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Oxygenation of Bisphenol A to Quinones by Polyphenol Oxidase in Vegetables

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To understand conversion of bisphenol A and its related compounds under some chemical and biological environments, oxidation of these compounds was performed. Bisphenol A was oxidized to monoquinone and bisquinone derivatives by Fremy's salt, a radical oxidant; but salcomine and alkali did not catalyze the oxidation by molecular oxygen. Bisphenol A, bisphenol B, and 3,4′-(1-methylethylidene)bisphenol were converted to their monoquinone derivatives in the presence of oxygen and polyphenol oxidase from mushroom at 25 °C at pH 6.5. Among crude enzyme solutions of fruits and vegetables, potato, mushroom, eggplant, edible burdock, and yacon showed remarkable oxidative activity on bisphenol A. The highest activity was observed in potato, and the main product obtained by the enzymatic oxygenation was the monoquinone derivative of bisphenol A, accompanied by a small amount of the bisquinone derivative. The oxidation reactions found here will be useful for developing techniques for elimination of phenolic endocrine disrupters from the environment.

KEYWORDS: Endocrine disrupting chemical; Fremy's salt; tyrosinase; quinone; bisphenol B; 3,4′-(1-methylethylidene)bisphenol; potato (*Solanum tuberosum* L.)

INTRODUCTION

Bisphenol A (4,4′-(1-methylethylidene)bisphenol) (**1**) is widely used as a material for polycarbonate resins and epoxy resins. Since the estrogenic activity of this compound was reported (*1*, *2*), its contamination (as an endocrine disrupting chemical) of canned food from the inner coating (*3*−*5*) drew the attention of consumers. Migration of this compound into food from polycarbonate tableware and plastic packages has also caused public concern, as well as pollution of soils, rivers, and lakes by this compound. Knowledge of potential pathways of chemical and biological conversion of bisphenol A and its related compounds is necessary to understand the fate of these compounds which are contaminating food and the environment. Toxicity and biological activity of the reaction products and metabolites should be investigated for risk assessment of the contaminants for human health and the environment. The information about the pathways of chemical and biological conversion is useful also for establishing techniques for environmental remediation.

It was reported by Pottenger et al. that bisphenol A can be converted to glucuronide conjugate and sulfate conjugate in rats (*6*). On the other hand, Atkinson and Roy showed evidence of oxidation of bisphenol A by peroxidase activation system and microsomal cytochrome P450 system in vitro, and by in vivo experiments using rats, based on the DNA adduct formation (*7*, *8*). It was also found that bisphenol A was metabolized by a Gram-negative aerobic bacterium via oxidation of the aliphatic methyl group (*9*, *10*). Recently, degradations of bisphenol A by manganese peroxidase and laccase from basidiomycetes were reported in research aiming at remediation of the environment polluted by the chemical (*11*−*13*). There are some reports on removal of bisphenol A from aqueous solution using peroxidases from other sources (*14*, *15*), too. These facts showed a possibility of oxidation of bisphenol A by oxidases from various sources. But a recent paper by Endo et al. (*16*) reported that bisphenol A was not oxidized in the presence of tyrosinase from mushroom, though *p*-octylphenol was.

Then we investigated the possibility of oxidation of bisphenol A in some chemical environments, and oxygenation of bisphenol A and its related compounds by polyphenol oxidase was also tried. The oxidation products were identified as quinone derivatives and reported in our preliminary communication (*17*). We proceeded to survey vegetables and fruits for the ability of oxygenation of bisphenol A to seek a good source of enzyme for removal of this type of pollutant from the environment. Results of our experiments on oxidation of bisphenol A and its related compounds obtained so far are summarized here with some discussion.

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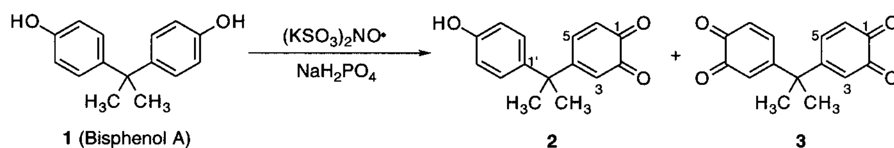
Scheme 1



MATERIALS AND METHODS

Materials. Bisphenol A, bisphenol B, and cobalt salcomine were purchased from Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan; 3,4′-(1-methylethylidene)bisphenol was obtained from Aldrich Chemical Co., Inc., Milwaukee, WI; and mushroom polyphenol oxidase (tyrosinase, monophenol, L-dopa:oxygen oxidoreductase: EC 1.14.18.1) was purchased from Worthington Biochemical Co., Lakewood, NJ and Sigma. Other reagents, including potassium nitrosodisulfonate (Fremy's salt) (*18*) and poly(vinylpyrrolidone) K90, and solvents were from Wako Pure Chemical Industries, Osaka, Japan. Vegetables and fruits were purchased commercially at local stores in Tsukuba, Ibaraki, Japan.

Oxygenation with Cobalt Salcomine. Bisphenol A (200 mg) was dissolved in 10 mL of *N*,*N*-dimethylformamide, and cobalt salcomine (330 mg) was added. They were stirred at room temperature for 24 h with bubbling of oxygen gas, and the mixture was extracted by diethyl ether.

Oxygenation in Alkaline Solution. Bisphenol A (10 mg) was dissolved in 100 mL of 4.5 mM KOH aqueous solution, stirred at 100 °C for 16 h with bubbling oxygen gas, and then stood at room temperature for 5 days. The mixture was extracted by chloroform.

Oxidation by Fremy's Salt. Ethereal solution of bisphenol A (0.5 g/11 mL) was added to cooled aqueous solution (330 mL) of Fremy's salt (6 g) and sodium dihydrogen phosphate (1 g) in a separatory funnel (1 L). The mixture was shaken for 20 min and then extracted with three 100-mL portions of chloroform. Combined extract was washed with 150 mL of water and dried over magnesium sulfate. After evaporating most of the solvent under reduced pressure, the residual matter contained reddish crystals. Removal of mother liquor and recrystallization from chloroform gave 22 mg (3.9%) of bisquinone as red crystals: mp >300 °C. After carrying out the same reaction using 1 g of bisphenol A without changing the other conditions, the chloroform extract was concentrated under reduced pressure and then column chromatographed on silica gel (50 g) with hexane/ethyl acetate (2:1). Concentration of reddish eluate and further purification with MPLC (SiO_2, hexane/ethyl acetate (2:1), 22 × 100 mm, Kusano Scientific Instrument, Tokyo, Japan) gave 80 mg (7.5%) of monoquinone as red powder. Under this purification procedure no bisquinone was obtained because of its strong absorption on silica gel. The purity of both quinone compounds was shown to be >97% by ^{1}H NMR and HPLC analyses.

Oxygenation with Polyphenol Oxidase. Bisphenol A (0.1 g), bisphenol B (0.1 g), or 3,4′-(1-methylethylidene)bisphenol (0.1 g) was dissolved in 3 mL of 0.5 M potassium phosphate buffer (pH 6.5), and polyphenol oxidase (tyrosinase) from mushroom (300 units) was added. After bubbling of oxygen gas for 5 min, the mixture was left for 1 h at 25 °C.

Kinetic Analysis of Enzymatic Reaction. Kinetic parameters were calculated by a nonlinear regression analysis program, Grafit Ver. 3 (Erithacus Softwear, London, U.K.), based on the equation $v = V_{max}/(1 + K_m/[S] + [S]/K_i)$, where v is the observed velocity at given substrate concentration [S], V_{max} is the maximum velocity at saturating concentration of substrate, K_m is the Michaelis constant, and K_i is inhibition constant.

Identification of Oxidation Products. Oxidation products were identified by high-resolution mass measurement on an electrospray ionization Fourier transformation ion cyclotron resonance mass (FT-ICRMS) spectrometer (Apex II 70e, Bruker Daltonics, Berlin, Germany) and by NMR spectrum measurement (DRX 600, Bruker, Karlsruhe, Germany).

Evaluation of Activity in Vegetables and Fruits for Oxygenation of Bisphenol A. Edible parts of fresh vegetables and fruits were cut into pieces, and 100 g was homogenized by a mixer (MX-L20GA, Toshiba, Tokyo, Japan) in 100 mL of 0.5 M potassium phosphate buffer (pH 6.5) with 20 g of poly(vinylpyrrolidone) K90 to eliminate phenols in the plant tissues. Supernatant was obtained by centrifugation at 21800*g* for 5 min at 4 °C and used as crude enzyme solution. Bisphenol A was dissolved in 1.9 mL of distilled water at the concentration of 0.5 mM and mixed with 1.0 mL of 0.5 M potassium phosphate buffer (pH 6.5). Oxygen gas was bubbled through this solution for 5 min in a cuvette prior to addition of enzyme. The crude enzyme solution (0.1 mL) was mixed with the bisphenol A solution, and increase of absorbance at 380 nm (A_{380}), which is an absorbance maximum (ϵ 2178 $M^{-1}cm^{-1}$) of the monoquinone derivative of bisphenol A, was monitored for 15 min at 25 °C on a Shimadzu UV-160A spectrometer (Kyoto, Japan). The oxidative activity was expressed by production rate of the monoquinone derivative calculated from the slope of linear portion of the graph plotting A_{380} against incubation time. Measurement of optimum temperature for the oxygenation with crude enzyme from potato tuber was done on a Beckman DU 640 spectrometer (Fullerton, CA).

RESULTS

Oxidation of Bisphenol A Using Chemical Catalyst or Radical Oxidant. Cobalt salcomine, one of the catalysts generally used for oxidation of phenols by molecular oxygen, was tested first. Almost all of the bisphenol A, however, was recovered unchanged from the reaction mixture. Oxygenation in aqueous alkaline solution at 100 °C was also tried, but bisphenol A was recovered intact as a main component.

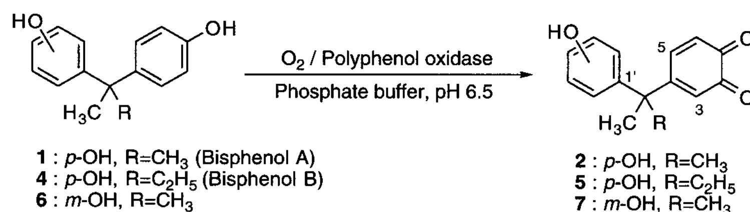
Oxidation of bisphenol A by Fremy's salt, a radical oxidant was done as the next trial (**Scheme 1**). Reaction products of this oxidation were identified as follows.

Monoquinone derivative (4-[1-(4-hydroxyphenyl)-1-methylethyl]-1,2-benzoquinone), ***2***. High-resolution FTICRMS calculated for $C_{15}H_{15}O_3$ ($[M + H]^+$), 243.1021; found, 243.1016. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 1.54 (6H, s, $2CH_3$), 4.97 (1H, s, OH), 6.22 (1H, dd, J = 10.3 and 0.7 Hz, H6), 6.50 (1H, dd, J = 2.4 and 0.7 Hz, H3), 6.64 (1H, dd, J = 10.3 and 2.4 Hz, H5), 6.84 (2H, d, J = 8.8 Hz, H3′, H5′), 7.14 (2H, d, J = 8.8 Hz, H2′, H6′). ^{13}C NMR (150.90 MHz, $CDCl_3$) δ 27.26, 43.03, 115.89, 123.76, 127.79, 128.99, 136.02, 141.60, 154.83, 161.46, 180.397, 180.404.

Bisquinone derivative (4,4′-(1-methylethylidene)bis(1,2-benzoquinone), ***3***. High-resolution FTICRMS calculated for $C_{15}H_{13}O_4$ ($[M + H]^+$), 257.0813; found, 257.0806. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 1.54 (6H, s, $2CH_3$), 6.44 (2H, dd, J = 10.3 and 0.7 Hz, H6), 6.48 (2H, dd, J = 2.4 and 0.7 Hz, H3), 6.85 (2H, dd, J = 10.3 and 2.4 Hz, H5). ^{13}C NMR (150.90 MHz, $CDCl_3$) δ 24.60, 44.21, 126.30, 130.89, 138.88, 155.88, 179.24, 179.30.

The ^{1}H NMR spectrum of the monoquinone derivative **2** showed a signal pattern of 1,2,4-trisubstitued benzene in addition to the A_2X_2 pattern of a 4-substituted phenol, which is observed in the ^{1}H NMR of bisphenol A as well. Besides these ^{1}H NMR signal patterns and the result of high-resolution mass analysis, two carbonyl signals in ^{13}C NMR confirmed that this oxidation product contained not a catechol (1,2-hydroquinone) moiety but a 1,2-quinone moiety. In the ^{1}H NMR spectrum of the derivative **3**, the signals of 4-substituted phenol were not observed, but only signals of a 1,2-quinone moiety and a singlet methyl group

Scheme 2



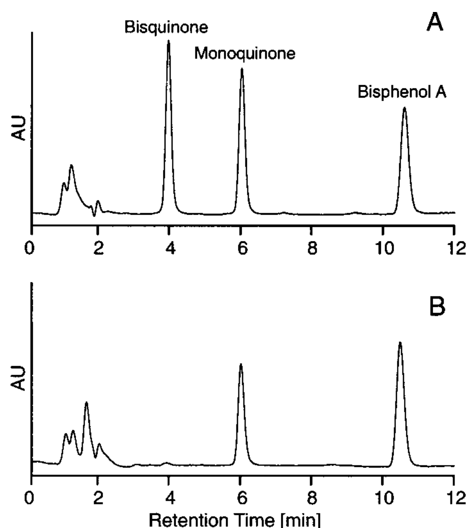


Figure 1. HPLC analysis of bisphenol A and its quinone derivatives. (A) 10 ppm Standard solution mixture; (B) mixture of bisphenol A and crude enzyme solution of potato (Irish Cobbler) after incubation at 25 °C for 15 min. Instrument, Alliance 2690 (Waters, Milford, MA); column, Symmetry Shield RP_{18} (3.5 μm, 2.1 × 150 mm) with a guard column (2.1 × 10 mm) (Waters); mobile phase, 40% acetonitrile, 0.2% formic acid; flow rate, 0.23 mL/min; temperature, 40 °C; detection, 280 nm; injection volume, 1 μL.

were observed. Together with data of the high-resolution mass measurement, this compound was identified as the bisquinone derivative of bisphenol A.

Bisphenol A and its quinone derivatives could be analyzed together on reversed-phase HPLC as shown in **Figure 1A**. The bisquinone **3** was eluted first at 4.0 min, followed by the monoquinone **2** at 6.0 min, and then bisphenol A at 10.6 min. Oxygenation of bisphenol A and its related compounds can be monitored quantitatively on HPLC under these analytical conditions.

Oxygenation of Bisphenol A and Its Related Compounds Using Mushroom Polyphenol Oxidase. For enzymatic oxidation, polyphenol oxidase (tyrosinase) from mushroom supplied by Worthington Biochemical was used. By the reaction for 1 h at 25 °C, almost all of the bisphenol A was oxidized to the monoquinone derivative **2** (**Scheme 2**). From the analysis of reaction rate and substrate concentration, strong substrate inhibition was found: $K_m = 12 \pm 7\ \mu$M, $K_i = 270 \pm 130\ \mu$M. Tyrosinase from a mushroom supplied by Sigma, however, did not oxidize bisphenol A.

Bisphenol B (4,4′-(1-methylpropylidene)bisphenol, **4**, a material for phenol resins, was converted to its monoquinone derivative (**5**). ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 0.81 (3H, t, J = 7.4 Hz, CH_2−CH_3), 1.26 (3H, s, CH_3), 2.06 (2H, q, J = 7.4 Hz, CH_2−CH_3), 6.21 (1H, d, J = 10.4 Hz, H6), 6.47 (1H, d, J = 2.3 Hz, H3), 6.60 (1H, dd, J = 10.4 and 2.3 Hz, H5), 6.82 (2H, d, J = 8.6 Hz, H3′, H5′), 7.11 (2H, d, J = 8.6 Hz, H2′, H6′) but not to bisquinone in the presence of the polyphenol oxidase (**Scheme 2**).

Table 1. Oxygenation of Bisphenol A with Crude Enzyme Solutions of Fruits and Vegetables

fruit, vegetable	monoquinone production (μM/min)
apple (*Malus pumila*)	0.00
banana (*Musa acuminata*)	0.03
potato (*Solanum tuberosum*)	4.96
eggplant (*Solanum melongena*)	0.83
tomato (*Lycopersicon esculentum*)	0.00
sweet pepper (*Capsicum annuum* var. *angulosum*)	0.06
head lettuce (*Lactuca sativa*)	0.00
edible burdock (*Arctium lappa*)	0.73
yacon (*Samallanthus sonchifolius*)	0.23
sweet potato (*Ipomoea batatas*)	0.00
celery (*Apium graveolens*)	0.00
lotus root (*Nelumbo nucifera*)	0.00
bean sprout (*Vigna radiata*)	0.00
chinese yam (*Dioscorea opposita*)	0.00
mushroom (*Agaricus bisporus*)	2.75
shiitake mushroom (*Lentinula edodes*)	0.00

3,4′-(1-Methylethylidene)bisphenol (**6**), an isomer of bisphenol A, was also oxidized by the polyphenol oxidase to the corresponding quinone derivative (**7**). ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 1.55 (6H, s, $2CH_3$), 6.23 (1H, d, J = 10.3 Hz, H6), 6.50 (1H, d, J = 2.2 Hz, H3), 6.64 (1H, dd, J = 10.3 and 2.2 Hz, H5), 6.75 (1H, d, J = 7.9 Hz, H4′ or H6′), 6.76 (1H, brs, H2′), 6.84 (1H, d, J = 7.9 Hz, H6′ or H4′), 7.23 (1H, dd, J = 7.9 and 7.9 Hz, H5′) (**Scheme 2**).

Screening of Oxygenation Activity of Vegetables and Fruits for Bisphenol A. As the polyphenol oxidase from mushroom showed ability for oxygenation of bisphenol A and its related compounds, such activity was surveyed in fresh vegetables and fruits. Among 2 fruits and 14 vegetables tested in this work, potato, mushroom, eggplant, edible burdock, and yacon showed remarkable oxidative activity for bisphenol A (**Table 1**). The highest activity was observed in potato. Proceeding of the oxygenation resulting in the formation of the quinone derivative from bisphenol A was monitored through increase of A_{380}, absorbance of quinone moiety, of the reaction mixture, which is absent in the original UV spectrum of bisphenol A (**Figure 2**).

Tubers of 10 potato cultivars, which were harvested at National Agricultural Research Center for Hokkaido Region in late September 2000, pre-stored at 10−15 °C for a month, and stored at 2 °C for 5 months, were tested for the oxidative activity of bisphenol A. Among them, Norin 1, which shows strong discoloration after peeling and/or cooking, had the highest activity (**Table 2**). On the other hand, Hokkai 87, Toyoshiro, and Hokkaikogane showed very low oxidative activity.

The optimum pH for the oxygenation with the crude enzyme from a tuber of Irish Cobbler, a common potato cultivar for

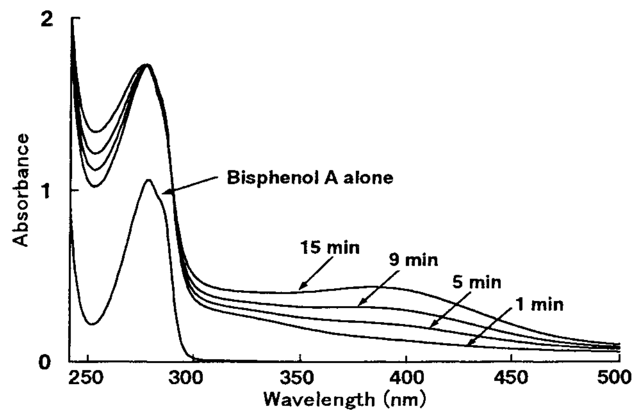


Figure 2. Change of UV spectrum during the oxygenation of bisphenol A with potato crude enzyme solution. Preparation of sample and reaction condition were as described in Evaluation of Activity in Vegetables and Fruits for Oxygenation of Bisphenol A in Materials and Methods. Times in the spectrum refer to time after addition of the crude enzyme solution to the bisphenol A solution.

Table 2. Comparison of Bisphenol A Oxidative Activity among Potato Cultivars

cultivar	monoquinone production[a] (μM/min)
Norin 1	11.60 ± 3.62a
Hokkai 86	7.87 ± 1.00b
Hokkai 87	1.52 ± 0.77de
Hokkai 88	4.66 ± 1.88c
Irish Cobbler	6.28 ± 1.82bc
Sayaka	6.15 ± 0.87bc
Touya	4.92 ± 0.89bc
Toyoshiro	1.18 ± 0.20e
Beniakari	4.35 ± 0.70cd
Hokkaikogane	1.29 ± 0.56e

[a] Each value is mean ± S. D. Values in a column not sharing the same superscript letter are significantly different at $P < 0.05$ by Tukey's multiple comparison test.

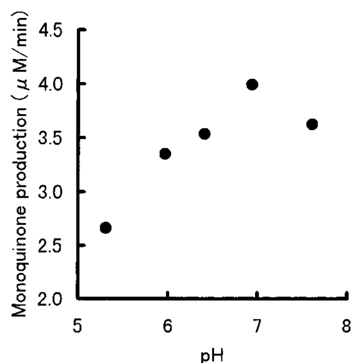


Figure 3. pH dependence of the oxygenation of bisphenol A by potato (Irish Cobbler) crude enzyme solution (reaction temperature was 25 °C).

culinary use in Japan, was 7 (**Figure 3**). As for the optimum temperature, highest activity was observed at the initial stage up to 5 min at 60 °C among the temperatures tested (**Figure 4**), but the activity was lost within 10 min at temperature higher than 55 °C, and the maximum production of the oxidation product was obtained at 45 °C after 15 min reaction.

Oxidation products of bisphenol A by the reaction with the crude enzyme from Irish Cobbler at 25 °C for 15 min were identified by HPLC. The reaction mixture was centrifuged at 21800*g* for 10 min, and the supernatant was analyzed under the condition described in the caption of **Figure 1**. The monoquinone derivative was the major product, and trace amount of the bisquinone derivative was detected (**Figure 1B**). In addition, black sediments which seem to be polymerized products derived from radical coupling reactions of bisphenol A and its quinone derivatives with phenolic compounds, amines, amino acids, and proteins in the potato tissue were present in the reaction mixture.

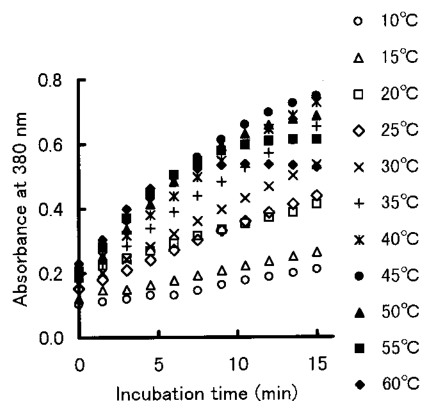


Figure 4. Temperature dependence of the oxygenation rate of bisphenol A by potato (Irish Cobbler) crude enzyme solution (buffer pH was 7.0).

DISCUSSION

Trials of oxidation of bisphenol A by molecular oxygen using cobalt salcomine at room temperature and using alkaline solution at 100 °C were not successful. These results suggested that bisphenol A was rather stable against these general oxidative conditions, which means that it will be rarely oxidized during the usual food processing and cooking conditions. But a radical oxidant, Fremy's salt, could oxidize bisphenol A to two quinone derivatives. Although formation of the monoquinone derivative **2** by oxidation of bisphenol A using Fremy's salt had been reported by Atkinson and Roy (*7*), we detected the bisquinone derivative **3** as a novel product in addition.

Then we used a commercial polyphenol oxidase for a trial of enzymatic oxygenation of bisphenol A. Polyphenol oxidase is a well-known enzyme in plants, including vegetables and fruits, which catalyzes oxygenation of phenolic compounds to quinones. The quinones can polymerize through radial coupling reactions, producing brown or black pigments, so that this enzyme is regarded as a key enzyme of browning of fresh vegetables and fruits. The enzyme called polyphenol oxidase includes three types of enzymes: monophenol monooxygenase (tyrosinase), diphenol oxidase (catechol oxidase, diphenol oxygen oxidoreductase), and laccase (*19*). These enzymes are different in substrate specificity, but the former two activities are often found on the same enzyme protein. Diphenol oxidase activity is usually much higher than tyrosinase activity in plants. Bisphenol A has two 4-substituted phenol moieties in the molecule and is expected to be a substrate of tyrosinase. The intermediate product of the oxygenation by tyrosinase will be a catechol-type derivative which is accepted as a substrate by diphenol oxidase and laccase. Thus, the oxygenation will proceed not only by tyrosinase but in a complex combination system including all polyphenol oxidases in the plants.

Polyphenol oxidase of mushroom supplied by Worthington Biochemical successfully converted bisphenol A to its monoquinone derivative at room temperature. An enzyme sold as tyrosinase of mushroom by Sigma, however, did not oxidize bisphenol A effectively as reported by Endo et al. (*16*), suggesting that the ability to accept bisphenol A as a substrate

varies among isozymes. The former polyphenol oxidase also catalyzed the oxygenation of bisphenol B and 3,4′-(1-methylethylidene)bisphenol to their monoquinone derivatives in the same condition used for bisphenol A. The bisquinone derivative **3** was not obtained in the enzymatic oxygenation of bisphenol A under the conditions used in this work. Bisquinone derivative of bisphenol B was not detected in the oxygenation, either. Product inhibition of the enzyme by the monoquinone derivatives may be a cause of the absence of the bisquinone products. Reaction of the chemically active quinone products to the enzyme resulting in irreversible damage on the structure and function of the enzyme molecule may also interfere with the production of the bisquinones. Catechol-type intermediates of the tyrosinase reaction were not detected in this work. It may be due to immediate oxidation of the catechols to quinones by the second oxidation step in the enzyme. If the catechol intermediates are released outside, it will also be oxidized by molecular oxygen in the medium.

Potato, mushroom, eggplant, edible burdock, and yacon had remarkable oxidative activity on bisphenol A, but some of the other tested vegetables and fruits did not show the oxidative activity. This difference should be derived from the difference in the tyrosinase activity of their polyphenol oxidase. The highest activity was observed in potato. Although there are some variations among the cultivars, Irish Cobbler, one of the common potato cultivars in Japan, showed promising level of the activity following Norin 1, suggesting it to be a good enzyme source for oxidative conversion of bisphenol A. The major product in the oxygenation with the crude enzyme from Irish Cobbler was the monoquinone derivative, and a small amount of the bisquinone derivative was also detected on HPLC analysis of the reaction mixture. The amount of the bisquinone derivative varied with the amount of the crude enzyme solution used, reaction temperature, and reaction time.

Hirano et al. reported that the oxidation products of bisphenol A by H_2O_2 with manganese peroxidase of the white-rot basidiomycete, *Pleurotus ostreatus*, were phenol, 4-isopropenylphenol, 4-isopropylphenol, and hexestrol, although several minor peaks on HPLC were not identified (*11*). The quinone derivatives found in this work were not detected in the oxidation with manganese peroxidase. Polymerization of the oxidation products by radical coupling, which produces black sediments found in the enzymatic oxygenation in this work, was also suggested in the work of Tsutsumi et al. using manganese peroxidase and laccase from lignin-degrading basidiomycetes (*13*) and Sakurai et al. (*15*) using *Coprinus cinereus* peroxidase.

Now we know the possibility of use of oxidases from plants and microorganisms for removal of bisphenol A from the environment. Next the toxicity and endocrine-disrupting activity of the oxidation products, including the quinone compounds reported here, should be investigated together with their reactivity to substances in the natural environment and in foods. The knowledge obtained in these studies will be useful in development of techniques for elimination of phenolic endocrine disrupters from the environment by using enzymatic reactions.

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LITERATURE CITED

(1) Krishnan, A. V.; Stathis, P.; Permuth, S. F.; Tokes, L.; Feldman, D. Bisphenol A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* **1993**, *132*, 2279−2286.

(2) Olea, N.; Pulgar, R.; Perez, P.; Olea-Serrano, F.; Rivas, A.; Novillo-Fertrell, A.; Pedraza, V.; Soto, A. M.; Sonnenschein, C. Estrogenicity of resin-based composites and sealants used in dentistry. *Environ. Health Perspect.* **1996**, *104*, 298−305.

(3) Brotons, J. A.; Olea-Serrano, M. F.; Villalobos, M.; Pedraza, V.; Olea, N. Xenoestrogens released from lacquer coatings in food cans. *Environ. Health Perspect.* **1995**, *103*, 608−612.

(4) Biles, J. E.; McNeal, T. P.; Begley, T. H. Determination of bisphenol A migrating from epoxy can coatings to infant formula liquid concentrates. *J. Agric. Food Chem.* **1997**, *45*, 4697−4700.

(5) Kawamura, Y.; Sano, H.; Yamada, T. Migration of bisphenol A from can coatings to drinks. *Shokuhin Eiseigaku Zasshi* **1999**, *40*, 158−165.

(6) Pottenger, L. H.; Domoradzki, J. Y.; Markham, D. A.; Hansen, S. C.; Cagen, S. Z.; Waechter, J. M., Jr. The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. *Toxicol. Sci.* **2000**, *54*, 3−18.

(7) Atkinson, A.; Roy, D. *In vitro* conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite(s). *Biochem. Biophys. Res. Commun.* **1995**, *210*, 424−433.

(8) Atkinson, A.; Roy, D. *In vivo* DNA adduct formation by bisphenol A. *Environ. Mol. Mutagen.* **1995**, *26*, 60−66.

(9) Lobos, J. H.; Leib, T. K.; Su, T. Biodegradation of bisphenol A and other bisphenols by a gram-negative aerobic bacterium. *Appl. Environ. Microbiol.* **1992**, *58*, 1823−1831.

(10) Spivack, J.; Leib, T. K.; Lobos, J. H. Novel pathway for bacterial metabolism of bisphenol A. *J. Biol. Chem.* **1994**, *269*, 7323−7329.

(11) Hirano, T.; Honda, Y.; Watanabe, T.; Kuwahara, M. Degradation of bisphenol A by the lignin-degrading enzyme, manganese peroxidase, produced by the white-rot basidiomycete, *Pleurotus ostreatus*. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 1958−1962.

(12) Tanaka, T.; Yamada, K.; Tonosaki, T.; Konishi, T.; Goto, H.; Taniguchi, M. Enzymatic degradation of alkylphenols, bisphenol A, synthetic estrogen and phthalic ester. *Water Sci. Technol.* **2000**, *42*, 89−95.

(13) Tsutsumi, Y.; Haneda, T.; Nishida, T. Removal of estrogenic activities of bisphenol A and nonylphenol by oxidative enzymes from lignin-degrading basidiomycetes. *Chemosphere* **2001**, *42*, 271−276.

(14) Caza, N.; Bewtra, J. K.; Biswas, N.; Taylor, K. E. Removal of phenolic compounds from synthetic wastewater using soybean peroxidase. *Water Res.* **1999**, *33*, 3012−3018.

(15) Sakurai, A.; Toyoda, S.; Sakakibara, M. Removal of bisphenol A by polymerization and precipitation method using *Coprinus cinereus* peroxidase. *Biotechnol. Lett.* **2001**, *23*, 995−998.

(16) Endo, Y.; Xuan, Y. J.; Fujimoto, K. The oxidation of *p*-octylphenol by mushroom tyrosinase. *Nippon Nogeikagaku Kaishi* **2000**, *74*, 1337−1341.

(17) Yoshida, M.; Ono, H.; Mori Y.; Chuda, Y.; Onishi, K. Oxidation of bisphenol A and related compounds. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1444−1446.

(18) Zimmer, H.; Lankin, D. C.; Horgan, S. W. Oxidations with potassium nitrosodisulfonate (Fremy's radical), the Teuber reaction. *Chem. Rev.* **1971**, *71*, 229−246.

(19) Mayer, A. M. Polyphenol oxidases in plants − recent progress. *Phytochemistry* **1987**, *26*, 11−20.

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