In vitro biotransformation of PBDEs by root crude enzyme extracts: Potential role of nitrate reductase (NaR) and glutathione S-transferase (GST) in their debromination

Honglin Huang, Shuzhen Zhang*, Sen Wang, Jitao Lv

State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China

highlights

- PBDEs could be debrominated and hydroxylated by the root crude enzyme extracts.
- Debromination was the main degradation pathway.
- In vitro and in vivo exposure to PBDEs produced the similar enzyme responses.
- NaR and GST were the key enzymes in the plant degradation of PBDEs.

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 10 October 2012
Accepted 13 October 2012
Available online 10 November 2012

Keywords:
PBDEs
Biotransformation
Root crude enzyme extracts
In vitro

ABSTRACT

In order to investigate the enzyme transformation of PBDEs and to track the key enzymes involved in PBDE degradation in plants, in vivo exposure of plants of ryegrass, pumpkin and maize and in vitro exposure of their root crude enzyme extracts to PBDEs were conducted. Degradation of PBDEs in the root crude enzyme solutions fit well with the first order kinetics ($R^2 = 0.52–0.97$, $P < 0.05$), and higher PBDEs degraded faster than the lower ones. PBDEs could be transformed to lower brominated PBDEs and hydroxylated-PBDEs by the root crude enzyme extracts with debromination as the main pathway which contributed over 90% of PBDE depletion. In vitro and in vivo exposure to PBDEs produced similar responses in root enzyme activities of which the nitroreductase (NaR) and glutathione-transferase (GST) activities decreased significantly, while the peroxidase, catalase and cytochrome P-450 activities had no significant changes. Furthermore, higher enzyme concentrations of NaR and GST led to higher PBDE debromination rates, and the time-dependent activities of NaR and GST in the root crude enzyme extracts were similar to the trends of PBDE depletion. All these results suggest that NaR and GST were the key enzymes responsible for PBDE degradation. This conclusion was further confirmed by the in vitro debromination of PBDEs with the commercial pure NaR and GST.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are an important class of additive brominated flame retardants (BFRs) that are now recognized as ubiquitous environmental contaminants. PBDEs have been detected in a wide variety of environmental samples, ranging from water, soil and sediment to biota (de Wit, 2002). In recent years, levels of PBDEs in the environment have been increasing significantly. They are of particular concern due to their persistence, long-range transport behavior and possible adverse effects on humans (McDonald, 2002; Richardson, 2010). However, the recent studies on PBDEs are mainly focused on their environmental distributions and animal and human exposures (Costa et al., 2008).

PBDEs can be transformed through debromination, hydroxylation and methoxylation reactions in various environmental media and biota. The heavy molecule congeners of PBDEs have a tendency to break down into lower brominated congeners (Betts, 2008). Hydroxylated PBDEs (OH-PBDEs) and methoxylated PBDEs (MeO-PBDEs) have emerged as environmentally relevant due to the reports of their natural production and metabolism (Malmvarn et al., 2005; Kelly et al., 2008; Wan et al., 2009). Derivatives of PBDEs, lower brominated PBDEs, OH-PBDEs and MeO-PBDEs, exert additional adverse influences to bear on the environment and human health (Hakk and Letcher, 2003; Hamers et al., 2008). Therefore, transformation of PBDEs is of significant importance.
Previous studies have indicated that PBDEs in soils can be taken up by plants (Mueller et al., 2006; Huang et al., 2010; Wang et al., 2011a). Metabolites of lower debrominated PBDEs and OH-PBDEs were also detected in plant tissues (Wang et al., 2011b), which demonstrates that plants are able to transform PBDEs from higher-to lower-brominated congeners as well as to OH-PBDE congeners. Enzymatic transformation, mainly as the result of biotic processes mediated by plants, is by far recognized as the major route of organic degradation (Van Eerd et al., 2003). However, it still remains unclear how plant enzymes response to the exposure of PBDEs and what particular plant enzymes are responsible for PBDE biodegradation.

 Peroxidase (POD), catalase (CAT), cytochrome P450 (CYP450), glutathione-transferase (GST) and nitroreductase (NAR) are known to be involved in plant metabolism of many xenobiotic pollutants. POD was found to play a pivotal role in the oxidation of PAHs (Durán and Esposito, 2000). CAT can catalyze the degradation of azo dyes (Paar et al., 2001). Hydroxylated metabolites of some herbicides in plants are predominantly formed via CYP450-mediated oxidation (Siminskiy, 2006). NAR and GST were reported to mediate the reductive degradative pathway of PCBs and some explosives (Blanchette and Singh, 2003; Anke et al., 2003; Medina et al., 2004; Magee et al., 2008). But the key enzymes involved in PBDE degradation in plants are virtually unknown. Metabolism of PBDEs is usual a two-step process of which bromine atoms are first reductively removed, and then the phenyl rings are oxidatively cleaved. Studies on degradation of PBDEs have indicated that reductive debrumination is the main metabolite pattern of PBDEs in the biota and plants (Stapleton et al., 2006; Huang et al., 2010). Dehalogenation by GST and NAR has previously been shown in some researches (Mackiewicz and Wiegel, 1998; Anandarajah et al., 2000; De et al., 2006). Therefore, we hypothesized the putative capability of NaR and GST to reductively debrominate PBDEs; however we are not aware of any evidence to support this hypothesis.

In this study, in vitro exposure of root crude enzyme extracts and in vivo plant exposure to PBDEs were performed to investigate the biotransformation of PBDEs and to track the key enzymes involved in degradation of PBDEs in plants. The most commonly detected congeners in the environment, BDE-28, -47, -99 and -209, were selected as the representatives of tri-, tetra-, penta- and deca-BDEs, respectively. Metabolites of PBDEs in the plant root crude enzyme solutions were identified. Responses of enzymes following in vitro and in vivo exposure to PBDEs were monitored, and commercial pure enzymes were further used to test their roles in degradation of PBDEs. The results of this study are expected to better understand transformation of PBDEs in plants.

2. Materials and methods

2.1. Chemicals and reagents

The following standards were purchased from AccuStandard (AccuStandard, New Haven, USA): BDE-28, -47, -99 and -209, a standard solution of PBDEs containing 39 native congeners (mono- through hepta-BDEs), OH-PBDE standards (2-OH-BDE3, 3’-OH-BDE7, 4’-OH-BDE17, 3’-OH-BDE28, 3-OH-BDE47, 5-OH-BDE47 and 6-OH-BDE47) and MeO-PBDE standards (2-MeO-BDE3, 3’-MeO-BDE7, 4’-MeO-BDE17, 3’-MeO-BDE28, 3-MeO-BDE47, 5-MeO-BDE47 and 6-MeO-BDE47). A standard solution of BDE-209 dissolved in tetrahydrofuran. They were then spiked into solutions of root crude enzyme extracts at concentrations of 30, 15 and 5 μg L⁻¹ for BDE-28, -47, -99 and -209, respectively, based on their water solubility. Acetone and tetrahydrofuran concentrations in the final solutions were always below 1% (v/v) to minimize cosolvent effects. Glass vials with Teflon-lined caps containing PBDE spiked root crude enzyme solutions were placed on a tumble shaker and shaken at 200 rpm for 4 h. The supernatants were taken at different time intervals up to 96 h. Enzyme activities and concentrations of PBDEs and their metabolites were monitored at each time interval.

For the in vitro plant exposure, PBDEs were spiked in the cultural solutions with the same concentrations as the ones in the in vitro exposure experiments. Plants with mature roots were then transplanted to the exposure solutions in a 150 mL glass-stoppered flask wrapped with aluminum foil to avoid possible evaporation and photo-degradation of PBDEs. Plants grown in non-spiked PBDE cultural solution were set as controls. Plants were taken out of the containers after incubation for 96 h for the measurements of enzyme activities.

2.2. Plant cultivation and preparation of crude enzyme extracts from plant roots

Italian ryegrass (Lolium multiflorum L.), pumpkin (Cucurbita pepo ssp. Pepo cv.), and maize (Zea mays L.) were chosen as the test plants in which transformation of PBDEs has been observed (Huang et al., 2011). Seeds were purchased from the Chinese Academy of Agricultural Sciences, Beijing, China. Method for plant cultivation has already been described in the previous study (Zhu et al., 2007). The growth period varied from 4 to 8 weeks for different plant species until they had developed mature roots.

Fresh roots were finely chopped with pruning shears and homogenized in an extraction buffer (pH 7.8) containing 0.05 M NaH₂PO₄, 0.5 M HPO₄₂⁻, 2 mM EDTA, 1% (w/v) polyvinylpyrrolidone and 2 mM DL-Dithiothreitol with a ratio of 1:8 (w/v) under liquid nitrogen. The homogenate was filtered through four layers of cheesecloth and the filtrates were centrifuged at 11 000 rpm for 30 min. All operations were performed at 4°C. The supernatants of root crude enzyme extracts were collected, separated in sterile glass vials (each 10 mL) and stored at −80°C before use.

2.3. Plant exposure experiments

Activities of the exposure solutions of root crude enzyme extracts are given in Table 1 in the Supplementary material. Solubility of PBDEs in water is low and decreases sharply with the increase of bromine atom number. Therefore, standard solutions were prepared with BDE-28, -47, -99 dissolved in acetone, and BDE-209 dissolved in tetrahydrofuran. They were then spiked into solutions of root crude enzyme extracts at concentrations of 30, 15, 10 and 5 μg L⁻¹ for BDE-28, -47, -99 and -209, respectively, based on their water solubility. Acetone and tetrahydrofuran concentrations in the final solutions were always below 1% (v/v) to minimize cosolvent effects. Glass vials with Teflon-lined caps containing PBDE spiked root crude enzyme solution were placed on a tumble shaker and shaken at 200 rpm at 4°C in the dark. The solutions were incubated for 4 d. Controls of plant enzymes denatured with heat by placing aliquots of enzyme solution in a water bath at 100°C for 10 min were conducted under the same conditions. Triplicate sample tubes were taken at different time intervals up to 96 h. Enzyme activities and concentrations of PBDEs and their metabolites were monitored at each time interval.

For the in vivo plant exposure, PBDEs were spiked in the cultural solutions with the same concentrations as the ones in the in vitro exposure experiments. Plants with mature roots were then transplanted to the exposure solutions in a 150 mL glass-stoppered flask wrapped with aluminum foil to avoid possible evaporation and photo-degradation of PBDEs. Plants grown in non-spiked PBDE cultural solution were set as controls. Plants were taken out of the containers after incubation for 96 h for the measurements of enzyme activities.

2.4. Reaction of PBDEs with commercial pure GST and NaR

BDE-28, -47, -99 and -209 at concentrations of 30, 15, 10 and 5 μg L⁻¹, respectively, were incubated for 96 h in the presence of commercial pure GST (purified from equine liver, 55 U mg⁻¹ protein, Sigma–Aldrich) or NaR (purified from Z. mays L., 20 U mg⁻¹ protein, Sigma–Aldrich). The reaction mixture consisted of 3.6 μg L⁻¹ GST with 0.05 M phosphate buffer and 0.5 μM GSH or HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water (18.2 MΩ) used was obtained by using a Milli-Q system (Milli-Q Advantage A10, Millipore Corporation, Billerica, MA, USA). All other chemicals and reagents used were of reagent grade.
10 mg L\(^{-1}\) NaR with 0.05 mM phosphate buffer (with 10 mM KNO\(_3\) and 0.05 mM EDTA), 2 mM NADH and 3 mM Na\(_2\)MoO\(_4\), respectively. Enzyme activity in the reaction mixture was 0.2 U mL\(^{-1}\), and pH was 7.8. Reaction vials were incubated at 4 °C in the dark under agitation at 200 rpm. Controls were conducted under the same conditions with enzyme denatured. Experiments were performed in triplicate.

### 2.5. Enzyme assays

Enzyme activities were measured at 25 °C using a spectrophotometer (Shimadzu UV-2100, Japan). Detailed information on the enzyme assays (POD, CAT, CYP450, NaR and GST) is available in the Supplementary material. All the activities are expressed as U g\(^{-1}\) of root fresh weight or U mL\(^{-1}\) of enzyme solution.

### 2.6. Analysis of PBDEs and their metabolites in enzyme solutions

Extraction and cleanup of PBDEs in solutions followed the method of López et al. (2009), and the details are provided in the Supplementary material. \(^{13}\)C-PCB-141 was added as surrogate standard to the samples prior to extraction and \(^{13}\)C-PCB-208 was added to the final solutions as an internal standard. An Agilent 6890II gas chromatograph equipped with a \(\mu\)-electron capture detector (GC-\(\mu\)ECD) was used for the analysis of PBDEs. An Agilent J&W 5 m DB-5 HT column (0.25 mm i.d. \(\times\) 0.10 \(\mu\)m) was used for the determination of BDE-209, and an Agilent J&W DB-5MS column (30 m \(\times\) 0.32 mm \(\times\) 0.25 \(\mu\)m) (J&W Scientific, Folsom, CA) was used for the other PBDEs. Quality assurance and quality control of the extraction and analysis are provided in the Supplementary material.

The extraction and analysis procedures for OH-PBDEs and MeO-PBDEs were based on the method of Verreault et al. (2005). A UPLC-MS/MS system was used for separation and quantification of OH-PBDEs (Waters, Milford, MA, USA). A reversed-phase chromatography was performed by Waters ACQUITY UPLC BEH C\(_{18}\) column (2.1 mm \(\times\) 100 mm, 1.7 \(\mu\)m) maintained at 40 °C. An Agilent 7890 GC-MS (5975 inert) (Agilent, Palo Alto, CA) were used for the analysis of MeO-PBDEs. Details are given in the Supplementary material.

### 2.7. Data analysis

All of the data represent the mean and standard deviation of triplicates. The data were subjected to statistical analysis by using the SPSS version 12.0 software package. Analysis of variance (ANOVA) and Duncan’s multiple range test were used to examine the significance of PBDE degradation and enzyme activities in root crude extracts between plant species or PBDE congeners. A 95% confidence limit (\(P < 0.05\)) was chosen to indicate differences between samples.

### 3. Results and discussion

#### 3.1. In vitro biodegradation of PBDEs by root crude enzymes

Time-dependent degradation of PBDEs in enzyme solutions of different plant species are given in Fig. 1. There was a slight decrease in PBDE concentrations in the controls (6.1–9.0%), and the plant enzymes were capable of significantly degrading all of the four PBDE congeners with the decrease of their concentrations in the range of 21.2–67.3% compared with the controls. Degradation of PBDEs in plant enzyme solution reached an apparent equilibrium after 72, 60, 48 and 36 h for BDE-28, -47, -99 and -209, respectively. The kinetics were well fit with the first-order kinetic model (\(R^2 = 0.52–0.97, P < 0.05\)). Degradation of different PBDE congeners occurred at rates corresponding to bromine substitution levels and the kinetic constant \(k\ (h^{-1})\) was in the following order: BDE-28 (0.00095–0.0078) < BDE-47 (0.0016–0.0079) < BDE-99 (0.0011–0.017) < BDE-209 (0.0016–0.019) (Table S2 in the Supplementary material). BDE-209 degraded faster than BDE-99 and BDE-99 faster than BDE-47, very similar to the results obtained for PBDE debromination in a biomimetic experimental system using coenzyme vitamin B12, in which PBDEs underwent reductive debromination reactions at rates corresponding to the number of bromine substitutions (Tokarz et al., 2008). Among plant species, enzyme solution from maize roots degraded PBDEs more obviously than the ones from pumpkin and ryegrass roots, which is speculated to be ascribed to the difference in metabolic enzyme activity of different plant species (Chhikara et al., 2010).

#### 3.2. Metabolism products of PBDEs in root crude enzyme solutions

Debrominated, hydroxylated and methoxylated metabolites of PBDEs were analyzed in the root crude enzyme solutions at 96 h reaction time. A variety of debrominated products were found for all the four PBDE congeners and all the plant species (Fig. 2). OH-PBDEs were found at low levels (0.01–0.06 ng mL\(^{-1}\), Table 1) and no MeO-PBDEs were detected in any of the treatments. The total debrominated PBDEs accounted for over 90% and the hydroxylated-PBDEs occupied only 1–3% of each PBDE depleted in the root crude enzyme solution, which was consistent with the results of previous research on plants and marine organisms (Kelly et al., 2008; Huang et al., 2010), and suggested that debromination was the major biotransformation route for PBDEs.

All the four PBDE congeners were found to exhibit similar debromination pathways in the root crude enzyme solutions and showed a stepwise loss of bromine, while patterns of debrominated products were slightly different between plant species. Here debromination of BDE-99 in the maize root crude enzyme solution was taken as an example in discussion. The consecutive debromination of PBDE congeners over time (0, 24, 48 and 72 h), including the formation and subsequent enzymatic debromination of the generated congeners, were evident (Fig. 3). The first step in the transformation of BDE-99 by root enzyme extract was the loss of one bromine atom to produce two tetra-BDEs (BDE-47 and -66) as shown in the chromatogram after 24 h of exposure. Less substituted congeners (mono- through tri-BDEs) appeared in succession as the exposure time increased. There was a clear trend that concentrations of the debrominated PBDEs after 24 h of exposure were in the following order: tetra-BDEs > tri-BDEs > di-BDEs > mono-BDEs. Then, concentration of debrominated di- and mono-BDEs increased with the exposure time increased. BDE-28, -47 and -99 investigated in this study were all at the ortho- or para-positions. Based on the debrominated PBDEs detected, removal of both para- and ortho- bromines occurred in the root enzyme solutions. BDE-28 was mainly debrominated to BDE-15, -7 and -3, and BDE-47 to BDE-28, -7, and -3. The dominant debromination products of BDE-99 included BDE-74, -66 and -47. Previous reports have demonstrated that bromine atoms are preferentially removed from the meta- and para-positions on the biphenyl structure by microbial degradation (Gerecke et al., 2005). For the fully brominated BDE-209, debromination products via removal of the ortho-, meta- and para- bromine atoms were all detected, and differences in their concentrations were small. BDE-7 was frequently detected in the debromination products of the four parent PBDEs, probably because of its low enthalpy of formation (Zeng et al., 2008). Detection of BDE-11 (3,3′-diBDE) in the BDE-47 and -99 exposed solutions indicated the possibility of bromine rearrangement (Zhao et al., 2012). Concentrations of the total debrominated products in the
Root crude extracts of different plant species were in the following order: maize > ryegrass > pumpkin. Except for enzyme solution of maize in which no OH-PBDEs was found, four OH-PBDEs including 20-OH-BDE3, 30-OH-BDE7, 40-OH-BDE17 and 30-OH-BDE28 of the seven OH-PBDE standards were detected in the root crude enzyme solutions of pumpkin and ryegrass (Table 1). No OH-PBDE was found in the blank controls (enzyme denatured with PBDE exposure), indicating that OH-PBDEs detected came from metabolism of PBDEs in the crude enzyme solutions. The types of OH-PBDE congeners in the pumpkin enzyme solution were more than those in the ryegrass enzyme solution. Among the OH-PBDEs, 30-OH-BDE7 was the most frequently detected congener, which was similar to the observation of previous study of in vivo hydroxylation of PBDEs in plants (Wang et al., 2011b).

### Table 1

<table>
<thead>
<tr>
<th>Plants</th>
<th>Exposure</th>
<th>OH-PBDE products*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumpkin</td>
<td>BDE-28</td>
<td>2’-OH-BDE3 (0.02), 3’-OH-BDE7(0.06)</td>
</tr>
<tr>
<td></td>
<td>BDE-47</td>
<td>3’-OH-BDE7(0.01), 3’-OH-BDE28(0.05)</td>
</tr>
<tr>
<td></td>
<td>BDE-99</td>
<td>2’-OH-BDE3(0.02), 4’-OH-BDE17(0.06), 3’-OH-BDE28(0.04)</td>
</tr>
<tr>
<td></td>
<td>BDE-209</td>
<td>3’-OH-BDE7 (0.02)</td>
</tr>
<tr>
<td>Ryegrass</td>
<td>BDE-28</td>
<td>2’-OH-BDE3(0.01), 3’-OH-BDE7(0.02)</td>
</tr>
<tr>
<td></td>
<td>BDE-47</td>
<td>2’-OH-BDE3(0.02)</td>
</tr>
<tr>
<td></td>
<td>BDE-99</td>
<td>3’-OH-BDE7(0.02)</td>
</tr>
<tr>
<td></td>
<td>BDE-209</td>
<td>2’-OH-BDE3(0.01)</td>
</tr>
<tr>
<td>Maize</td>
<td>None</td>
<td>None of OH-PBDE products was detected in all the treatments (&lt;LODb).</td>
</tr>
</tbody>
</table>

Data in the parenthesis were the concentration of OH-PBDEs (ng mL\(^{-1}\)).

b LOD, limit of detection.

**Fig. 1.** The time-depended degradation of PBDEs in the root crude enzyme extracts of ryegrass, pumpkin and maize. (\(C/C_0\), PBDE concentration at any time/PBDE initial concentration).

**Fig. 2.** In vitro debromination products of PBDEs in the root crude enzyme extracts of ryegrass, pumpkin and maize.
3.3. Responses of root enzymes to the exposure of PBDEs

Responses of root enzymes to both in vivo (direct plant exposure) and in vitro (root crude enzyme solution exposure) exposures of PBDEs were tested. When plants exposed to PBDEs for 96 h, there were not significant changes in enzyme activities of CAT, POD and CYP450, indicating they were not inhibited by plant exposure to PBDEs or by metabolism of PBDEs inside plants. However, exposure of plants to PBDEs significantly induced the reductions of NaR (13.8–82.4%) and GST (5.3–42.4%) (Fig. S1, in the Supplementary material). Similarly, no significant loss of enzyme activity was observed for CAT, POD and CYP450 in the vitro exposure experiment; however significant decreases in the activities of NaR and GST occurred, especially in the maize root crude enzyme solution at the exposure time of 96 h (Fig. 4). Exposure to BDE-28 induced the most inhibition of the activities of NaR and GST, likely attributed to its higher solubility and therefore higher exposed concentration which caused a relatively higher toxicity to enzymes than the other congeners. On the other hand, although liable to degradation, BDE-209 exposure did not result in a significant inhabitation of NaR activity due to its low solubility and low concentration in the root extract solutions.

NaR may utilize halogen as electron acceptor (Anandarajah et al., 2000), and NaR was found to mediate reductive dechlorination of PCBs even in aerobic condition (Magee et al., 2008). GST had also been reported to be involved in the reductive dechlorination of PCBs (Gilmartin et al., 2003). We thus hypothesized that NaR and GST might also exhibit activity for PBDE debromination. Higher enzyme concentrations of NaR or GST led to higher PBDE debromination rates. Furthermore, degradation percentages of PBDEs and NaR activities exhibited significant positive relationship ($R^2$ ranged from 0.85 for BDE-209 to 0.94 for BDE-99, $P < 0.05$, Fig. S2 in the Supplementary material). The time-dependent activities of NaR and GST in the PBDE degradation process were measured in the pumpkin root crude enzyme solution (Fig. 5). NaR and GST activities decreased rapidly during the initial period (the first 24 h for BDE-28, -47 and -99, and the first 12 h for BDE-209) and then changed smoothly, showing a similar trend to the depletion of PBDEs in the root crude enzyme solution. The NaR activity showed the fastest decrease after exposure to BDE-209, while GST activity decreased the fastest after exposure to BDE-99. Difference in the changes of NaR and GST responding to different PBDEs may be as the result of congener-specific action of PBDEs on NaR and GST activities. Similar phenomena were observed in the effects of PBDEs on GST, deiodinase, and CYP17 activities in porcine ovarian follicles (Karpeta et al., 2011) and fishes (Roberts et al., 2011). The degradation reaction of PBDEs stopped gradually with the NaR and GST became inactive. These results suggested that NaR and GST were the key enzymes involved in the degradation of PBDEs in the root crude extracts.

Functions of enzymes to catalyze the degradation of xenobiotic compounds are diverse from each other. The CYP450 enzymes are the important enzymes in phase I metabolism and generally mediate the initial oxidative step in metabolism of organic contaminants such as PAHs and PCBs (Harford-Cross et al., 2000; Jones et al., 2001). CYP450 enzyme-mediated oxidation is recognized as the general metabolic pathway of PBDEs to form hydroxylated metabolites (Stapleton et al., 2006). POD and CAT, which often function in Phase III metabolic enzymes, are also responsible for oxidative degradation of xenobiotic compounds. Low concentrations of OH-PBDEs detected and no MeO-PBDEs identified in the root crude enzyme solutions was consistent with the evidence that
the changes in CYP-450, POD and CAT activities were not significant after exposure to each PBDE congener, suggesting the weak potential of these enzymes in oxidation of PBDEs. GST, which is an inducible phase II enzyme, plays an important role in conjugation of electrophilic compounds and is capable of catalyzing reductive dehalogenation reactions (Anandarajah et al., 2000). Previous work has convinced the involvement of GST in the reductive debromination of PBDEs in fish (Benedict et al., 2007; Noyes et al., 2010). This study suggested the role of GST in PBDE reductive debromination in plants.

3.4. Degradation of PBDEs by pure NaR and GST

To further confirm the roles of NaR and GST in PBDE debromination, reactions of PBDEs with commercially available pure NaR and GST were conducted. A total of four to six debrominated PBDEs were determined in the enzyme solutions after exposure to BDE-
28, -47, -99 and -209 for 96 h (Fig. 53 in the Supplementary material), which are similar to those found in the root crude enzyme extracts, validating the conclusion that NaR and GST in root crude enzyme solutions play important roles in PBDE debromination. Furthermore, concentrations of the total debrominated PBDEs were 1.3–2.6 times higher in NaR enzyme solution than in GST enzyme solution, suggesting that NaR (at the same unit activity) contributed more than GST to PBDE debromination (Fig. 6). Debromination rate (Fig. 6) was higher for BDE-209 than the other congeners in pure enzyme solutions, consistent with the faster decrease of NaR in root crude enzyme solution with the exposure of BDE-209 than the other congeners.

4. Conclusions

This study provides for the first time the evidence of PBDE biotransformation by plant enzymes. PBDE congener in root crude enzyme solutions degraded at different rates with higher brominated PBDEs faster than the lower ones. Debrominated PBDEs and OH-PBDEs were detected, indicating the occurrence of debromination and hydroxylation in root crude enzyme solution. Decreases in NaR and GST activities and depletion of PBDEs in the root crude enzyme solution shared a similar time-dependent trend, indicative of the roles of NaR and GST in PBDE debromination. This conclusion was further verified by using the pure NaR and GST to debrominate PBDEs.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (Projects 21177139 and 2021063) and the National Basic Research Program of China (Project 2009CB421603).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.10.013.

References


References


