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Distribution and Biodegradation of Bisphenol A in Water Hyacinth

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Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane; CAS Registry No. 80-05-7) is made by combining acetone and phenol and is widely used as a material for the production of epoxy resins, phenol resins, polycarbonates, polyacrylates, polyesters, and lacquer coatings on food cans (Staples et al., 1998). Possible exposure of human and animals to BPA, which is leached from these plastic products and food- and drink- packaging containers, has been demonstrated (Brotons et al., 1995; Biles et al., 1997; Kang and Kondo, 2003).

BPA is regarded as one of endocrine disruptors and has an acute toxicity to aquatic organisms in the range of about 1 to 10 mg/L for a number of freshwater and marine species (Alexander et al., 1988). In rodents, the major metabolite in urine is BPA glucuronide, and free BPA and hydroxylated BPA are identified in feces. BPA absorbed through the intestine is glucuronidated by an isoform of UDP-glucuronosyltransferase in rat liver and excreted as BPA glucuronide in urine (Kang et al., 2006b).

BPA can be contained in effluent from landfills or wastewater from its production factories because it is partially removed during leachate or wastewater treatment (Kang and Kondo, 2006). On the other hand, BPA can be degraded by bacteria distributed in river water under aerobic conditions (Dorn et al., 1987; Klecka et al., 2001; Kang and Kondo, 2002a, b; Kang et al., 2004; Kang and Kondo, 2005), but not under anaerobic conditions (Kang and Kondo, 2002b). Moreover, lignin-degrading enzymes such as manganese peroxidase and laccase, which are produced by white rot basidiomycetes fungi, can degrade BPA and remove its estrogenic activity (Hirano et al., 2000; Tsutsumi et al., 2001). Recently, the oxidative degradation of BPA by crude enzyme of potato and by polyphenol oxidase in vegetable was reported (Xuan et al., 2002; Yoshida et al., 2002; Kang et al., 2006a). The present study investigated the distribution and biodegradation of BPA in plant, water hyacinth (*Eichhornia crassipes*).

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MATERIALS AND METHODS

Bisphenol A (BPA) (>99%), polyvinylpyrrolidone and anhydrous sodium sulfate were purchased from Nacalai Tesque (Kyoto, Japan). Oricinol monohydrate, phenylhydrazine, and tropolone were purchased from Sigma-Aldrich (St. Louis, MO). A solid-phase extraction cartridge, OASIS HLB cartridge (60 mg), was obtained from Waters (Millipore Co., Milford, MA). Water used in this study was distilled and purified with a Milli-Q water purification system (Nihon Millipore, Yonezawa, Japan). Acetonitrile and methanol were purchased from Kanto Chemical (Tokyo, Japan).

BPA in standards and sample extracts was determined using a Waters 600E multisolvent delivery system (Millipore) equipped with a Waters 717plus Autosampler, a Waters Model U6K Universal Liquid Chromatograph Injector (Millipore), and a CR6A Chromatopac integrator (Shimadzu, Kyoto, Japan). The analytical column was a Symmetry Shield RP_{18} 3.5 μm (4.6 × 150 mm) with a 3.5-μm guard column (2.1 × 10 mm) (Millipore). Fluorescence detector (Model F-1050, Hitachi, Tokyo, Japan) was set with excitation at 275 nm and emission at 300 nm. The mobile phase was acetonitrile-water (40:60, v/v) with a flow rate of 1 ml/min under isocratic conditions. The column temperature was 40℃ at a Column heater U-620 Type 30 (Sugaichemi, Osaka, Japan).

Water hyacinth plants collected from the Kiyotake River of Miyazaki city in Japan were washed with water and were kept in polyethylene plastic tanks containing tap water for 10 days. After 10 days, plants were maintained in the nutrient solution (1/1000 Hyponex solution in water) (Hyponex, Ohio) for a week prior to the test. Plants were transferred to glass bottles containing 1000 ml of the nutrient solution with BPA (1 or 10 mg/L). The bottles were covered with foil and placed in a chamber. Lighting in the chamber was set for 16 h light and 8 h dark, and temperature was set at 25 ± 1℃. Humidity in the chamber was 20 ± 5%.

Ten grams of shoots or roots were homogenized with 10 ml of 0.1 M sodium phosphate buffer (pH 7) and 2 g of polyvinylpyrrolidone to remove phenol compounds from the shoot or root tissues. The mixture was centrifuged at 10000 rpm for 10 min at 4℃ (KUBOTA 6900, Kubota co., Tokyo, Japan). The supernatant was transferred to Eppendorf tubes and again centrifuged at 15000 rpm for 15 min. The supernatant obtained from the second centrifuging was used as crude enzyme solution (Kang et al., 2006a).

A typical assay solution (pH 7) contained 5 ml of 0.1 M sodium phosphate buffer, BPA (at 10 mg/L) in 0.1 M sodium phosphate buffer, and 0.5 ml of crude enzyme solution. The control sample with no crude enzyme solution was also prepared. The samples were incubated at 37℃ for 60 min.

The optimal pH and temperature of crude enzyme solution were tested from pH 6 to 9 and from 20 to 60℃, respectively. The highest BPA degradation was identified at pH 7 and at a 40 temperature (data not shown). In the present study, the assay solution was set at pH 7 and was incubated at a 37℃ temperature.

The desired inhibitors (at 0.01 M) of oxidative enzymes (orcinol, tropolone, and phenylhydrazine) in 0.1 M sodium phosphate buffer (pH 7) were added to the assay solution to identify the effect of oxidative enzymes on BPA degradation. The mixture solution was incubated at 37℃ for 60 min. The assay solution with no inhibitors described above was used as the control sample and was incubated at same conditions.

At each sampling time (1, 2, 4, 7, 10, 15, and 24h), plants were washed with water and divided into shoots and roots. In the case of roots, two types of washed and unwashed root samples were prepared. One gram of shoots or roots was homogenized with 20 ml of methanol and 2 g of anhydrous sodium sulfate, and centrifuged at 10000 rpm for 20 min. The supernatants were transferred to a glass tube. The residues were mixed by a vortex mixer with 20 ml of methanol and centrifuged at the above condition. The supernatants were combined with the first extract in the tube. The extracts were evaporated to dryness under nitrogen in a 40℃ water bath. The dried extracts were dissolved in 1 ml mobile phase, transferred to Eppendorf tubes, and then centrifuged at 15000 rpm for 15 min. After filtering of the supernatant through a filter of 0.45 μm pore size (Millipore), aliquots of 50 μl were injected into HPLC system for the detection of BPA.

The solid-phase extraction method by an OASIS HLB cartridge was used for the extraction of BPA from nutrient solution and mixed assay solution with inhibitors or no inhibitors. One milliliter solution was introduced into the OASIS HLB cartridge, which had previously been conditioned with 5 ml of methanol and 5 ml of water. The cartridge was washed with 5 ml of water, and BPA was eluted with 3 ml of methanol. The methanol extract was evaporated just to dryness under nitrogen and dissolved in 1 ml of mobile phase. Aliquots of 50 μl were used for the analysis of BPA.

RESULTS AND DISCUSSION

Absorption, translocation, and degradation of BPA in roots and shoots are presented in Figure 1A and B. The highest concentration of BPA (about 250 μg/L in 1 mg/L and 4980 μg/L in 10 mg/L BPA solution) in roots was found at 1 h following the immersion in 1 and 10 mg/L BPA solution, but in shoots was at4 h (about 17 μg/L in 1 mg/L and 80 μg/L in 10 mg/L solution). The levels of BPA in shoots were smaller compared with roots, but BPA in shoots continued longer in existence than in roots (Figure 1A and B). On the other hand, a rapid decrease of BPA in the nutrient solution containing both 1 and 10 mg/L BPA was identified and half-lives for BPA degradation were below 1 h (Figure 1A and B).

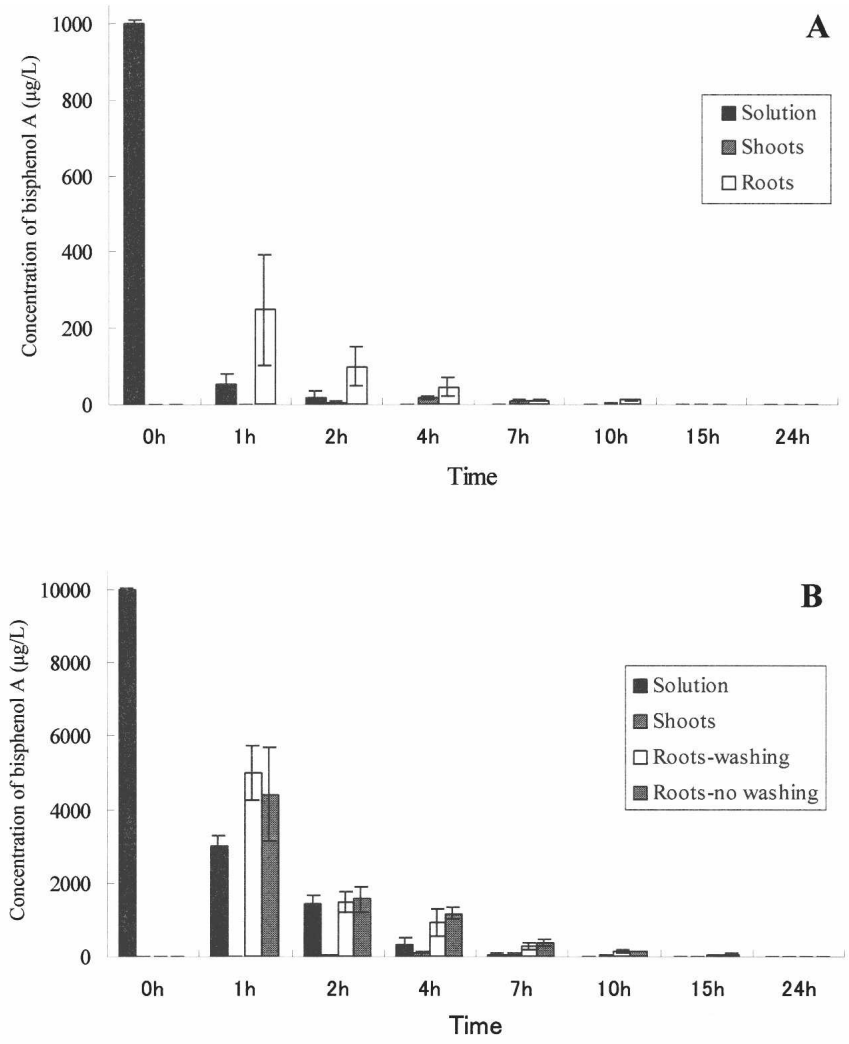

Figure 1. Changes of BPA concentration in roots and shoots of water hyacinth immersed in the nutrient solution with 1 (A) and 10 mg/L BPA (B). In the case of roots, two types of washed and unwashed samples were prepared to identify whether BPA is accumulated on the surface of roots or not (B).

Two samples of roots (washed and unwashed root samples) were prepared to identify whether BPA is accumulated on the surface of roots or not. The differences in BPA levels were not found between the washed roots and the unwashed roots (Figure 1B).

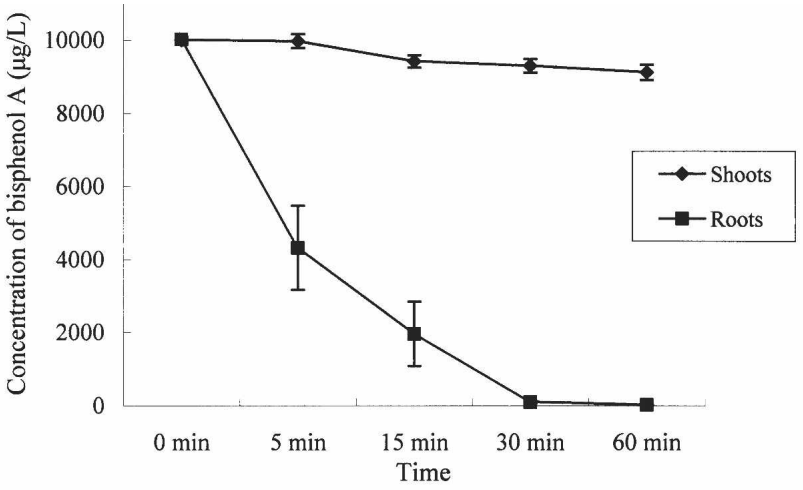

Figure 2. BPA degradation by crude enzyme of roots and shoots. A typical assay solution (pH 7) contained 5 ml of 0.1 M sodium phosphate buffer, BPA (at 10 mg/L) in 0.1 M sodium phosphate buffer, and 0.5 ml of crude enzyme solution. The samples were incubated at 37℃ for 60 min.

This result means that BPA can't be accumulated on the outsides of roots and quickly absorbed from them.

The crude enzyme solutions with 10 mg/L BPA were prepared to examine the effect of enzyme on BPA degradation. In the enzyme solution of roots, BPA was rapidly decreased. Above 50% of BPA was removed at 5 min after incubation at 37℃ and only its 1% was detected at 30 min. However, about 10% of BPA was degraded in the enzyme solution prepared from shoots during the incubation of 60 min (Figure 2). Moreover, no BPA degradation was identified from the control sample for 60 min (data not shown).

Plants can rapidly absorb BPA from water through their roots and metabolize it to several glycosidic compounds. Nakajima et al. (2002) found that the BPA absorbed through root systems was metabolized to its β-glucoside and the metabolites were translocated to their leaves. On the other hand, Noureddin et al. (2004) suggested that BPA metabolites are detected as the BPA base at *ca.* 10% in the roots, some in the stems, but none in the leaves. In our study, most BPA absorbed through roots was eliminated in roots, and only small amounts of BPA are translocated to shoot tissues and degraded in shoot. From these studies, the distribution of BPA and its metabolites in plant may be variable according to plant species.

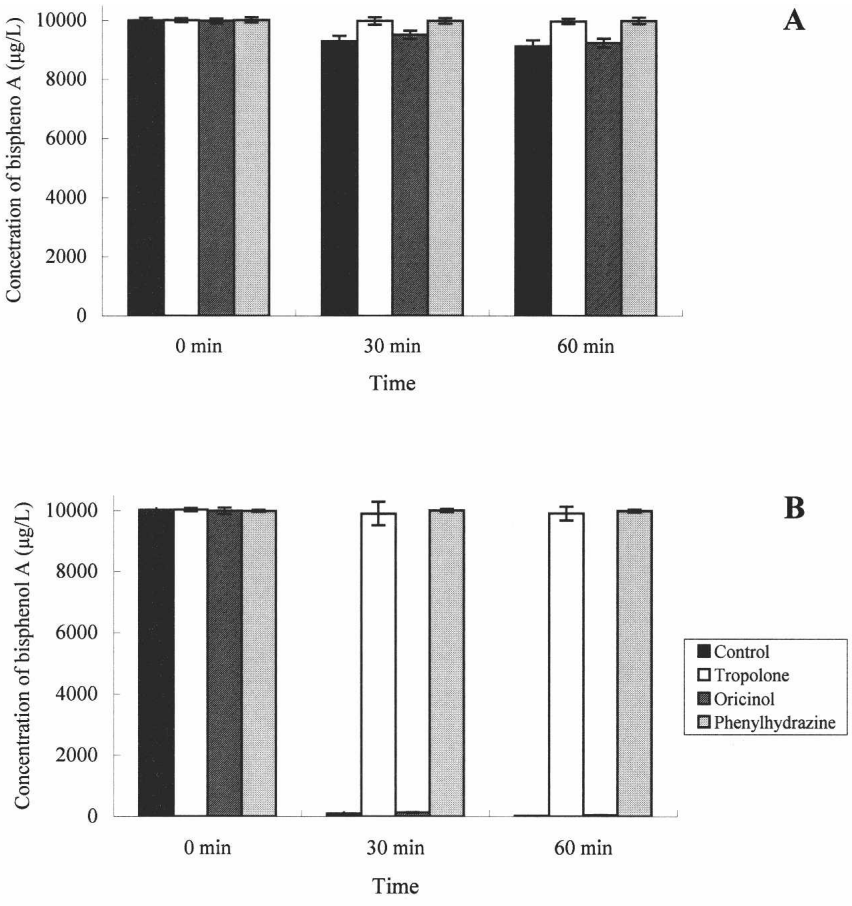

Figure 3. Effect of inhibitors of oxidative enzyme on BPA degradation. The desired inhibitors (at 0.01 M) of oxidative enzymes (orcinol, tropolone, and phenylhydrazine) in 0.1 M sodium phosphate buffer (pH 7) were added to the assay solution with crude enzyme solution of shoots (A) and roots (B), and BPA (10 mg/L). The mixture enzyme solution was incubated at 37℃ for 60 min.

Three inhibitors (orcinol, tropolone, and phenylhydrazine) were used to evaluate the effect of two main oxidative enzymes, polyphenol oxidase (EC 1.14.18.1) and peroxidase (EC 1.11.1.7), on BPA degradation. Some peroxidase inhibitors (e.g., cathechol, hydroquinone, and chlorogenic acid) have an ability to increase polyphenol oxidase activity. However, orcinol is a peroxidase inhibitor with no effect on polyphenol oxidase (Srivastava and van Huystee, 1977). Moreover, tropolone is a

very effective inhibitor of polyphenol oxidase, but has no influence on peroxidase activity (Kahn, 1985). Phenylhydrazine can inhibit both polyphenol oxidase and peroxidase (van Doorn and Vslier, 2002).

BPA level in the root assay solution with orcinol following the incubation of 60 min was twice higher than that in the control solution containing no inhibitors, but was below 50 μg/L. Peroxidase had a minor effect on BPA degradation. In the root solution with tropolone, however, about 1% loss of BPA was found for 60 min. A decrease in BPA level in the root solution with phenylhydrazine was less than 1%. These results suggest that most BPA absorbed in root tissues is degraded by polyphenol oxidase (Figure 3A). On the other hand, BPA degradation was hardly found (<1%) in the shoot assay solution with tropolone or phenyhydrazine. The degradation of BPA was <10% in the solution with oricinol and the control sample (Figure 3B).

In plants, physiological stress, wounding, microbial or viral infection result in increased activity of polyphenol oxidase and peroxidase for self-protection. Two enzymes relate to enzymatic browning and off-flavor generation in fruit or vegetable products, but the primary enzyme in the formation of pigments such as melanins is polyphenol oxidase (Vámos-Vigyázó, 1981). In our study, polyphenol oxidase was shown to have a high BPA degradability. Similarly, a previous study reported that polyphenol oxidase could convert BPA into monoquinone and bisquinone derivatives (Yohsida et al., 2002).

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