Use of Chitosan for Removal of Bisphenol A from Aqueous Solutions Through Quinone Oxidation by Polyphenol Oxidase

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ABSTRACT: In this study, a combined use of biopolymer chitosan and oxidoreductase polyphenol oxidase (PPO) was applied to the removal of bisphenol A (BPA) as an endocrine disrupting chemical from aqueous solutions. The optimum conditions for the enzymatic quinone oxidation of BPA were determined to be pH 7.0 and 40°C. Quinone derivatives generated were chemisorbed on chitosan beads, and BPA was completely removed at 4–7 h. The removal time was shortened with an increase in the amount of dispersed chitosan beads or the PPO concentration. In addition, the initial velocity of quinone oxidation increased with an increase in the amount of chitosan beads. The use of chitosan in the form of porous beads was more effective than the use of chitosan in the form of solutions or powder. It was found that an important factor for this procedure was a high-specific surface area of chitosan beads and heterogeneous reaction of quinone derivatives enzymatically generated with chitosan. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 796–804, 2012

Key words: polyphenol oxidase; bisphenol A; quinone oxidation; quinone adsorption; chitosan

INTRODUCTION

The substances that can mimic or antagonize the effects of endocrine hormones are called endocrine disrupting chemicals. Recently, the environmental pollution by endocrine disrupting chemicals has aroused the public concerns. Unfortunately, their environmental distribution is being increasingly widespread. It is well known that bisphenol A [2,2-bis(hydroxylphenyl) propane, BPA] used as the starting material for synthesis of epoxy resins and polycarbonate plastics is one of the endocrine disrupting chemicals. The annual production of BPA stood at 490,000 ton in 2001 in Japan. Also, BPA is frequently detected in effluent samples of wastewater treatment plants and found in sediments and fish.[1] Another potential source is leachate from hazardous waste landfills. When waste plastics produced from BPA are buried in a landfill, a hydrolytic or leaching process may occur to release BPA to the leachate.[2,3]

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Chemical procedures such as adsorption,[4–6] nanofiltration,[7] polymerization,[8] photolysis,[9,10] photooxidation,[9] or biological procedures by use of microalgae and bacteria[11,12] have been constructed to detoxify or degrade BPA. In some cases, however, BPA cannot be treated effectively by the above procedures or are left in waste streams. As an alternative procedure, much attention has been paid to the use of enzymes to specifically catalyze the conversion of pollutants and toxic compounds to less toxic or more reactive intermediates.[13–16] Oxidoreductases such as peroxidase[17,18] and tyrosinase[13–15,19] have been used to detoxify or degrade phenolic compounds such as alkylphenols,[13–15] chlorophenols,[14] and BPA.[17–19] Of them, peroxidases have been more frequently used to treat BPA. Peroxidases catalyze the radical formation of phenolic compounds in the presence of hydrogen peroxide (H_2O_2). Because the phenoxy radicals generated spontaneously react with each other to form water-insoluble oligomers,[16] phenolic compounds can be removed by filtering out the oligomers. The studies have been also widely carried out on quinone oxidation of alkylphenols and chlorophenols with tyrosinase.[19]

We reported that BPA was effectively quinone-oxidized by mushroom tyrosinase in the presence of H_2O_2.[19] In addition, because most of the quinone

derivatives have a high reactivity, they can undergo various chemical reactions.[14,15,20–22] Therefore, the second step after tyrosinase-catalyzed quinone oxidation is the use of chitosan as a amino group-containing biopolymer for the removal of BPA from aqueous solutions. Chitosan in different forms has been used to remove phenolic compounds. The use of chitosan was divided into two ways; homogeneous reaction with chitosan solutions[23] and heterogeneous reaction with chitosan gels, porous beads, and powder.[19,21] When porous chitosan beads were dispersed in a BPA solution containing tyrosinase and H_2O_2, BPA was effectively removed through nonenzymatic quinone adsorption on chitosan beads.[19] Consequently, BPA was completely removed by this procedure. However, the issue of concern is a possible remaining of H_2O_2 and a high enzyme dose.

We focused attention on the combined use of an enzyme polyphenol oxidase (PPO) and biopolymer chitosan for the removal of BPA because in our preliminary experiment, BPA successfully underwent quinone oxidation by PPO in the absence of H_2O_2. Therefore, in this study, we investigated the removal of BPA from aqueous solutions through the two steps; PPO-catalyzed quinone oxidation of BPA and subsequent quinone adsorption on chitosan beads. First, the process parameters such as the pH value, temperature, and enzyme dose were determined to convert BPA to quinone derivatives. In particular, the use of chitosan in the form of porous beads for the two-step removal of BPA was closely followed up in comparison with the use of chitosan in the form of solution and powder.

MATERIALS AND METHODS

Chemicals

PPO (EC 1.14.18.1) of the specific activity of 820 U/mg-solid (activity determined by the supplier) was purchased from Worthington Biochemical (Lakewood, NJ). A chitosan bead, chitopearl AL-01 (particle size, 70–210 μm; specific surface area, 70–100 m^2/g) used as an absorbent was purchased from Fuji Spinning (Tokyo, Japan) and has the density of amino groups of 0.25 $mmol/cm^3$-resin in water. These nominal values were obtained from the manufacturer. The water content of the chitosan beads was 92.5% in water. The chitosan beads were stored in phosphate buffers at different pH values before use. BPA and a chitosan sample, chitosan 300, were purchased from Wako Pure Chemical Industry (Tokyo, Japan). Chitosan powder (diameter, 74–100 μm) was supplied from Dainichiseika Color and Chemicals Mfg. Co. (Tokyo, Japan).

Quinone oxidation and removal of BPA

Phosphate buffers (ionic strength: 0.01*M*) were prepared with HCl/KH_2PO_4 at 4.0 and with $NaOH/KH_2PO_4$ at 5.0–10.0. Stock solutions of BPA at 0.35 m*M* and PPO at 800 U/cm^3 were prepared in the buffers at pH 4.0–10.0. The enzymatic reaction was initiated by adding PPO to a BPA solution (35 cm^3) in a 50-cm^3 Erlenmeyer flask, just after the temperature of the solutions was adjusted in a temperature-controlled bath. The initial concentration in the solutions was [BPA] = 0.30 m*M* and [PPO] = 100 U/cm^3 unless otherwise noted. The BPA concentration was fixed at 0.30 m*M*, because the optimum values of the parameters for PPO-catalyzed treatment of BPA can be determined with a high accuracy from the generation of quinone derivatives using absorbance measurements and from the determination of the concentration of remaining BPA using high-performance liquid chromatography (HPLC) measurements. The solutions were continuously stirred during the reaction, and the absorbance at 385 nm was measured at predetermined time intervals on a Shimadzu UV–vis recording spectrophotometer UV-260. For removal experiments, a given amount of chitosan beads stored in buffers were dispersed in BPA solutions with a bullet for titration, and then the enzymatic reaction was initiated by adding PPO.[13–15,19] During the reaction, the supernatants were taken from the reaction solutions not so as to contain chitosan beads, and the absorbance was measured at 385 nm.

Quantitative assay of BPA

The concentration of remaining BPA in the reaction solutions was determined by a Hitachi L-7000 HPLC system equipped with a UV-spectrophotometer and an integrator. A reverse phase column, Inertsil ODS-3 (5 μm, 4.6 mm i.d. × 15 cm), was used. The volume composition of aqueous acetonitrile solutions as the mobile phase was adjusted to 45 vol % of acetonitrile, and the mobile phase was flowed at 1.0 cm^3/min. The retention time of BPA was 7.2 min. Aliquots of 0.2–0.3 cm^3 taken from the reaction solutions at predetermined time intervals were heated in hot water at about 80°C for about 3 min to deactivate PPO. Then, a solution of 20 mm^3 was injected to the HPLC system, and the absorption spectra were measured at 278 nm as the maximum absorption wavelength of BPA. A good linear relationship was made between the BPA concentration and the peak intensity by HPLC measurements at the initial BPA concentration of 0.30 m*M*. The conversion % values were calculated from the peak areas of BPA before enzymatic

reaction and time t ($Area_0$ and $Area_t$) using eq. (1).[13–15,19]

$$\text{Conversion}\% = \frac{\text{Area}_0 - \text{Area}_t}{\text{Area}_0} \times 100 \qquad (1)$$

As control experiments, removal of BPA was also estimated in a homogeneous system with chitosan and PPO and in a heterogeneous system with PPO in the presence of chitosan powder. A chitosan solution (~ 1 w/v %) was prepared by adding 1.0 g chitosan flakes to 100-cm^3 distilled water and intermittently adding 2*M* HCl to keep the pH value of 3–4. After most of the added chitosan flakes were dissolved, the solution was filtered under reduced pressure with a G3 glass filter to remove insoluble parts. The amino group concentration of the stock chitosan solution prepared was determined from the weight concentration of the chitosan solution and the degree of deacetylation of the chitosan sample used. Then, the chitosan solution was diluted with a pH 6.0 buffer to the amino group concentrations of 1–30 m*M*.[24,25] Here, the pH value was adjusted to 6.0, because chitosan was insoluble in a pH 7.0 buffer. Chitosan solutions were added to a BPA solution containing PPO at pH 6.0 so as to reach the final amino group concentrations from 0.1 to 10 m*M* ([—NH_2]/[BPA] = 0.33–3.33), and then the solutions were moderately stirred at 40°C. After stirred for 24 h, the water-insoluble aggregates were filtered out with a 5C filter paper, and then the absorbance of the filtrates was measured at 385 nm. As another control experiment, chitosan powder was dispersed in a BPA solutions containing PPO at pH 7.0 in place of chitosan beads, and then PPO was added to the solution.

Instrumental analysis of chitosan/quinone reaction

Chitosan films were prepared by pipetting a chitosan solution of 3.0 g into a Petri dish of diameter 3.2 cm. The solution was allowed to dry in the oven at 50°C. The chitosan films (average thickness : 24 μm) separated from the Petri dishes were thoroughly washed with 1*M* NaOH and water to neutralize the amino groups on the surfaces and then dried under reduced pressure. Chitosan films were incubated in a BPA solution containing PPO (100 U/cm^3) for 0.5–3 h at pH 7.0 and 40°C. After incubation, the chitosan films were washed with water and then dried under reduced pressure. The incubated chitosan films were placed perpendicular to the light path, such that the light passed directly through the films in the chamber of the spectrophotometer. The UV–vis spectra were recorded in the range of wavelength from 200 to 600 nm.[14]

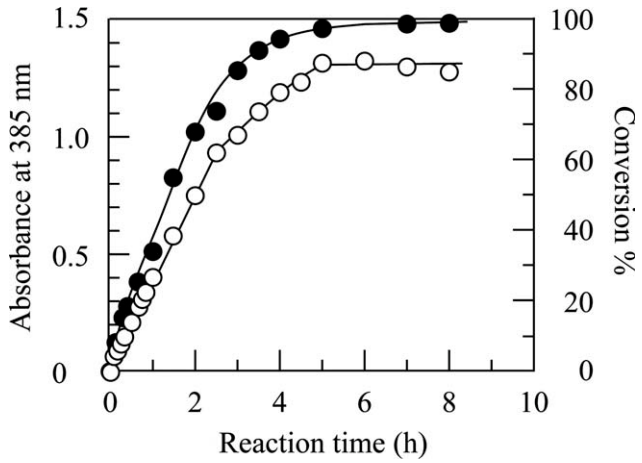

Figure 1 The time course of the absorbance at 385 nm (●) and conversion % value (○) for PPO-catalyzed (100 U/cm^3) quinone oxidation of BPA at pH 7.0 and 40°C.

RESULTS AND DISCUSSION

Determination of optimum conditions for quinone oxidation by PPO

Figure 1 shows the time course of the absorbance at 385 nm and conversion % value for PPO-catalyzed quinone oxidation of BPA at pH 7.0 and 40°C. The absorbance sharply increased 3 h after PPO was added to a BPA solution. Concurrently, the color of the solution turned yellow, then orange-red, and finally brown. The conversion % value also increased over the enzymatic reaction time and reacted 85.7% at the reaction time of 5 h. Finally, most BPA was enzymatically quinone-oxidized at 7 h. The peak position of the quinone derivative generated from BPA by PPO in this study was in good agreement with those of the quinone derivatives from alkylphenols[14,15] and BPA[19] by mushroom tyrosinase. The appearance of the peak at 385 nm and color development of the solution supports the fact that BPA effectively underwent PPO-catalyzed quinone oxidation in the absence of H_2O_2.

The enzyme most frequently used for the treatment of phenolic compounds is peroxidase. However, H_2O_2 is required to catalyze radicalization of phenolic compounds such as alkylphenols,[26] chlorophenols,[27,28] and BPA,[17,18] because peroxidase has no activity in the absence of H_2O_2. Another candidate to treat BPA is tyrosinase. We reported in a previous article that mushroom tyrosinase-catalyzed quinone oxidation of BPA was enhanced by the addition of H_2O_2.[16] On the other hand, PPO effectively catalyzed quinone-oxidation of BPA in the absence of H_2O_2 or any additives. The conversion % value of 86% obtained at 5 h for PPO at 100 U/cm^3 was a little higher than 78% at 3 h for tyrosinase at 200 U/cm^3. The PPO dose was half as mush as

tyrosinase dose for enzymatic quinone oxidation of BPA. Espín et al.[29] have reported that the cresolase activity of mushroom tyrosinase toward *N*-acetyl-L-tyrosine or 4TBP is enhanced by adding hydroxyanthranilic acid (HAA). However, because HAA is referred to as a carcinogen, the use of HAA is infavorable in the viewpoint of environment. Therefore, the effects of the pH value, temperature, and enzyme dose on the PPO-catalyzed quinone oxidation of BPA were investigated in the next section.

Effects of pH value, temperature, and enzyme dose

The optimum values of the pH and temperature of the enzymatic activity are slightly different from one article to another and depend on the experimental conditions such as the kind and concentration of the substrates and kind and salt concentration of buffers in addition to the source of enzymes. First, the effect of the pH value on PPO-catalyzed quinone oxidation of BPA was investigated at 40°C. The initial velocity was calculated from the slope of the absorbance at 385 nm against the reaction time in the initial stage. Figure 2(a) shows the changes in the initial velocity and the absorbance and conversion % at 3 h with the pH value at 40°C. As the pH value increased, the absorbance and conversion % value increased. Although the initial velocity had the maximum value at pH 9.0, the absorbance tended to level off from 40 min after the enzymatic reaction was started due to gradual deactivation during the enzymatic reaction. Although the conversion % value was almost the same in the range of pH 7.0–10.0, the absorbance gradually decreased with an increase in the pH value. In addition, the initial velocity at pH 7.0 was a third as high as that at pH 9.0. However, the absorbance increased over the reaction time at pH 7.0. Therefore, the optimum pH value was determined to be 7.0. Consequently, the effect of the temperature on PPO-catalyzed quinone oxidation of BPA was investigated at pH 7.0.

Figure 2(b) shows the changes in the initial velocity and the absorbance and conversion % at 3 h with the reaction temperature. The initial velocity increased with an increase in the reaction temperature from 20 to 55°C, but both absorbance and conversion % had the maximum value at 40°C. At temperatures higher than 40°C, PPO was gradually deactivated probably due to thermal denaturation. Although the initial velocity at 40°C was lower than those at 50 and 55°C, the absorbance increased over the reaction time, and the conversion % value at 40°C was higher. A decrease in the enzymatic activity at temperatures higher than the optimum temperature was observed for treatment of BPA by other enzymes such as mushroom tyrosinase,[19] horseradish peroxidase,[17] and soybean peroxidase.[18]

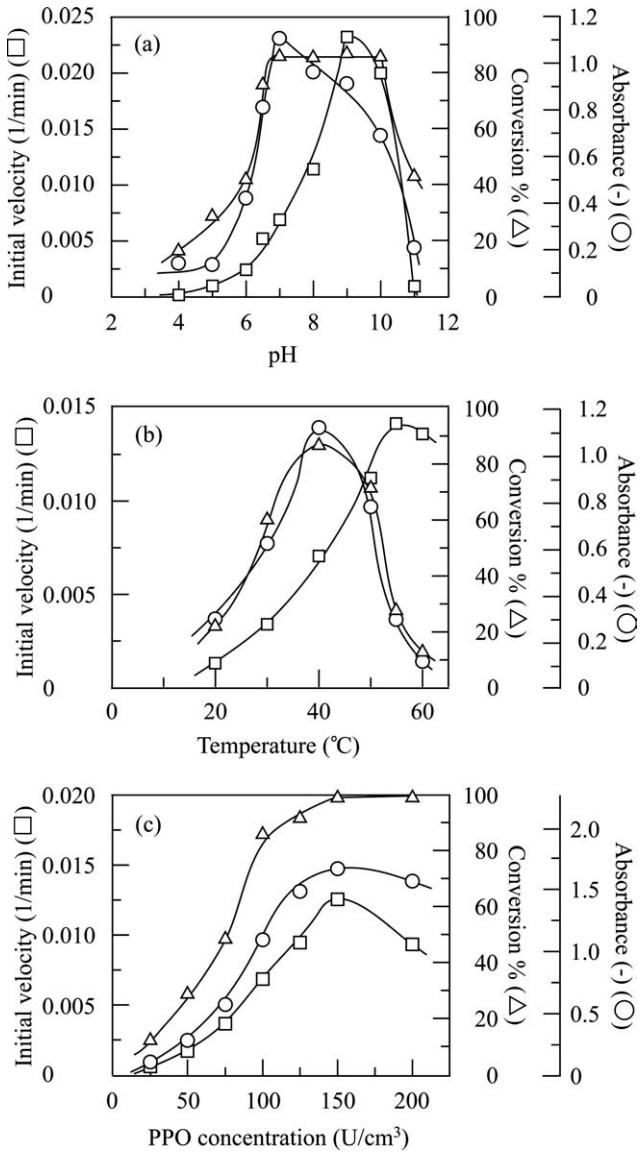

Figure 2 The effects of the (a) pH value, (b) temperature, and (c) enzyme concentration on PPO-catalyzed quinone oxidation of BPA. The parameters except for the factor in the *X* axis were constant, such as pH = 7.0, temperature = 40°C, and [PPO] = 100 U/cm^3.

From the above results, the optimum temperature was determined to be 40°C.

Finally, PPO-catalyzed quinone oxidation of BPA was investigated at different PPO concentrations at pH 7.0 and 40°C as the optimum conditions determined earlier. Figure 2(c) shows the changes in the initial velocity and the absorbance and conversion % at 3 h with the PPO concentration at pH 7.0 and 40°C. The initial velocity and absorbance increased with an increase in the enzyme concentration and had the maximum value at the PPO concentration of 150 U/cm^3. The conversion % value also increased with an increase in the enzyme concentration, and BPA was completely converted into quinone

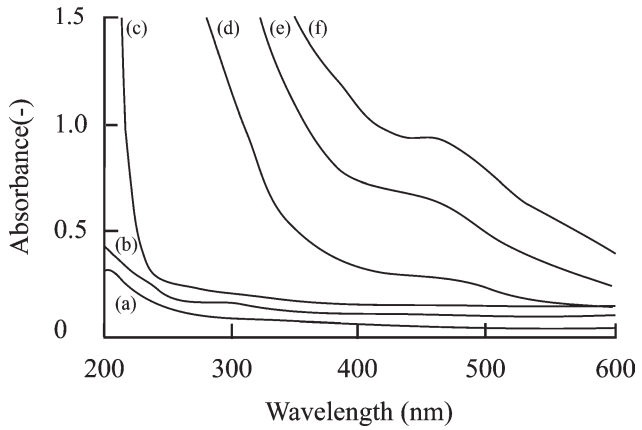

Figure 3 UV–vis spectra of chitosan films incubated in a BPA solution containing PPO (100 U/cm^3) and in different control solutions at pH 7.0 and 40°C. Incubation conditions (a) unreacted chitosan film, (b) chitosan film in BPA without PPO, (c) cellulose film in BPA with PPO, (d–f) chitosan films in BPA with PPO. Incubation time (h): (d) 0.5, (e) 2, and (b), (c), and (f) 3.

derivatives for 3 h at 200 U/cm^3. At 125 and 150 U/cm^3, the complete conversion was obtained by prolonging the reaction time to 6 and 4 h, respectively (not shown). The amount of PPO required for complete quinone oxidation of BPA by PPO in the presence of chitosan beads will be discussed in the following section.

Reaction of quinone adsorption with chitosan

The reactivity of quinone derivatives enzymatically generated from BPA with amino groups was investigated with a chitosan film. Figure 3 shows the UV–vis spectra of chitosan films incubated in a BPA solution containing PPO and other control films. A peak emerged at 460 nm for the chitosan films incubated in a BPA solution containing PPO. The brown coloration was developed, and the peak intensity increased with an increase in the incubation time. On the other hand, for a cellulose film incubated under the same conditions, this peak disappeared.[15,19]

Because cellulose has a —OH group in the C3 position in place of a —NH_2 group for chitosan, an amino group is considered to be involved in the quinone reaction with chitosan.[19] In addition, by use of the two-step process, PPO-catalyzed quinone oxidation of BPA in the absence of H_2O_2 and quinone reaction of chitosan's amino groups under relatively mild conditions, we estimated the removal of BPA with porous chitosan beads in the next section.

Removal of BPA through quinone adsorption on chitosan beads

Figure 4 shows the time course of the absorbance in the case where PPO was added to BPA solutions so as to reach the concentration of 100 U/cm^3 in the presence of different amounts of chitosan beads at pH 7.0 and 40°C. An increase in the absorbance at 385 nm was considerably depressed by dispersing the chitosan beads in the BPA solutions containing PPO. This means that quinone derivatives enzymatically generated were chemisorbed on the chitosan beads and the quinone concentration in the solution sharply decreased. At the amount of chitosan beads of 0.025 cm^3/cm^3, the portion of 11.4% of BPA was left unreacted in the solution at 3 h. In addition, the solution remained a little colored, because a small amount of quinone derivatives were present in the solution. When the amount of added chitosan beads was increased to 0.050 cm^3/cm^3, the conversion % value increased to 94.3%. When the reaction time was prolonged to 7 h at 0.025 and 0.050 cm^3/cm^3, enzymatically generated quinone derivatives disappeared by adsorption on chitosan beads, but the portion of 1.4 and 1.1% of BPA was left unreacted in the solutions, respectively.

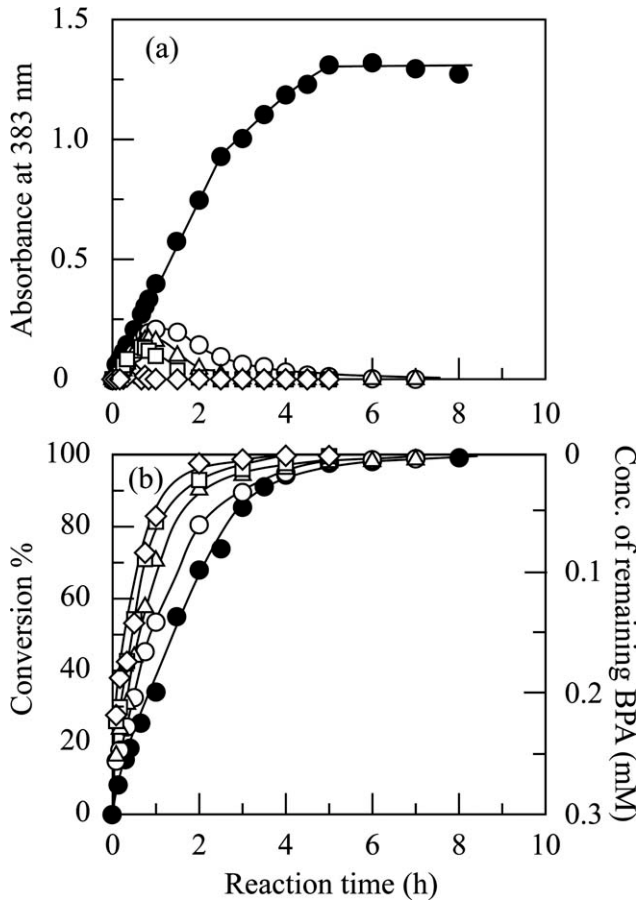

Figure 4 The effect of the amount of added chitosan beads on removal of BPA through PPO-catalyzed (100 U/cm^3) quinone oxidation and subsequent quinone adsorption on chitosan beads at pH 7.0 and 40°C. The amount of added chitosan beads (cm^3/cm^3): ●: without; ○: 0.025; △: 0.050; □: 0.10; ◇: 0.20.

TABLE I
Determination of Initial Velocity of PPO-Catalyzed Quinone Oxidation in the Absence and Presence of Chitosan Beads and Removal of BPA at pH 7.0 and 40°C

Enzyme dose (U/cm^3)	Initial velocity[a] (mM/min)	Amount of chitosan beads (cm^3/cm^3)	Initial velocity[b] (mM/min)	Removal time (h)	Removal (%)
100	1.36×10^{-3}	0.025	2.01×10^{-3}	7	98.7
		0.050	2.73×10^{-3}	9	99.4
		0.10	3.35×10^{-3}	5	100
		0.20	3.60×10^{-3}	2.5	100
75	7.80×10^{-3}	0.10	1.51×10^{-3}	7	100
		0.15	1.58×10^{-3}	5	100
50	3.38×10^{-4}	0.10	1.11×10^{-3}	5	68.5

[a] The values of initial velocity of PPO-catalyzed quinone oxidation in the absence of chitosan beads.
[b] The values of initial velocity of PPO-catalyzed quinone oxidation in the presence of chitosan beads.

At 0.10 cm^3/cm^3, the absorbance disappeared at 3 h and BPA was completely converted into quinone derivatives at 5 h. This result indicates that BPA was completely removed from an aqueous solution through the PPO-catalyzed quinone oxidation and subsequent nonenzymatic quinone adsorption on chitosan beads. When the amount of chitosan beads was further increased to 0.20 cm^3/cm^3, the removal time was shortened to 4 h. It was found from these results that BPA was completely removed through quinone adsorption on chitosan beads, and an increase in the amount of chitosan beads added to the BPA solutions containing PPO led to a decrease in the removal time. A decrease in the removal time was also observed for the removal of alkylphenols, chlorophenols, and BPA by the combined use of mushroom tyrosinase and chitosan beads.[15,19]

As a control experiment, only chitosan beads (0.10 cm^3/cm^3) were dispersed in a BPA solution without PPO at pH 7.0 and 40°C. A small amount of BPA was physically adsorbed on chitosan beads. Even when the reaction time was prolonged to 5 h, the adsorption % value was limited to be only 15%. It was verified from this result that the combined use of the PPO-catalyzed quinone oxidation of BPA and subsequent nonenzymatic quinone adsorption on chitosan beads is a good procedure to remove BPA from aqueous solutions. In other words, a decrease in the quinone concentration in the solutions through quinone adsorption on chitosan beads enhanced PPO-catalyzed quinone oxidation of BPA. This suggests that quinone adsorption on chitosan beads suppresses the unfavorable quinone-related inactivation of PPO such as contact between quinone derivatives and PPO molecules or their active sites.

Enhancement of PPO-catalyzed quinone oxidation by addition of chitosan beads

The effect of the addition of chitosan beads on the PPO-catalyzed quinone oxidation was estimated from the initial velocity, because it was found that quinone oxidation became faster with an increase in the amount of added chitosan beads from Figure 4. The initial velocity of PPO-catalyzed quinone oxidation was calculated from the slope of the BPA concentration in solutions determined by the HPLC measurements against the reaction time in the early stage in the presence of chitosan beads. The initial velocities of quinone oxidation of BPA in the presence of PPO are summarized in Table I with the removal of % values. The initial velocity of quinone oxidation was calculated to be 1.36×10^{-3} mM/min at pH 7.0 and 40°C in the absence of chitosan beads from Figure 1. The initial velocity increased with an increase in the amount of added chitosan beads and reached 3.35×10^{-3} mM/min at 0.10 cm^3/cm^3 at which BPA was completely removed. This value was 2.47 times higher than the initial velocity in the absence of chitosan beads. This indicates that the decrease in quinone concentration in the solutions would depress potential unfavorable interactions between quinone derivatives and PPO.

Effects of enzyme dose on BPA removal

As shown in Figure 4 and Table I, when chitosan beads were added to a BPA solution containing PPO at 100 U/cm^3, BPA was completely removed and the initial velocity of enzymatic quinone oxidation was enhanced. Therefore, it can be expected that complete removal of BPA is attained at lower PPO concentrations. The effect of the enzyme concentration on the removal of BPA through quinone adsorption on chitosan beads was investigated at pH 7.0 and 40°C. As shown in Table I, the absorbance at 385 nm quite disappeared by stirring for 4 h at 75 U/cm^3. However, the portion of 8.6% of BPA was left unreacted. When the reaction time was prolonged to 7 h, remaining BPA was converted into quinone derivatives and BPA was completely removed. The initial velocity of quinone oxidation at 75 U/cm^3 was lower than that at 100 U/cm^3. When the amount of added chitosan

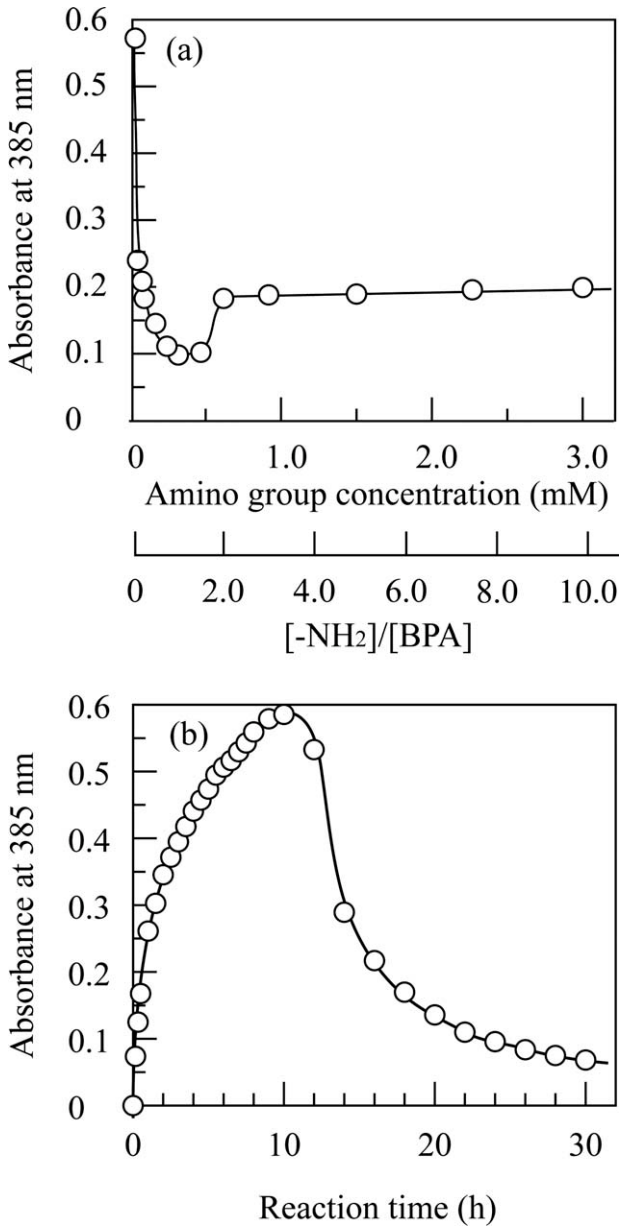

Figure 5 Removal of BPA through PPO-catalyzed quinone oxidation (100 U/cm^3) and subsequent homogeneous quinone reaction with chitosan at pH 6.0 and 40°C. (a) The effect of amino group concentration on the absorbance at 385 nm. (b) The time course of the absorbance at [—NH_2]/[BPA] = 1.0 where the minimum absorbance was obtained.

beads was increased to 0.15 cm^3/cm^3 at 75 U/cm^3, the initial velocity was a little increased, and the removal time was shortened to 5 h. On the other hand, the conversion % value was limited to be 68.5% at 5 h, although the conversion % value increased over the reaction time at 50 U/cm^3. It was found from the above results that the enzyme dose was decreased to 75 U/cm^3 to completely remove BPA. Consequently, the PPO dose for the complete removal of BPA was half as much as or less than half the mushroom tyrosinase dose.

Removal of BPA with chitosan solutions in homogeneous system

Removal of BPA was estimated in the homogeneous system with chitosan in the form of solution. Chitosan solutions were mixed with BPA solutions at pH 6.0 containing PPO at 100 U/cm^3 so as to reach the amino group concentrations from 0.03 to 3.0 m*M*, and then the solutions were mildly stirred at 40°C for 24 h. Here, the pH value was adjusted to 6.0, because chitosan was insoluble in a pH 7.0 buffer. The BPA solution containing PPO alone was gradually color-developed through enzymatic quinone generation. Even when the solution was stirred for 24 h, no water-insoluble quinone oligomers were generated, and the solution remained highly colored. On the other hand, for the BPA solutions containing both PPO and chitosan, the solutions were highly decolorized, and water-insoluble aggregates were formed through the reaction of quinone derivatives with chitosan's amino groups at higher than 0.03 m*M*.[23] Then, the aggregates generated were filtered out with a 5C filter paper, and the UV–vis spectra of the filtrates were measured.

Figure 5(a) shows the change in the absorbance at 385 nm with the chitosan's amino group concentration. The absorbance sharply decreased with an increase in the amino group concentration, indicating that the concentration of remaining quinone derivatives in solutions decreased. The solution was fairly decolorized at the amino group concentration of 0.3 m*M* or the concentration ratio of amino groups to BPA of 1.0 ([—NH_2]/[BPA] = 1.0). However, when the amino group concentration further increased from 0.3 m*M*, the absorbance gradually increased and the generation of aggregates decreased. Aggregates generated are settled down in the solutions. However, at higher amino group concentrations, some chitosan chains with unreacted free amino groups remained soluble in the solutions.[19]

Figure 5(b) shows the time course of the absorbance at [—NH_2]/[BPA] = 1.0, where the minimum absorbance was observed in Figure 5(a). The absorbance gradually decreased after going through the maximum value. No aggregates were formed in the solution, and the absorbance at 5 h was 0.474. This indicates that quinone derivatives enzymatically generated were present in the solutions together with unreacted BPA. It was found from Figure 5(a,b) that stirring for 20–24 h was required to generate water-insoluble aggregates. However, the absorbance of 0.096 was obtained for the filtrate after removing the aggregates, indicating that a small amount of quinone derivatives was left in the solutions. The generation of water-insoluble aggregates was much later than we expected for the reaction in the homogeneous system with chitosan and PPO solutions. In contrast, BPA was completely removed from

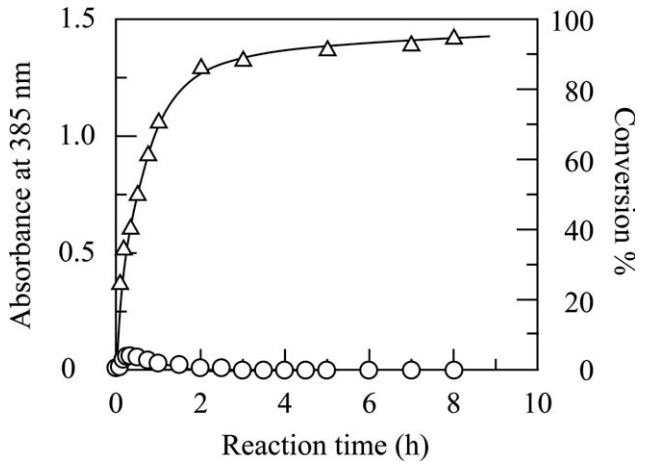

Figure 6 The time course of the absorbance at 385 nm (○) and conversion % value (△) for the removal of BPA through PPO-catalyzed (100 U/cm^3) quinone oxidation and subsequent quinone adsorption on chitosan powder at pH 7.0 and 40°C.

aqueous solutions at 4–7 h depending on the PPO concentration and amount of added chitosan beads for this procedure as shown in Table I. The comparison between both experimental results indicates that the procedure in the heterogeneous system with chitosan beads is very effective in removing BPA through quinone oxidation.

Removal of BPA with chitosan powder

As another control experiment, 0.30 g of chitosan powder (particle size: 75–100 μm), which corresponded to the quantity of 4.0 cm^3 of chitosan beads equilibrated in a pH 7.0 buffer, was dispersed in a BPA solution containing PPO in place of chitosan beads. Figure 6 shows the time course of the absorbance at 385 nm and conversion % value for PPO-catalyzed quinone oxidation and subsequent quinone adsorption on chitosan powder at pH 7.0 and 40°C. The conversion % value increased over the reaction time. The absorbance gradually decreased after passing through the maximum value at 10 min. The conversion % value and absorbance reached 91.3% and 0.02, respectively, at 5 h when BPA was completely removed at the amount of added chitosan beads of 0.10 cm^3/cm^3. This means that although most of the quinone derivatives enzymatically generated was removed, a small amount of BPA remained in the solution. On the supposition that the chitosan powder used is a perfect sphere with the diameter of 100 μm and the density of chitosan is 1.0 g/cm^3, the surface area of 0.30 g of the chitosan powder is calculated to be 180 cm^2, which corresponds to the specific surface area of 0.060 m^2/g. This value is much lower than that of the chitosan beads used in this study. Therefore, the porosity of the chitosan beads used, or the large specific surface area, also plays an important role in enhancing the removal efficiency.

CONCLUSIONS

In this study, the availability of chitosan was investigated for the removal of BPA through the PPO-catalyzed quinone oxidation and subsequent nonenzymatic quinone oxidation on chitosan beads. The optimum conditions for PPO-catalyzed quinone oxidation of BPA at 0.3 m*M* were determined to be pH 7.0 and 40°C. When chitosan beads were dispersed in BPA solutions containing PPO, quinone derivatives enzymatically generated from BPA were adsorbed on chitosan beads. The removal time was shortened, as the amount of added chitosan beads or the PPO concentration was increased. A decrease in the quinone concentration in the reaction solution through quinone adsorption on chitosan beads depress a decrease in the activity of PPO, resulting in an increase in the initial velocity of PPO-catalyzed quinone oxidation. The removal in the heterogeneous system with chitosan beads was much effective and faster compared to the homogeneous system with chitosan solutions, in which quinone derivatives enzymatically generated were a little left in the solutions at the optimum amino group concentration of 0.3 m*M* or [—NH_2]/[BPA] = 1.0 after stirring for 24 h. When chitosan powder was used, quinone adsorption occurred, but the removal efficiency was a little lower than that for the heterogeneous system with chitosan beads and unreacted BPA was left in the solution. The results mentioned earlier indicate that BPA was completely removed by the procedure constructed in this study.

It should be noted that neither H_2O_2 nor any additives were required for the removal of BPA by the procedure constructed in this study, and this procedure is comparable to or better than conventional procedures, which would be operationally complex and require capital investments. In addition, a variety of bisphenol derivatives have come into wide use for synthesis of specialized epoxy and polycarbonate resins with more high-performance compared to ordinary ones. These bisphenol derivatives have two phenol groups in common, but the chemical structure between phenol groups is different. Some of them are reported to exhibit endocrine-disrupting effects. Therefore, our next purpose is to apply this procedure to remove these bisphenol derivatives.

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