

A computational study on enzymatically driven oxidative coupling of chlorophenols: An indirect dehalogenation reaction



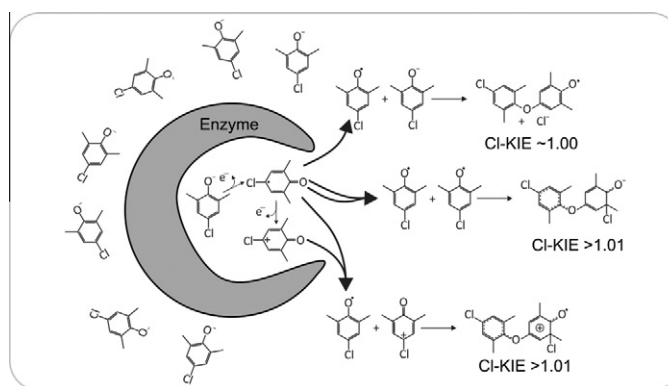
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HIGHLIGHTS

- ▶ Coupling of chlorophenoxy radicals by HRP proceeds via radical–anion/radical/cation mechanisms.
- ▶ No condensation reaction leading to dibenzodioxins is observed.
- ▶ Chloride elimination is not accompanied by chlorine kinetic isotope effect.

GRAPHICAL ABSTRACT



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ABSTRACT

Density functional theory calculations have been used to describe the mechanism of the dimerization reaction catalyzed by peroxidases and predict whether it could be accompanied by chlorine isotopic fractionation when various chlorophenols are used as their substrates. Since free radicals formed during the catalytic cycle of peroxidases can undergo coupling reactions (either radical–radical or radical–molecule) that can lead to dimers and also polymers formation four different pathways have been considered: radical–anion, radical–cation, radical–radical (singlet state) and radical–radical (triplet state). The following substrates have been investigated: 2-chlorophenol, 4-chlorophenol, 2,4,6-trichlorophenol and 4-chloro-2,6-dimethylphenol. Based on the obtained energetic profiles radical–cation and radical–radical (singlet) coupling seem to be the most probable. Radical–anion coupling although energetically more expensive should not be disregarded taking into account the excess of the anionic form of the substrate being provided in the course of enzymatic reaction. Upon dimer formation halogen is released during radical–anion and radical–radical triplet coupling. However only the latter pathway exhibits chlorine isotopic fractionation. Radical–cation and radical–radical singlet couplings are two-step reactions where the second step comprises intramolecular chlorine transfer accompanied by large chlorine isotope effect.

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1. Introduction

Chlorinated phenols (CPs) have been widely used for decades as wood impregnation agents, and due to past work practices or

accidental releases the soil, surface and ground water has been contaminated (Laine et al., 2003). Their presence in the groundwater causes a threat to public health, and the environment. These recalcitrant compounds can be (bio)transformed by soil microbes via hydroxylation, methoxylation or polymerization reactions. Several mineralization pathways of CPs have been adopted by bacteria, and fungi present in the polluted areas (Fetzner and Lingens, 1994; Mannisto et al., 1999). One group of the species capable of

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converting CPs are enzymes from white rot fungi (Field, 2003). Chlorophenols ranging from penta- to monochlorophenols can be mineralized to CO₂ and chloride ions by respective enzymes which adopted a number of mechanisms among which the most important are: (i) oxidation, (ii) methylation, (iii) benzoquinone reduction, (iv) hydroxylation and (v) reductive dehalogenation.

The major reaction catalyzed by ligninolytic enzymes is oxidation. The products of the oxidation can vary from quinones to polymerized species (Dec and Bollag, 1990). Laccases and plant peroxidases have been proposed to be the *in vivo* generators of the phenoxy radicals, which then can link up to form dimers, trimers, and higher oligomers. Since the oxidation of phenols proceeds fast in aqueous solution, and the oligomeric products are easily formed peroxidases have found their place as efficient catalysts of functional polymeric materials synthesis (Kobayashi et al., 2001; Ritter et al., 2006). For example, horseradish peroxidase (HRP) has been shown to be active in a number of organic solvents and solvent mixtures, thus provides mild reaction conditions without the use of toxic reagents (environmentally benign process).

The catalytic cycle of HRP-catalyzed polymerization of phenols is carried out in the presence of H₂O₂ and comprises two one-electron transfer steps, resulting in the formation of a radical during the first step, and a cationic form of substrate in the second step, respectively. And this is the only enzyme-dependent step of the overall polymerization reaction. The subsequent coupling reactions of the resulted free radicals can produce dimers and eventually lead to the formation of polymer (Danner et al., 1973; Ward et al., 2001). While the formation and subsequent precipitation of higher molecular weight products have been observed (Nakamoto and Machida, 1992; Buchanan and Nicell, 1997; Huang et al., 2005), the details of possible reactions of formed phenoxy radicals remain unknown.

In the case of unsubstituted phenols the polymers are formed via C–C bridges mostly. In *para*-substituted phenols a recombination reaction takes place mainly at the *ortho* position. Mechanistic studies of the polymerization of 4-hydroxybenzenesulfonic acid have provided the evidence of C–O–C coupling which can be a dominant linkage structure of the resulting polymers during the polymerization of phenols with electron-withdrawing groups (like COOH or Cl) (Liu et al., 2001). And although, in general, phenols with electron-withdrawing substituents are considered to be poor substrates for the HRP-catalyzed polymerization there have been some attempts to use them as starting compounds for new functional materials (Ikeda et al., 2000). And it was not the only

motivation. Peroxidases-catalyzed polymerization of halogenated phenols is also of interest because this method as such has some potential to be applied to remove the resulting polymers from the wastewater and hence help to clean the polluted areas (Nicell, 1994; Ghiourelotis and Nicell, 1999).

Apart from polymers formation oxidative coupling in the case of substituted phenols has another effect – release of substituents (Dec and Bollag, 1994). Several mechanistic possibilities have been proposed for coupling reactions between free radicals generated during enzymatic oxidation of chlorophenols (Park et al., 2000; Dec et al., 2003). And although substituent release from organic compounds has been found to be a common phenomenon caused either by microorganisms or some physical processes taking place in the environment, the fact that it has been also observed during oxidative coupling reactions of chlorophenols may constitute another possible way of removing these toxic compounds. Furthermore, if adopted by the same enzyme, for instance HRP, it may additionally constitute another way of removing halogenated compounds applied by the same biocatalyst, as peroxidases are capable of removing chlorines via direct water attack on the aromatic carbon of the substrate (Wiese et al., 1997).

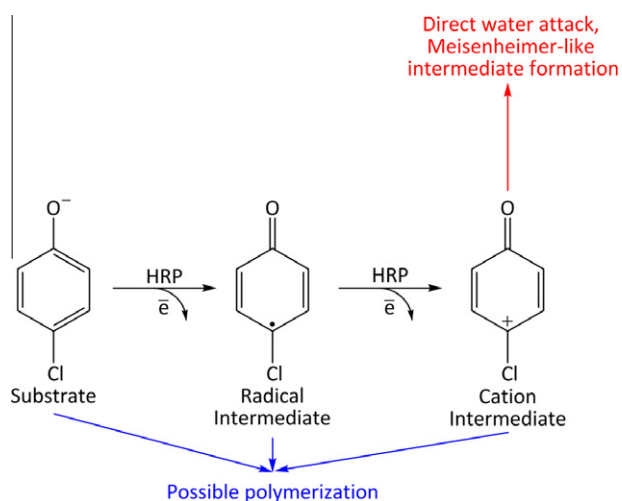
Eliminating halogens due to coupling reactions will be then indirect process leading to the same effect. Such two possible dehalogenation pathways are shown in Scheme 1. Since the direct (via water attack) and indirect (via oxidative coupling) dehalogenation constitute of distinct reaction channels catalyzed by HRP a detailed mechanistic information is of interest in order to estimate (quantify) peroxidases efficiency in eliminating toxic compounds from the environment. Some efforts have been already made in order to describe the mechanism of direct dehalogenation (Ferrari et al., 1999). Most recently it has been shown that the dehalogenation step occurs in a similar fashion as the uncatalyzed reaction in water environment and the enzyme role is to convert the substrate into a form which will undergo dehalogenation reaction more facile. Based on chlorine isotope effects determined both experimentally and theoretically the cationic form of the substrate has been pointed out as a form from which the chlorine substituent is expelled via preceding Meisenheimer complex formation (Szatkowski et al., 2011).

In our continuing efforts to better understand the dehalogenation process taking place during the reaction catalyzed by peroxidases we have decided to investigate the dimerization channel of the reaction in more detail, and hence provide better insight into the removal process of toxic halogenated phenols. To this end since the dehalogenation process and its extent during oxidative coupling of formed radicals are of interest we have employed theoretically predicted chlorine kinetic isotope effects (Cl-KIEs) tool to describe a concurrent channel of reaction occurring during oxidation of selected chlorophenols.

2. Methods

2.1. General approach

All electronic structure calculations have been carried out using the Gaussian09 package (Frisch et al., 2009). Initial geometries of studied complexes have been optimized to respective minima at the M05-2X/6-31+G(d,p) level of theory (Frisch et al., 1984; Zhao et al., 2006). Based on our previous results for oxidation reaction of trichloroethylene by permanganate (Adamczyk et al., 2011) and other communications on E2 or S_N2 reactions of X[−] + CH₃CH₂X (X = F, Cl) (Bento et al., 2008; Zhao and Truhlar, 2010) we have assumed that such level of theory along with the IEF-PCM (Miertus et al., 1981) representation of solvent (water) will be adequate for treating the reactions of interest. Water environment can be additionally justified by the fact that the reactions of interest occur when generated phenoxy radicals escape from a



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