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Biodegradation of bisphenol A and its halogenated analogues by *Cunninghamella elegans* ATCC36112

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Abstract Bisphenol A and its halogenated analogues are commonly used industrial chemicals with strong toxicological effects over many organisms. In this study, metabolic fate of bisphenol A and its halogenated analogues were evaluated with *Cunninghamella elegans* ATCC36112. Bisphenol A and related analogues were rapidly transformed into several metabolites by *C. elegans* within 2–4 days. Detailed analysis of metabolites reveals that both phase I and II metabolism occurred in *C. elegans*. Cytochrome P450-dependent hydroxylation was observed in BPA. However, major reaction with bisphenol A and analogues with 1-2 halogen atoms were the formation of glucose-conjugate, not being inhibited by cytochrome P450 inhibitor. Overall metabolic rates decreased with increasing number of substitution at 2- and 6-position of BPA structures, which may be consequences of limited bioavailability or steric hindrance to conjugate-forming reaction. Information from the current study will provide detailed insights over the fungal metabolism of BPA and analogues.

Keywords *Cunninghamella elegans* · Bisphenol A · Analogue · Glycoside

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Introduction

Bisphenol A (BPA) and its halogenated analogues (BPAs) are widely used industrial chemicals for the production of resins, plastics, flame retardants and many others (Kang et al. 2006a). These chemicals are considered as ubiquitous environmental contaminants over several different matrixes (e.g., soils, sediments, sea waters, etc.). Recent survey has proven that large portions of human population are exposed to BPA (Calafat et al. 2005). In addition, higher frequency of BPA-exposure from urban area than rural population indicates that BPA contamination is definitely related to human activities (Calafat et al. 2005). Kang et al. (2006a) has reviewed the human exposure to BPA.

Among several different toxicological responses, their endocrine disruptor-activity has been proven from many organisms, including mammals, amphibians, and fishes (Alexander et al. 1988; Lindholst et al. 2003; Newbold et al. 2007; Ramakrishnan and Wayne 2008; Vandenberg et al. 2007). For example, BPA induce deformation/malfunction of spermiogenesis in mice and rat (Toyama et al. 2004). Estrogenic

effects of BPA are in part by the transcriptional inhibition of some isozyme of cytochrome P450, being the most important genes of steroidal hormone metabolism (Huang and Leung 2009; Niwa et al. 2001).

Numerous researches have been performed to develop efficient method to remove BPA from environmental matrix. For example, photochemical methods, including Fenton reaction was proven to be promising methods but some limitation (Sajiki and Yonekubo 2004). More advanced technology with novel catalyst is also devised (Chen et al. 2006; Venkatachalam et al. 2006).

BPA readily undergoes metabolic dissipation in natural environment. Many organisms, including animals, plants, and microorganisms can metabolize BPA or its halogenated analogues (Kang et al. 2006b). As of many phenolic chemicals, major metabolic pathway of BPA is conjugate formation (e.g., glucuronides, glycoside, sulfate). However, some plants and fungi can transform BPA to simple alkylphenols or benzoates (Chai et al. 2003, 2005; Kang et al. 2006b).

In this study, metabolic fate of BPA and its analogues with different number of halogens were evaluated with *Cunninghamella elegans*, a well-known xenobiotic degrading fungus. Possible role of cytochrome P450 or other enzymes were tested for the dissipation of these estrogenic contaminants. In addition, the stability of BPA conjugate against enzymatic hydrolysis was evaluated.

Materials and methods

Chemicals and media

BPA, tetrachloroBPA (TCBPA), and tetrabromoBPA (TBBPA) were obtained from Tokyo Kasei Chemicals. Acetobromoglucose, cetyltrimethylammonium bromide, sodium hydrogen carbonate, iodine, β-glucosidase from *Aspergillus niger*, bis(trifluoromethylsilyl)acetamide-trimethylsilyl chloride (BSTFA-TMCS), and *N*-bromosuccinimide (NBS) were from Sigma-Aldrich Korea (Seoul, Korea). Potato dextrose agar (PDA) and broth (PDB) were purchased from BD Korea Ltd (Seoul, Korea). Solvents were HPLC grade or higher. Other reagents were of highest grade available.

Synthesis of partially halogenated BPA analogues

Partially halogenated analogues of BPA were prepared by halogenations with *N*-bromosuccinimide (for brominated-BPA) or catalytic iodination (iodinated-BPA). For the preparation of mono- to di-bromo-BPA, BPA (1 mmol) was dissolved in glacial acetic acid (20 ml) and NBS (1.5 mmol) was added portion-wise. The mixture was stirred at 70°C for 2 h. Then the mixture was poured into water (200 ml). The precipitate was filtered, washed with water (100 ml × 4), and dried under vacuum.

Iodinated BPA was prepared by literature method, with some modification (Holzapfel and Williams 1995). To a mixture of BPA (1 mmol) and sodium hydrogen carbonate (1.5 mmol) in tetrahydrofuran (30 ml), was added iodine (1.5 mmol) in one portion. The mixture was stirred for 4 h at 45°C. The cooled solution was diluted with water (100 ml) and extracted with ether (200 ml). The organic solution was repetitively washed with aqueous solution of sodium sulfite (5%, 100 ml × 2) and water (100 ml). After removing solvent, the residue was purified with silica gel column chromatography.

Synthesis of BPA-glycoside

BPA-monoglycoside was prepared from BPA and acetobromoglucose under basic condition with the aid of phase transfer catalyst. BPA (1 mmol), suspended in aqueous potassium carbonate (1.3 mmol, 20 ml) were vigorously stirred. A solution of acetobromoglucose (1.3 mmol, 20 ml chloroform) was added, followed by catalytic amount of cetyltrimethylammonium bromide (1–2 mg). The mixture was refluxed for 16 h. To a cooled reaction mixture, was added distilled water (100 ml) and extracted with chloroform (100 ml × 2). After removing organic solvent, the oily residue was re-suspended in methanolic KOH (1 N, 100 ml) and stirred for 20 h at room temperature. The reaction mixture was concentrated to 20 ml and diluted with distilled water (50 ml). After acidification (approximately pH 3), the mixture was saturated with NaCl. Glycoside was extracted with ethyl acetate (EtOAc, 200 ml × 3). After removal of solvent under reduced pressure, the residue was purified by silica gel column chromatography.

Culture conditions

Cunninghamella elegans ATCC36112 was obtained from American Type Culture Collection (Manassas, VA). Fungal cultures were typically maintained on PDA, while the corresponding liquid culture was performed on PDB at 27°C, 200 rpm. For the degradation experiment with *C. elegans*, seed culture was prepared on PDB for 2 days. After removal of supernatants, mycelia (approximately 2 g) was re-suspended in fresh PDB (500 ml), supplemented with BPAs (2 mg/0.5 ml of methanol), and cultured at 27°C and 200 rpm for a pre-defined period.

To study the effects of cytochrome P450 (CYP)inhibitor, stock solution of piperonyl butoxide (PB, 2 mg/l) was added and pre-incubated for 6 h, followed by addition of BPA (2 mg/0.5 ml of methanol). For large scale preparation of metabolites, three cultures (500 ml × 3) were combined and extracted after 4 days.

For sterilized control experiment, cultures after 3 days were sterilized at 120°C for 30 min, followed by addition of stock solutions of BPA or analogues (2 mg/0.5 ml of methanol). The control was also maintained at the same conditions. Triplicate experiments were performed for all treatments.

Extraction and derivatization of metabolites

Aliquots of culture with mycelium (80 ml) were diluted with saturated NaCl solution (50 ml) and pH was adjusted approximately 2 with 6 N hydrochloric acid. The mixture was extracted with ethyl acetate (150 ml × 2) and dried over anhydrous sodium sulfate. After removal of solvent under reduced pressure, residue was re-suspended in dry pyridine (1 ml) and derivatized with BSTFA-TMCS (200 µl) at 70°C for 1 h.

Hydrolysis of conjugates

To determine the chemical structures of conjugates, aliquots of crude organic extracts from batch cultures were concentrated to dryness and re-dissolved in a mixture of ethyl acetate and methanol (2 ml, 1/1, v/v). An half of the solution was derivatized with BSTFA-TMCS, while another half was suspended in sodium acetate buffer (30 ml, 5 mM, pH 5.2). After addition of β-glucosidase (1 mg) from *Aspergillus niger*, the mixture was incubated at 37°C, 100 rpm for a defined period. The solution was then extracted with ethyl acetate (100 ml). After removing organic solvent, residue was derivatized with BSFTA-TMCS.

Gas chromatography-mass spectrometry

BPAs and their metabolites were routinely analyzed with gas chromatography-mass spectrometry (GC-MS, Agilent GC 6890 with 5972 MSD), equipped with VF-5MS column (30 m, 0.25 µm film thickness, 0.25 mm i.d.; Varian Inc., CA, USA). Helium was a carrier gas at a flow rate of 1 ml/min. The column temperature were programmed as follows: 130°C (10 min) and raised to 290°C at a rate of 5°C/min and held for 40 min. Temperatures of injection port and interface were set at 285 and 280°C, respectively. The mass spectrometer was operated at electron impact (EI) mode at 70 eV. For the analysis of halogenated analogues and their metabolites, the same GC-MS conditions were used, except temperatures of injection port and column. Injection port temperature was set at 300°C and the column temperature was programmed as follows: 170°C (10 min), raised to 300°C at a rate of 4°C/min and held for 40 min.

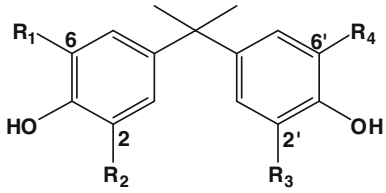
Estimatation of octanol–water partition coefficient (Log*P*)

Octanol–water partition coefficient was estimated using EPISuite from Environmental Protection Agency USA.

Results and discussion

Synthesis of partially halogenated BPAs and BPA monoglycoside

Bromination of two equivalents of NBS gave a mixture of mono- and di-brominated BPA. Approximate ratio was 2:3 for mono- to di-bromo BPA. In general, halogenation of phenol by *N*-halogenated succinimide is preferentially occurred in *para*-, followed by *ortho*-position. Because *para*-position of BPA is blocked by dimethylmethylene group, 2-bromo or 2,2′-dibromo derivatives are more plausible structures of bromination products (IV and V, Fig. 1). Catalytic iodination also gave a mixture of



BPA (I): R1=R2=R3=R4= H
TCBPA (II): R1=R2=R3=R4= Cl
TBBPA(III): R1=R2=R3=R4= Br
BrBPA1 (IV): R1=R2=R3= H, R4 = Br
BrBPA2 (V): R1=R3= H, R2=R4= Br
IBPA1 (VI): R1=R2=R3= H, R4= I
IBPA2 (VII): R1=R3= H, R2=R4= I

Fig. 1 Structure of bisphenol A (BPA) and its halogenated analogues with abbreviations

mono- and di-iodoBPA (1:2, peak area based) (VI and VII, Fig. 1).

Reaction yield for BPA glucoside synthesis was approximately 40% after isolation. Because of the strong hydrophilic properties of glucoside, practically pure glucoside can easily be separated from unreacted BPA to give white powder.

Synthetic trials for the preparation of TCBPA/TBBPA glucoside were unsuccessful (data not shown). Lower reactivity of acetobromoglucose to these derivatives may be the consequence of steric hindrance of substituents at 2- and 6-positions.

Metabolism of BPA

Residual BPA and its halogenated analogues (BPAs) were rapidly dissipated with different degree (Fig. 2). Several kinetic parameters of BPAs degradation were estimated from pseudo-first-order kinetics (Table 1). The half-life ($t_{1/2}$) of BPA was approximately 1 day with concomitant accumulation of several metabolites (Fig. 3). Major metabolite (Retention time, Rt, 69.306 min) in BPA-treated culture gave characteristic mass spectral fragmentation pattern of trimethylsilyl (TMS)-derivatives of glucose and those of BPA (Fig. 4a). For example, fragment ions of m/z 147, 204, 217, and 361 are commonly found in all conjugates, originating from TMS-glucose (or other hexose). Ions with m/z 171, 191, 207, 357, and 372 indicate that the metabolite contains BPA-like structure. Comparative analyses with synthetic standard confirmed the metabolite as BPA-monoglucoside. The concentration of glucoside was gradually increased till the end of experiments (Fig. 5). It has to be mentioned that carbohydrate conjugation can strongly reduce the estrogenic activity of BPA (Morohoshi et al. 2003). Molecular ion of peak 2 and its fragmentation pattern (*Rt*, 44.071 min) suggest that the metabolite may be a hydroxylated BPA. Hydroxylation is commonly found metabolic pathways of BPA. However, the exact position can be varied. For example, 3-hydroxy BPA, a catecholic metabolite was observed in the experiment with human cells (Nakagawa and Suzuki 2001). Hydroxylation on methyl groups, rather than phenyl ring is reported during the bacterial metabolism (Ike et al. 2002).

Because no authentic standards were available, it was not able to determine the structure of the hydroxylated metabolite. It is noteworthy that oestrogenic activity of BPA is reduced by hydroxylation (Nakagawa and Suzuki 2001). A peak at glucoside elution region (Rt, 71.454 min) gave characteristic mass fragmentation of TMS-derivatives of glucose and hydroxylated BPA (peak 4, Fig. 3b).

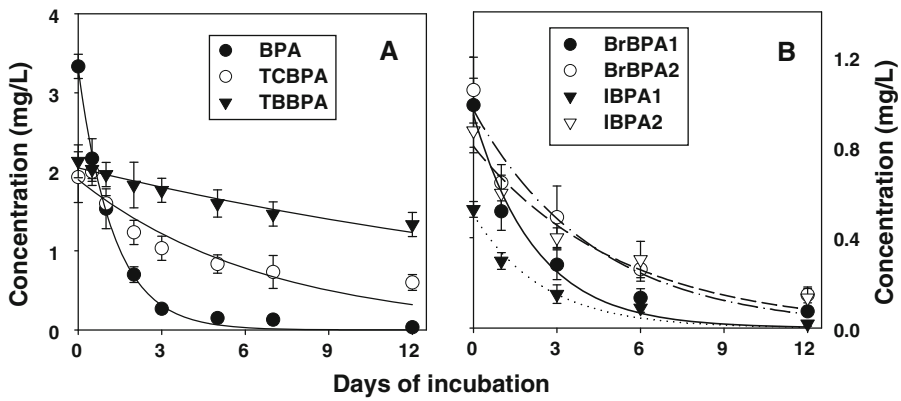


Fig. 2 Degradation kinetics of BPA, TCBPA, and TBBPA (**a**), mono- and di-halogenated analogues, brominated- and iodinated-BPA (**b**). *BrBPA1* 2-bromoBPA, *BrBPA2* 2,2′-dibromoBPA, *IBPA1* 2-iodoBPA, *IBPA2* 2,2′-diiodoBPA

Table 1 Kinetic parameters of degradations of BPA and its halogenated analogues by *C. elegans*

Chemicals	Rate constant (k'/day)[a]	Halflife ($t_{1/2}$, day)	R^2	Log P[c]
BPA	0.7892	0.9	0.9985[b]	3.64
TCBPA	0.1483	4.7	0.9474	6.22
TBBPA	0.0423	16.4	0.9740	7.20
BrBPA1	0.4442	1.6	0.9816	4.53
BrBPA2	0.2302	3.0	0.9693	5.42
IBPA1	0.4068	1.7	0.9871	4.81
IBPA2	0.1900	3.7	0.9731	5.98

BrBPA1 2-bromoBPA, *BrBPA2* 2,2′-dibromoBPA, *IBPA1* 2-iodoBPA, *IBPA2* 2,2′-diiodoBPA

[a] Pseudo-first-order reaction rate in/day

[b] Regression coefficient

[c] Estimated Log *P* with EPISuite

This metabolite was tentatively identified as glucosides of hydroxylated BPA. In addition, some minor peaks (Rts, 63.071 and 67.547) also gave similar mass spectra with that of peak 4. However, because of the high background noise, it was difficult to confirm the identities.

To find out the possible presence of other metabolites, especially those of dimethylmethylene bridge dissociation, chromatograms were carefully investigated. However, no traces of metabolites other than hydroxylation or glucoside formation were detected. Recent studies with several organisms gave many metabolites with simple alkylphenol structure (Chai et al. 2003, 2005; Hirano et al. 2000; Lobos et al. 1992). Further transformation to benzoic acid is also commonly found in white rot fungi and bacteria (Chai et al. 2005; Kang et al. 2006b).

According to these results, BPA metabolisms in C. elegans are highly resembled with those of plants and some fungi. For example, *Aspergillus fumigatus* can produce the same metabolites (BPA β-D-glucoside) as in current study (Yim et al. 2003).

Metabolism of halogenated analogues of BPA

The most noticeable difference between the metabolism of BPA and tetra-halogenated analogues were limited metabolic rates and concomitant formation of glucosides (Figs 2, 5). Half-lives of TCBPA and TBBPA were 4.7 and 16.4 days, respectively

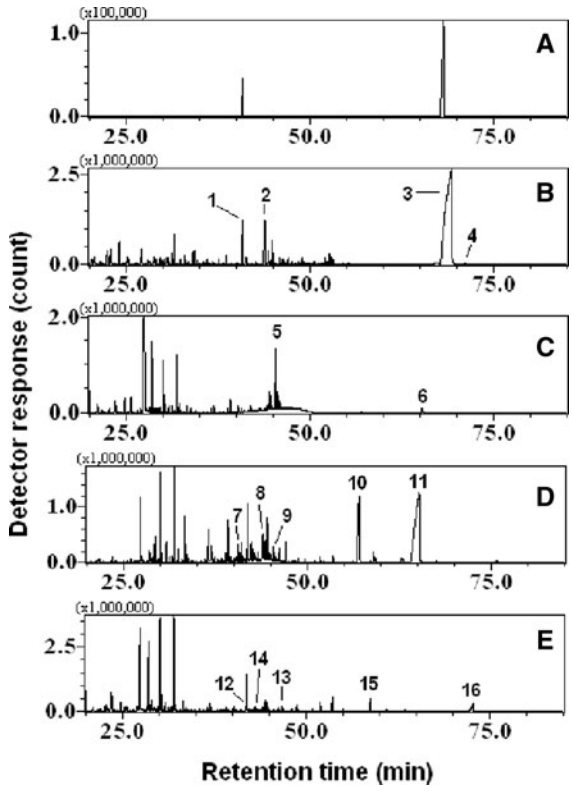


Fig. 3 Representative GC-MS total ion chromatograms of TMS-derivatized mixture of BPA and synthetic mono-glucoside (**a**) and culture extracts of BPA (**b**), TCBPA (**c**), BrBPA (**d**), and IBPA (**e**)

(Table 1). However, mono- and di-halogenated analogues were degraded much faster than tetrahalogenated analogues. Among several physicochemical properties, Log *P* (or octanol–water partition coefficient) are generally related to bioavailability of xenobiotics. Extremely high Log *P* of chemical indicates limited bioavailability, by which the biodegradation is retarded. Log *P*s of TCBPA and TBBPA (6.2 and 7.2) were much larger than those of BPA or lower halogenated analogues (Table 1). Slow degradation of TCBPA and TBBPA may be related to low bioavailability. Although the degradation was retarded, one metabolite (Rt, 65.449 min) was gradually accumulated in TCBPA-treated culture (Fig. 3c). Its mass spectrum indicates the presence of the structural fragments of TCBPA and glucose (Fig. 4b). The metabolite was tentatively identified as TCBPA monoglucoside.

Degradations of mono- and di-brominated or iodinated BPA (IV-VII, 1.6-3.7 days) were much

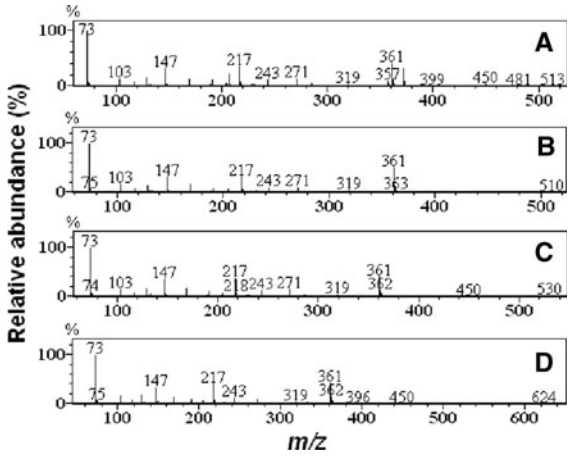


Fig. 4 Representative mass spectra of representative glucosides of BPA (**a**), TCBPA (**b**), 2,2′-dibromoBPA (**c**), and 2,2′-diiodoBPA (**d**)

faster than those of TCBPA/TBBPA (Fig. 2b). In general, half-lives of mono-substituted BPA were shorter than di-halogenated derivatives (Table 1). During the metabolism of IV–VII, continuous accumulation of some metabolites were observed (peak 10, 11, 15, and 16, Fig. 3d, e). Their mass spectra also indicate the presence of TMS dertivatized fragment of glucose and substituted BPA (Fig. 4, Table 2). These metabolites were tentatively identified as glucose conjugates of IV–VII. Among several analogues (IV–VII), Log *P* of VII was close to that of TCBPA. However, its half-life was shorter than TCBPA (3.7 vs. 4.7 days). In addition to bioavailability-related properties (e.g., Log *P*s), the presence of bulky substituent may also determine the rate of conjugate formation.

Detailed analysis of GC-MS TIC indicates that several additional metabolites were present in culture supernatant, treated with IV–VII. Peaks 9 and 14 in brominated- and iodinated BPA treated culture were tentatively identified as hydroxylated metabolites of IV and VI, respectively. In overall, however, major metabolites were glucosides of IV–VII.

Voordeckers et al. has reported that extensive dehalogenation of TCBPA and TBBPA to BPA in anaerobic sediments (Voordeckers et al. 2002). Because reductive removal of halogens from polyhalogenated chemical can increase the bioavailability of highly hydrophobic chemicals, such transformation can potentiate the toxicity of BPA analogue. However, no reductive dehalogenation products were observed in current study.

Hydrolysis of BPA glycoside by fungal β-glucosidase

In general, conjugation of xenobiotics metabolites to primary metabolite (e.g., sugar, amino acids, glucuronic acids) or phase II reaction is considered to be final detoxification reaction (Pottenger et al. 2000; Volkel et al. 2002). Numerous phenolic contaminants can easily be transformed to their glucoside or glucuronide. The conjugates can be further transformed to unextractable residues (plant) or excreted (animal). For example, rapid elimination of BPA from serum is well-correlated with the activity of UDP-glucuronosyltransferase (Takeuchi et al. 2006). However, it has to be mentioned that several microorganisms can produce enzymes to regenerate the aglycone from the conjugates. Domoradzki et al. (2004) has reported that dissipation rate of BPA in neonatal rat is faster than adults, which may be a results of reduced microbial β-glucuronidase activity

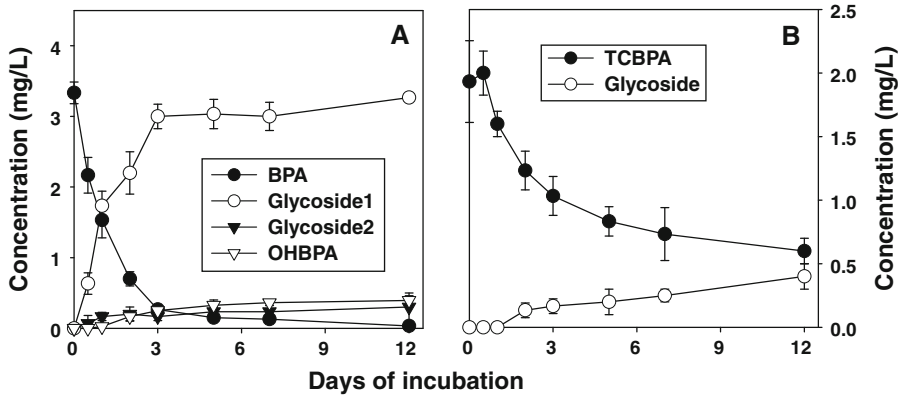


Fig. 5 Time-dependent change of the concentrations of BPA and metabolites (**a**) and TCBPA and metabolites (**b**). Concentrations of hydroxylated BPA, glucoside 2 of BPA (hydroxylated BPA glucoside), and TCBPA glucoside were estimated from the peak area of BPA, BPA glucoside, and TCBPA, respectively

Table 2 GC-MS properties of BPAs and their metabolites

Peak ID[a]	Chemicals	Retention time[b] (*Rt*, min)	Mass/charge of fragment ion (% relative abundance)[b]
1	BPA (I)	41.270	372(M^+, 18), 357(100), 207(17), 191(13), 151(8), 73(69)
2	Hydroxylated BPA	44.071	460(M^+, 24), 445(69), 371(2), 357(18), 207(44), 191(30), 73(100)
3	BPA glycoside	69.306	513(1), 450(10), 372(45), 361 (59), 271(30), 217(50), 147(44), 73(100)
4	Hydroxylated BPA glycoside	71.454	460(25), 445(6), 361(48), 271(30), 217(45), 147(40), 73(100)
5	TetrachloroBPA (II)	43.586	512(4), 510(6), 508(M^+, 5), 495(28), 275(31), 240(18), 217(11), 73(100)
6	TetrachloroBPA glycoside	65.449	512(1), 510(3), 508(2), 495(2), 361(70), 217(50), 204(19), 147(29), 73(100)
7	2-BromoBPA (IV)	40.440	452(11), 450(M^+, 12), 435(72), 355(2), 341(8), 207(15), 191(20), 73(100)
8	2,2′-DibromoBPA (V)	43.986	530(8), 532(6), 528(M^+, 5), 515(34), 287(19), 250(18), 138(40), 73(100)
9	Hydroxylated 2,2′-dibromoBPA	45.566	620(4), 618(7), 616(M^+, 5), 603(10), 515(3), 449(2), 285(14), 73(100)
10	2-BromoBPA glycoside	57.174	519(2), 450(12), 452(11), 361(56), 271(20), 217(50), 204(11), 73(100)
11	2,2′-DibromoBPA glycoside	65.141	532(3), 530(5), 528(4), 513(1), 361(68), 217(57), 147(50), 73(100)
12	2-IodoBPA (VI)	42.075	498(M^+, 4), 483(33), 277(28), 73(100)
13	2,2′-DiiodoBPA (VII)[b]	46.880	624(M^+, 5), 609(11), 483(1), 333(35), 297(41), 226(20), 73 (100)
14	Hydroxylated 2-iodoBPA	43.471	586(M^+, 8), 571(14), 483(7), 73(100)
15	2′-IodoBPA glycoside	58.967	498(23), 483(5), 450(5), 361(54), 319(20), 217(64), 147(45), 73(100)
16	2,2′-DiiodoBPA glycoside	72.861	624(4), 450(7), 361(64), 319(20), 271(19), 217(45), 204(9), 147(39), 73(100)

Roman numeral in parenthesis notes the structure in Fig. 1

[a] Peak ID in Fig. 3

[b] Retention time and mass spectral fragment ion patterns of TMS-derivatives

in neonatal intestines. Although the toxicity of BPA is reduced through the conjugate formation, BPA can be regenerated from its conjugates through glucuronidase or glucosidase.

In this study, synthetic BPA-glucoside was treated with β-glucosidase from *A. niger*. More than 99% of conjugate was hydrolyzed to BPA within 5 h (Fig. 6). Similar results were obtained by treatment of crude extracts of BPA-amended culture with the same enzyme (data not shown). These indicates that detoxification through conjugate formation can be reverted by the presence of hydrolytic enzymes. It has to be mentioned that several fungi produce extra- or intra-cellular β-glucosidase. For example, *Phanerochaete chrysosporium*, a well-known xenobiotic degrading fungus produces β-glucosidase (Smith and Gold 1979). These finding again suggests that glucoside formation may not assure the detoxification of xenobiotics in natural environment, where complex microflora, producing hydrolytic enzymes exists (Iwashita 2002).

Inhibition of BPA metabolism by PB

To evaluate the possible involvement of cytochrome P450 (CYP) in BPA metabolism, degradation behavior of BPA with fungal mycelia, treated with piperonylbutoxide (PB) was determined. Half-life of BPA was almost identical with and without PB. However, the concentrations of hydroxylated metabolites were negligible in PB-treated cultures while several hydroxylated metabolites were found in cultures without inhibitor (Fig. 7). As it was previously confirmed, the major transformation pathways of BPA is through the glucoside formation, while the fraction of oxidative transformation was highly limited (5–10% of hydroxyl metabolites vs. 80–90% of glucoside). The results indicate that CYP plays a minor role of BPA detoxification. Similar metabolic fate has been reported in other organism. For example, major metabolic pathways in plants and some fungi are glucoside formation (Nakajima et al. 2002 and 2004; Noureddin et al. 2004; Yim et al.

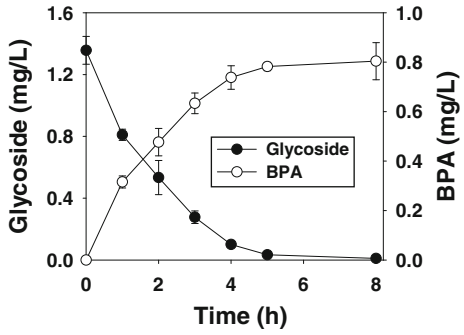


Fig. 6 Hydrolysis of synthetic BPA monoglucoside by β-glucosidase from *Aspergillus niger*

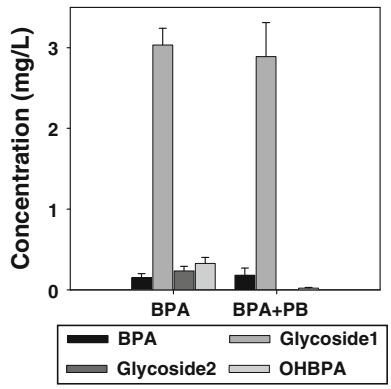


Fig. 7 Dissipation of BPA and accumulation of metabolites with and without piperonylbutoxide (PB) after 5 days of incubation

2003). Results with mammalian tissues or whole organism also give similar result. In this case, glucuronide is the major metabolite rather than glucoside (Inoue et al. 2001).

However, it has to be mentioned that some fungi (especially white-rot fungi) can mineralize or transformed BPA to lower-molecular metabolites (Chai et al. 2005). In addition, Chai et al. (2003) has reported that *Caragana chamlagu* can produce numerous non-conjugate type metabolites which are commonly found in bacterial metabolism of BPA.

In summary, the results indicate that *C. elegans* can metabolize BPAs through the formation of glucoside, with minor portion of CYP-catalyzed formation of hydroxylated BPA.

Both metabolic reactions of BPAs were retarded by the halogen-substitution at 2- and 6-position to phenolic OH groups. The conjugates were labile to hydrolytic cleavage by fungal β-glucosidase. Regeneration of BPA by fungal enzyme suggests that the toxicological risk of BPA may last much longer than expected in natural environment.

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