Chemosphere 72 (2008) 485–490

Contents lists available at ScienceDirect

Chemosphere

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

Chloroperoxidase-mediated transformation of highly halogenated monoaromatic compounds

Adriana Longoria, Raunel Tinoco, Rafael Vázquez-Duhalt *

Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apartado Postal 510-3, Cuernavaca, Morelos 62250, Mexico

ARTICLE INFO

Article history:
Received 6 October 2007
Received in revised form 2 March 2008
Accepted 3 March 2008
Available online 24 April 2008

Keywords:
Chloroperoxidase
Halogenated compounds
Pentachlorophenol
Pentachloroaniline
Tetrachlorophenol
Tetrachloroaniline

ABSTRACT

Peroxidase transformations of widely distributed pollutants, tetra- and penta-chlorinated phenols and anilines, were studied using different peroxidases. Chloroperoxidase from *Caldariomyces fumago* was able to transform tetra- and penta-chlorinated phenols and anilines, while horseradish peroxidase, lignin peroxidase from *Phanerochaete chrysosporium* and versatile peroxidase from *Bjerkandera adusta* were able only to transform the halogenated phenols. Chloroperoxidase showed a specific activity on pentachlorophenol two orders of magnitude higher than lignin peroxidase and horseradish peroxidase, and one order of magnitude higher than versatile peroxidase. The main product from peroxidase oxidation in all cases was a polymeric and insoluble material. The insolubilization of halogenated phenols and anilines permits their removal, reduces their bioavailability, and thus reduces their environmental impact. The other minor products from the enzymatic transformation of highly chlorinated compounds were determined by mass spectrometry. Tetrachloroquinone, dimers and trimers of halogenated compounds were also identified. Chloroperoxidase was able to halogenate tetrachloroaniline to form pentachloroaniline.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Highly halogenated monoaromatic compounds, including tetra- and penta-halogenated phenols and anilines, are currently used for a wide range of domestic, agricultural and industrial purposes. Chlorophenols are among the most widely distributed pollutants. Their toxicity increases as the degree of chlorine substitution increases (Zhao et al., 1995). Pentachlorophenol (PCP) and the lower chlorinated phenol, tetrachlorophenol (TCP), are intensively used as fungicides, herbicides, insecticides, and precursors in the synthesis of other pesticides. The ban on PCP production in USA since 1992 and in Europe since 2000, and the current production in countries such as China and Mexico, makes it difficult to find data about total world production of chlorophenols. However, the world production of these compounds was estimated to be over 200000 tons per year in 1980 (Ahlborg et al., 1980). Specifically, the PCP worldwide production has been estimated between 25000 and 90000 tons per year (Ullmanns Encyklopadie der technischen Chemie, 1983), and no data is available thereafter. PCP is the major synthetic wood preservative currently used and it is used in a variety of applications for its biocidal properties, as an additive for shoe leather, drilling mud, paper products, and certain food packaging. Despite its uses PCP has been banned in many countries and its use has been severely restricted in others, however it is still widely used and remains an important pollutant from a toxicological perspective. The toxicology and environmental impact of PCP has been reviewed (Ahlborg et al., 1980; Jensen, 1996; Proudfoot, 2003). PCP is a stable and persistent compound, that can be adsorbed by ingestion, inhalation and, to a lesser extent, by the skin. It is metabolized and eliminated slowly. Severe exposure results in acute effects mediated by uncoupling oxidative phosphorylation. Occasionally, fatal illness occurs with tachycardia, tachypnoea, hyperthermia, sweating, and convulsions (Proudfoot, 2003). PCP has been classified as a weak mutagen: however, it is able to form a DNA adduct (Dai et al., 2003, 2005), and thus should be considered as a potential carcinogen.

Chloroanilines are used as intermediates in the synthesis of dyestuffs, agricultural chemicals, pharmaceuticals, and others, and they can be released into the environment through industrial activity. They can also occur in the environment as biodegradation products of aniline-based pesticides. Pentachloroaniline (PCA) is a major metabolite of the widely used funguicide quintozene (pentachloronitrobenzene, PCNB) (Renner, 1980; Fushiwaki et al., 1990). This pesticide is converted to PCA in moist soil, estuarine sediments (Tas and Pavlostathis, 2005), and by animal metabolism (Renner, 1980; Larsen et al., 1998). Clary and Ritz (2003) found increased pancreatic cancer mortality among long-term residents in areas with high application rates of PCNB. Highly chlorinated anilines can be lethal for many organisms (De Wolf et al., 1991). In general, chloroaniline toxicity increases with the number of chlorine atoms, although this trend is less marked for highly

* Corresponding author. Tel.: +52 555 6227619; fax: +52 777 3172388.
E-mail address: vazqduh@ibt.unam.mx (R. Vázquez-Duhalt).

0045-6535/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2008.03.006

substituted compounds. Tetrachloroaniline (TCA) shows only slightly increased toxic effects with respect to trichloroanilines, while for pentachloroaniline (PCA), the trend of increasing toxicity is reversed since this compound was found to be less toxic than tetra-substituted anilines (Argese et al., 2001). Its toxicity potential was also estimated with the Microtox test and with other bacterial toxicological assays (Ribo and Kaiser, 1984). Chlorinated anilines are commonly classified as polar narcotic chemicals in most Quantitative structure–activity relationship (QSAR) studies focused on acute toxicity to aquatic organisms (Sixt et al., 1995).

Ligninolytic fungi are able to degrade PCP. *Phanerochaete chrysosporium* (Reddy and Gold, 2000; Shim and Kawamoto, 2002), *Gloeophyllum striatum* (Fahr et al., 1999), *Panus tigrinus* (Leontievsky et al., 2002), and *Trametes versicolor* (Walter et al., 2004) are able to mineralize PCP to CO_2. The proposed fungal PCP metabolism is a multi-step pathway starting with an oxidative dehalogenation mediated by extracellular peroxidases to form tetrachloro-1,4-benzoquinone (TCBQ). TCBQ is further degraded by reductive dehalogenations, and then hydroxylated. *In vitro* ligninolytic peroxidases and other peroxidases are able to transform PCP. Lignin peroxidase from *P. chrysosporium* (Hammel and Tardone, 1988; Chung and Aust, 1995; Rüttimann-Johnson and Lamar, 1996), horseradish peroxidase (Samokyszyn et al., 1995; Choi et al., 1999), myeloperoxidase (Wittsiepe et al., 2000), lactoperoxidase (Oberg and Paul, 1985), and the peroxidase model microperoxidase-8 (Osman et al., 1998) transform PCP to TCBQ by an oxidative dehalogenation in the presence of hydrogen peroxide. However, significant amounts of the highly toxic polychlorinated dibenzo-*p*-dioxins and dibenzofurans have been reported after peroxidase-catalyzed oxidation (Wittsiepe et al., 1999).

P. chrysosporium has also been shown to mineralize lignin conjugates of chloroanilines and free chloroanilines (Arjmand and Sandermann, 1985), but no products were identified. This ability is important in order to remove these conjugates from the environment. 4-Chloroaniline and 4-chlorophenol have been transformed by oxidoreductases in presence of humic acids and oligomerization of the substrates or their binding to organic matter was found (Park et al., 2000). Mono- and di-chlorinated anilines have been studied but there is no information about highly halogenated anilines.

In this work, the enzymatic transformation of highly chlorinated monoaromatic phenols and amines by chloroperoxidase from *Caldariomyces fumago* is studied. Chloroperoxidase from *C. fumago* is a versatile and unusual heme-peroxidase. *In vitro*, chloroperoxidase shows halogenase-, peroxidase-, catalase- and cytochrome P450-like activities (Colonna et al., 1999), and has been shown to be the most active peroxidase in the transformation of polycyclic aromatic hydrocarbons (Vazquez-Duhalt, 1998).

2. Materials and methods

2.1. Enzyme and chemicals

Purified chloroperoxidase (CPO) from *C. fumago* was a gift from Prof. Michael A. Pickard (University of Alberta, Canada). Lignin peroxidase form *P. chrysosporium* was purchased from Tienzyme (Salt Lake City, UT), versatile peroxidase from *Bjerkandera adusta* was obtained and purified as previously described (Pogni et al., 2005), and horseradish peroxidase was obtained from Sigma–Aldrich (Milwaukee, WI). Pentachlorophenol (PCP), 2,3,5,6-tetrachlorophenol (TCP), and 2,3,5,6-tetrachloraniline (TCA) were purchased from Sigma–Aldrich (Milwaukee, WI). Pentachloroaniline (PCA) was obtained from Alfa-Aesar (Ward Hill, MA). HPLC-grade organic solvents were from Fisher Scientific (Springfield, NJ). All the other chemicals were obtained from J.T. Baker (Phillipsburg, NJ) as reagent grade.

2.2. Enzymatic transformation of halogenated compounds

The enzymatic oxidation of highly halogenated monoaromatic compounds with CPO was carried out in a 1 ml reaction mixture containing 20% isopropanol in 60 mM acetate buffer (pH 3.0), 20 mM KCl, and with different concentrations of the corresponding halogenated compound. The reaction mixture contained between 31.4 and 314 pmol of CPO and the reaction was started by adding hydrogen peroxide. The transformation rate was determined as substrate depletion monitored by HPLC. The catalytic constants were determined varying the concentrations of halogenated substrate and hydrogen peroxide until catalytic saturation. Values were obtained using the EnzFitter software (Biosoft, Cambridge, UK) following a Michaelis–Menten model.

Activity was also measured with other peroxidases in a buffer containing 20% isopropanol. Lignin peroxidase from *P. chrysosporium* and versatile peroxidase from *B. adusta* were assayed in a 40 mM succinate buffer pH 3.0, while horseradish peroxidase was assayed in a reaction mixture containing 60 mM phosphate buffer pH 6.0. The concentration of halogenated compounds was set at 100 μM except for PCA which was at 20 μM because of its insolubility in 20% isopropanol buffer.

HPLC analysis was performed with a Perkin Elmer system equipped with a 235C diode array detector, using a C_5 column (15 cm $\times$ 4.6 mm, 5 μm particle size; Supelco, Inc., Bellefonte, PA). The mobile phase was 40% acetonitrile:60% H_2O containing 0.1% trifluoroacetic acid. TCP was detected at 210 nm, PCP at 215 nm and TCA and PCA at 220 nm.

2.3. Identification of reaction products

To have enough material for chemical analysis, 100 ml CPO-enzymatic reactions were carried out. Successive additions of enzyme and hydrogen peroxide were made until the substrate was exhausted. Reaction mixtures were extracted with dichloromethane and the extract evaporated under vacuum. When polymer was formed, the reaction mixture was centrifuged, the pellet washed with methanol, and the methanolic solution evaporated under vacuum. The volume of samples was reduced under N_2 flow and then redissolved in 50 μl of dichloromethane. The chemical nature of the products was determined by gas chromatography coupled to a mass detector (GC–MS). One microliter was injected on a GC–MS (Agilent 6890N gas chromatograph with 5973 mass selective detector). For insoluble samples, electron impact spectra were obtained using a direct insertion probe (Scientific Instrument Services). Samples were added to the probe as solids.

3. Results and discussion

Four highly halogenated monoaromatic compounds: tetra- and penta-chlorophenol and tetra- and penta-chloroaniline, were assayed for enzymatic transformation with four different peroxidases. The catalytic activity of CPO from *C. fumago*, lignin peroxidase from *P. chrysosporium*, versatile peroxidase from *B. adusta*, and horseradish peroxidase was determined on these halogenated compounds (Table 1). All the peroxidases tested were able to transform the halogenated phenols, however only CPO was able to transform the halogenated anilines. In addition, the specific activity of CPO was one order of magnitude higher than that of versatile peroxidase, and two orders of magnitude higher that those of lignin peroxidase and horseradish peroxidase, confirming that CPO is an interesting enzyme for environmental purposes (Vazquez-Duhalt, 1998).

The kinetic constants for CPO and the four halogenated substrates were determined (Table 2). The k_{cat} values vary from

Download English Version:

https://daneshyari.com/en/article/4414240

Download Persian Version:

https://daneshyari.com/article/4414240

Daneshyari.com