Chemical Engineering Journal 303 (2016) 529–538

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Differential enzymatic degradation of thiazole pollutants by two different peroxidases – A comparative study

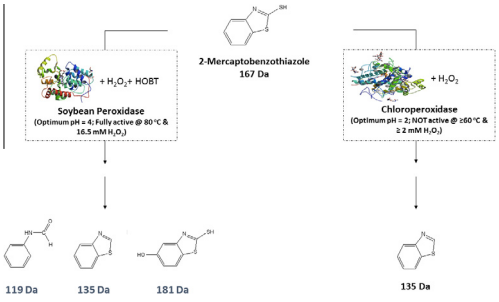
Aysha Hamad Alneyadi [a], Syed Salman Ashraf [b,*]

[a] Department of Biology, College of Science, UAE University, United Arab Emirates
[b] Department of Chemistry, College of Science, UAE University, United Arab Emirates

HIGHLIGHTS

- Detailed comparative study of two thiazole pollutants degradation by different peroxidases.
- Related peroxides have different pH optima, H_2O_2 and redox mediator requirements.
- Soybean- and Chloro-peroxidases exhibit remarkably different thermal stabilities.
- Related peroxidases show very different degradation pathways for the two thiazole pollutants.
- First report of the water pollutant, 2-mercaptobenzothiazole, degradation by two peroxidases.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 31 May 2016
Accepted 3 June 2016
Available online 4 June 2016

Keywords:
Emerging pollutants
Thioflavin T
2-Mercaptobenzothiazole
Soybean peroxidase
Chloroperoxidase
Bioremediation
Pollutant degradation

ABSTRACT

Enzyme based degradation of organic pollutants, including emerging pollutants, is a promising remediation approach due to the promiscuous nature of the technique and the wide range of available enzymes. However, it is generously assumed that all peroxidase enzymes behave similarly and may produce similar degradation products. In the present study, we have carried out detailed degradation studies on a model thiazole compound (Thioflavin T) using two of the most commonly used peroxidases – Soybean peroxidase (SBP) and Chloroperoxidase (CPO). In addition, these two peroxidases were also used to degrade a recalcitrant water pollutant, 2-mercaptobenzothiazole. Our studies show that these two enzymes have remarkably different optimum conditions for the decolorization of Thioflavin T. For example, SBP required a redox mediator for the decolorization of Thioflavin T, whereas CPO had no such requirement. Furthermore, SBP and CPO had very different thermal stabilities, with SBP showing full activity up until 80 °C, which was very different than CPO, which was almost completely inactive at 60 °C. HPLC analyses confirmed that both SBP and CPO transformed Thioflavin T into different compounds, which was further confirmed by LC–MS-MS studies. Degradation studies with 2-mercaptobenzothiazole also showed that SBP was much more efficient in degrading the pollutant and produced numerous breakdown products, whereas CPO was not as effective and generated only 2 intermediates. Our results show that related peroxidases may behave very differently and points to the need for detailed mechanistic studies to confirm the structures of the degradation intermediates produced during enzymatic remediation of emerging pollutants and other organic compounds.

* Corresponding author at: Dept. of Chemistry, COS, PO Box 15551, UAE University (UAEU), Al Ain, United Arab Emirates.
E-mail address: salman.ashraf@uaeu.ac.ae (S.S. Ashraf).

http://dx.doi.org/10.1016/j.cej.2016.06.017
1385-8947/

1. Introduction

One of the unfortunate consequences of our modern lifestyle is the release of various kinds of organic pollutants in the environment. Traditionally, the sources of aquatic pollution include industrial discharge, illegal dumping of untreated wastewater, as well as agricultural fertilizer runoffs. All of these contribute to the contamination of water bodies with large amounts of organic pollutants, heavy metals, as well as inorganic chemicals. For example, it is estimated that up to 280,000 ton of synthetic dyes per year are discharged into the environment (mostly rivers and seas) [1]. In addition to these traditional sources of pollution, increased environmental awareness has highlighted concerns arising from the prevalent presence of "emerging pollutants" or "contaminants of emerging concern" in our water supply [2–4]. These emerging pollutants are broadly defined as synthetic chemicals or microbes in the environment that are normally not monitored but have the potential to adversely affect various living organisms. A significant portion of these emerging environmental pollutants are due to the inappropriate use and subsequent disposal of personal care pharmaceuticals, including antibiotics, over-the-counter drugs, birth control hormones, etc., [3,4]. Not surprisingly, environmental activists and scientists are increasingly concerned by the alarmingly high concentrations of these physiologically active chemicals in our water supply. For example, up to 0.2 ppm of the reproductive hormone, 17 β-estradiol, and 10 ppm of acetaminophen (Paracetamol) have been detected in US water streams [5].

Several traditional techniques have been successfully used to treat polluted wastewater, and remove various contaminating organic pollutants - such as chemical and physical methods. Yet, most of them have several limitations, such as overall cost, inefficiency, and difficulty in complete mineralization of the pollutants [6–8]. Biological remediation methods are recent biotechnology techniques in which the organic pollutants are degraded using either microorganisms or enzymes. Various microorganism such as bacteria and fungi have been used to degrade different organic pollutants [9–11]. The enzymatic approaches for pollutant degradation is a relatively new and promising area of bioremediation research that has attracted much interest due to its relaxed substrate specificity, efficiency and the ease of handling [12,13]. The oxidoreductase enzymes such as peroxidases, laccases and tyrosinase have been mainly used in dye degradation, as they can generate free radicals that eventually facilitate bond-cleavage reactions and lead to pollutant degradation [14]. The most commonly used peroxidases for the degradation of organic pollutants are Soybean peroxidase (SBP), Chloroperoxidase (CPO), Horseradish peroxidase (HRP), Lignin peroxidase (LiP), Manganese peroxidase (MnP), and Lactoperoxidase (LPO). The catalytic cycle of peroxidase enzymes involve reaction with hydrogen peroxide to form a Fe (IV) cation radical form of the enzyme (called Compound I), which then reacts with the substrate (organic pollutant-RH) to form a Fe (IV) cation form called Compound II and pollutant radical. The Compound II form of the peroxidase can react with another molecule of the pollutant to return to the resting form of the enzyme and generate another pollutant radical. The pollutant radicals can then further react with themselves or other species in the reaction mixture to eventually be degraded [15], as shown below:

$$\text{Peroxidase} + H_2O_2 \longrightarrow \text{Compound I} + H_2O$$

$$\text{Compound I} + RH \longrightarrow \text{Compound II} + R^{\cdot}$$

$$\text{Compound II} + RH \longrightarrow \text{Peroxidase} + R^{\cdot} + H_2O$$

Despite numerous studies that have been published on the use of different enzymes for the degradation of organic compounds, systematic comparative degradation studies showing the behavior of different peroxidases on the same compound have not been carefully carried out, and only a few examples can be found in the published literature, with none of them reporting the identity of the degradation products. For example, van de Velde and colleagues have reported on the ability of CPO, LPO, SBP, HRP, and catalase to oxidize phenol [16]. Although, CPO, LPO, SBP, and HRP were all able to oxidize phenol, catalase showed very poor activity. Additional experiments carried out showed that although both CPO and HRP were able to efficiently degrade indole and dihydroxyfumaric acid (DHF), CPO was able to oxidize indole much more efficiently than DHF, whereas HRP showed the opposite trend [16]. Another study comparing LiP and HRP showed while both LiP and HRP were able to oxidize Methylene Blue and Azure B dyes, LiP required much less H_2O_2 than HRP [17]. The lack of extensive and careful comparative studies on different peroxidases have led many researchers to assume that "all peroxidases are equal" in their abilities to degrade organic pollutants.

A relatively new class of emerging pollutants is thiazole-based compounds that are increasingly being used for industrial, as well as personal/medical use. Fig. 1 shows a sample of thiazole compounds which include a vulcanizing agent (2-mercaptobenzothiazole, MBT), anti-parasitic agent (2-Amino-5,6-dichloroben zothiazole), fungicides (Thiabendazole and Tricyclazole), and a non-steroidal anti-inflammatory drug (Meloxicam) – all of which have unfortunately been found in various water bodies [18–22].

In the present study, we wanted to focus on these thiazole-based emerging contaminants and to explore the possibility of using peroxidases to degrade them and to test our hypothesis that related peroxidases may not degrade a given pollutant equally well or via the same mechanism. In this regard, we carried out extensive optimization studies on the degradation of a model thiazole compound (Thioflavin T dye - ThT) using two different (yet-related) peroxidases – namely, SBP and CPO. Furthermore, we report for the first time, degradation of the recalcitrant water pollutant 2-mercaptobenzothiozole [19] by these two peroxidases as well. In addition, we have used HPLC–MS-MS to identify the products formed during the enzymatic oxidation of Thioflavin T and 2-mercaptobenzothiazole by both SBP and CPO.

2. Materials and methods

2.1. Reagents

Thioflavin T was purchased from AnaSpec (California, USA). and was found to be 95% pure as judged by HPLC analysis and the molecular weight of the compound as judged by MS was 283 Da. Hydrogen peroxide (30% w/v) and 2-mercaptobenzothiazole (MBT) were purchased from Sigma-Aldrich (USA). Soybean peroxidase (SBP), with a specific activity of 2700 IU/mg (1 mg/ml), 26 μM, and Chloroperoxidase (CPO), with a specific activity of 1296 IU/mg (17 mg/ml), 405 μM were purchased from Bio-Research Products (Iowa, USA).

2.2. Thioflavin T decolorization

Thioflavin T decolorization studies were carried out as previously described for other dyes [23,24]. Briefly, Thioflavin dye (ThT), in specified buffer, along with hydrogen peroxide was exposed to either SBP or CPO and the decolorization was