ (μmol g^{-1})	R^2
10% VPY-MIP	0.84	793	6.3×10^4	0.968
10% VPY-NIP	0.78	1197	5.0×10^4	0.916

criteria of the catalytic efficiency, the 10% VPY-MIC seems to present the best characteristics to mimic CPO in catalyzing the oxidative dehalogenation of TCP. Nevertheless, the imprinting effect was also demonstrated by substrate binding and selectivity studies.

3.4. Binding properties

The binding parameters of the polymer synthesized with 10% VPY were obtained from equilibrium experiments, using the Freundlich isotherm to fit the data (Table 3). This model gives a good mathematical approximation of the binding characteristics of non-covalently imprinted polymers, and it is widely used to describe the heterogeneity of MIPs in the subsaturation region [24,25]. The heterogeneity index (m) is similar for both imprinted and non-imprinted polymers, although a slightly increase was observed for the MIPs. It could be due to the presence of specific TCP binding sites apart from the unspecific ones present in both types of polymers. The binding parameters were estimated in the (K_{min}, K_{max}) intervals (1.5×10^4, 5.4×10^5) and (1.7×10^4, 2.0×10^5) for 10% VPY-MIP and 10% VPY-NIP, respectively. The average number of binding sites ($N_{k\,min-k\,max}$) was lower for the imprinted polymer compared to the control one. However, 10% VPY-MIP exhibited more affinity for TCP than 10% VPY-NIP regarding the affinity constant ($K_{k\,min-k\,max}$). This could be related to the presence of specific TCP recognition sites, which had been created during the imprinting process.

3.5. Selectivity

For the selectivity studies we have selected 2,4-dichlorophenol (2,4-DCP) and 4-chloro-3-methylphenol (4-C-3-MP), two structurally similar compounds that could compete against TCP for the active sites of the polymers, and undergo oxidative dehalogenation since they have a chlorine atom in the *para* position. Additionally, we considered 2,4-dinitrophenol (2,4-DNP), which can block the binding/catalytic sites although it cannot undergo the catalytic reaction.

Table 5
Initial rate of the oxidative dehalogenation of DCP and 4C-3MP with 10% VPY-MICs as catalysts.

Interferent	Polymer	$v_o \times 10^{-5}$ (M min^{-1})
DCP	10% VPY-MIP	1.4 ± 0.4 (6)
	10% VPY-NIP	1.9 ± 0.5 (6)
4C-3MP	10% VPY-MIP	1.2 ± 0.3 (7)
	10% VPY-NIP	1.4 ± 0.5 (6)

DCP, 2,4-dichlorophenol; 4C-3-MP, 4-chloro-3-methylphenol.

Hill equation was used to analyse the kinetic data obtained for increasing amounts of TCP and a fixed concentration (10^{-4} M) of each interferent, and these results were displayed in Table 4. When 2,4-DCP and 4-C-3-MP were in the reaction media, the Hill index decreases until no cooperative effects took place, whereas a substantial increase of n is observed with 2,4-DNP. No significant changes were observed in v_{max} and k_{cat} with respect to the absence of interferents, and the pseudo-Michaelis constant doubled its value with 2,4-DCP and 4-C-3-MP, which indicated that the affinity of the catalyst to TCP decreased in the presence of these compounds. Besides, the catalytic efficiencies obtained for TCP with 2,4-DCP and 4-C-3-MP in the reaction media decreased respect to TCP alone. These facts indicated that these two compounds significantly interfere with TCP, and they both could compete for the active sites of MIPs. Finally, k_{cat}/K_s^* increased in the presence of 2,4-DNP with a more marked increase when MIPs acted as catalysts. This implied that this compound activates the oxidative dehalogenation of TCP under the described conditions.

To confirm the interference of 2,4-DCP and 4-C-3-MP in TCP reaction, these compounds were taken as substrates instead of TCP, and their main reaction products (2-chloro-1,6-benzoquinone and 3-methyl-1,6-benzoquinone, respectively) were monitored by HPLC-UV/VIS. Unexpectedly, at the concentration of polymer assayed these compounds exhibited constant substrate conversion rate, which is independent of the substrate concentration in the range 10^{-6}–10^{-3} M. This fact was observed for 2,4-DCP or 4-C-3-MP substrates with MIPs and NIPs as catalysts. Therefore a mean v_o was obtained in each case, as shown in Table 5.

These facts seem to indicate that 2,4-DCP and 4-C-3-MP have competed with TCP, and they acted as inhibitors of TCP oxidative dehalogenation. However, these interferents were bound to the MIPs in different sites from the specific ones created for TCP during the imprinting process, although these binding sites were also hemin-containing because the catalytic reaction could also take place. Nevertheless, their presence did not totally limit the

Table 4
Kinetic parameters for TCP with 10% VPY-MICs as catalyst and a fixed concentration (10^{-4} M) of interferent.

Interferent	Polymer	n	$v_{max} \times 10^{-5}$ (M min^{-1})	k_{cat} (min^{-1})	$K_s^* \times 10^{-4}$ (M)	$k_{cat}/K_s^* \times 10^3$ (M^{-1} min^{-1})
DCP	MIP	1.0 ± 0.2	1.3 ± 0.3	0.6 ± 0.2	3 ± 1	1.9 ± 0.8
	NIP	2.0 ± 0.2	1.2 ± 0.3	0.35 ± 0.08	1.19 ± 0.07	2.9 ± 0.7
4C-3MP	MIP	1.0 ± 0.3	1.1 ± 0.3	0.5 ± 0.2	2 ± 1	2 ± 1
	NIP	1.3 ± 0.4	1.4 ± 0.2	0.39 ± 0.06	1.2 ± 0.4	3 ± 1
DNP	MIP	2.7 ± 0.7	1.2 ± 0.5	0.52 ± 0.02	0.83 ± 0.08	6.2 ± 0.6
	NIP	2.2 ± 0.6	1.32 ± 0.07	0.37 ± 0.02	0.9 ± 0.1	4.1 ± 0.5

DCP, 2,4-dichlorophenol; 4C-3-MP, 4-chloro-3-methylphenol; DNP, 2,4-dinitrophenol.

oxidative dehalogenation of TCP, and even more, the oxidative dehalogenation of TCP was the preferred route, over those of the interferents.

4. Conclusions

We have synthesized polymers that mimic chloroperoxidase for the oxidative dehalogenation of 2,4,6-trichlorophenol using hemin as the catalytic centre and simulating the aminoacidic residues of the enzyme with a combination of neutral and basic functional monomers (methacrylamide, MA; 4-vinylpyridine, VPY, respectively). On the basis of the chemical nature of the aminoacidic residues in the catalytic centre of the natural CPO (3:1 acid:base character), three different ratios of MA to VPY were tested: 3:1 (25% VPY), 9:1 (10% VPY) and 1:1 (50% VPY). The appearance of 2,6-dichloro-1,4-benzoquinone as main oxidation product revealed that the polymers produce regioselective transformation of the substrate, thus acting as artificial mimics of natural chloroperoxidase. Unlike the natural enzyme, in this case the polymer with the lower VPY content (9:1 ratio) exhibited the highest catalytic efficiency. Substrate selectivity was introduced by the use of molecular imprinting technology. This molecularly imprinted polymer is easy to prepare, and its low cost allows its use in large quantities in degradation processes, competing with enzymes in bioremediation routes.

Acknowledgments

This research was financially supported by the Spanish Government, Ministerio de Ciencia y Tecnología (CTQ2008-02429/BQU) and the European Social Fund (ESF). G. Díaz-Díaz also thanks the Spanish Government Ministerio de Educación y Ciencia and the ESF for a FPI Fellow grant, and the Principado de Asturias Government Fundación para el Fomento en Asturias de la Investigación Científica Aplicada y la Tecnología (COF 09-20 FICYT) for a postdoctoral contract.

References

[1] M. Ahmaruzzaman, Adv. Colloid Interface Sci. 143 (2008) 48–67.
[2] C.D. Murphy, Biotechnol. Lett. 29 (2007) 45–49.
[3] E. Laurenti, E. Ghibaudi, G. Todaro, R.P. Ferrari, J. Inorg. Biochem. 92 (2002) 75–81.
[4] C.E.H. La Rotta, E. D'Elia, E.P.S. Bon, Electron. J. Biotechnol. 10 (2007) 24–30.
[5] A. Longoria, R. Tinoco, R. Vázquez-Duhalt, Chemosphere 72 (2008) 485–490.
[6] G. Lente, J.H. Espenson, New J. Chem. 28 (2004) 847–852.
[7] G. Lente, J.H. Espenson, Green Chem. 7 (2005) 28–34.
[8] P. Zucca, G. Mocci, A. Rescigno, E. Sanjust, J. Mol. Catal. A: Chem. 278 (2007) 220–227.
[9] P. Zucca, F. Sollai, A. Garau, A. Rescigno, E. Sanjust, J. Mol. Catal. A: Chem. 306 (2009) 89–96.
[10] P. Zucca, C. Vinci, A. Rescigno, E. Dumitriu, E. Sanjust, J. Mol. Catal. A: Chem. 321 (2010) 27–33.
[11] M. Fukushima, J. Mol. Catal. A: Chem. 286 (2008) 47–54.
[12] M. Fukushima, S. Shigematsu, J. Mol. Catal. A: Chem. 293 (2008) 103–109.
[13] O. Ramström, K. Mosbach, Curr. Opin. Chem. Biol. 3 (1999) 759.
[14] G. Wulff, Chem. Rev. 102 (2002) 1.
[15] G. Díaz-Díaz, Y. Diñeiro, M.I. Menéndez, M.C. Blanco-López, M.J. Lobo-Castañón, A.J. Miranda-Ordieres, P. Tuñón-Blanco, Polymer 52 (2011) 2468–2473.
[16] Z.Y. Cheng, Y.Z. Li, J. Mol. Catal. A: Chem. 256 (2006) 9–15.
[17] W.D.R. Santos, P.R. Lima, C.R.T. Tarley, N.F. Hoehr, L.T. Kubota, Anal. Chim. Acta 631 (2009) 170–176.
[18] G. Díaz-Díaz, M.C. Blanco-López, M.J. Lobo-Castañón, A.J. Miranda-Ordieres, P. Tuñón-Blanco, Appl. Catal. B 96 (2010) 51–56.
[19] M. Sundaramoorthy, J. Terner, T.L. Poulos, Structure 3 (1995) 1367.
[20] G. Díaz-Díaz, M.C. Blanco-López, M.J. Lobo-Castañón, A.J. Miranda-Ordieres, P. Tuñón-Blanco, J. Mol. Catal. B: Enzym. 66 (2010) 332.
[21] R.L. Osborne, G.M. Raner, L.P. Hager, J.H. Dawson, J. Am. Chem. Soc. 128 (2006) 1036.
[22] R.L. Osborne, M.K. Coggins, J. Terner, J.H. Dawson, J. Am. Chem. Soc. 129 (2007) 14838.
[23] I.H. Segel, Enzyme Kinetics, Behaviour and Analysis of Rapid Equilibrium and Steady-state Systems, John Wiley & Sons, USA, 1993.
[24] A.M. Rampey, R.J. Umpleby II, G.T. Rushton, J.C. Iseman, R.N. Sah, K.D. Shimizu, Anal. Chem. 76 (2004) 1123.
[25] R.J. Umpleby II, S.C. Baxter, M. Bode, J.K. Berch Jr., R.N. Sha, K.D. Shimizu, Anal. Chim. Acta 435 (2001) 35.