ELSEVIER

Chemosphere xxx (2004) xxx–xxx

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Leaching of bisphenol A (BPA) from polycarbonate plastic to water containing amino acids and its degradation by radical oxygen species

Junko Sajiki [a,*], Jun Yonekubo [b]

[a] *The Public Health Laboratory of Chiba Prefecture, 666-2 Nitona-cho, Chuo-ku, Chiba City, Chiba 260-8715, Japan*
[b] *Nihon Waters K.K., Katokichi Shin-Osaka Bldg., 5-14-10 Nishi-nakajima, Yodogawa-ku, Osaka 532-0011, Japan*

Received 16 April 2003; received in revised form 5 September 2003; accepted 4 December 2003

Abstract

In this study, (1) the change in the concentration of bisphenol A (BPA) leached from polycarbonate plastic (PCP) tube to water samples containing phosphate, sodium barbital, glycine, methionine or albumin at 37 °C as a function of time, and (2) the degradation rate of BPA leached from PCP tube to amino acid solutions in the presence of radical oxygen species (ROS) were investigated. The BPA leaching velocity (BPA-LV) from PCP tube to 50 mM glycine at pH 6 or 7 was twice that to control water, and the leaching was enhanced above pH 8. At pH 11, BPA-LV was significantly higher in 50 mM glycine and methionine solutions than in 50 mM NaOH. These results indicate that basic pH and amino acids contained in water could accelerate BPA leaching. The BPA-LV in phosphate buffer was different from the BPA-LVs in other buffers (barbital and glycine) at the same pH. BPA leached to the glycine or methionine solutions at pH 11 was degraded time dependently in a similar manner as the control water in the presence of ROS. The degradation of leached BPA was inhibited in the glycine solution, but was accelerated in the methionine solution. However, degradation of BPA added to freshly prepared methionine was inhibited in a similar manner to BPA in glycine. BPA degradation could be influenced by some kinds of amino acids, but glycine and methionine might be involved in BPA degradation in different ways.

Keywords: Bisphenol A (BPA) leaching; Polycarbonate plastic; BPA degradation; Amino acid; BPA–*o*-quinone

1. Introduction

Bisphenol A (BPA) is widely used as the monomer for the production of polycarbonate plastic (PCP) which is used in products such as baby bottles, and as a major component of epoxy resin, which is used for lining food cans and in dental sealants (Staples et al., 1998). To date, many reports on BPA detected in water (Gonzalez-Casado et al., 1998; Staples et al., 1998), baby food bottles (Mountfort et al., 1997), plastic waste (Yamamoto and Yasuhara, 1998), and living organisms including humans (Olea et al., 1996; Miyakoda et al., 1999) have been published. In particular, the release of resin components such as BPA and BPA dimethacrylate into the oral environment from dental composites, and the safety of these compounds in humans, have been subjects of controversy and concern in dentistry (Soderholm and Mariotti, 1999).

We observed a significant volume of BPA leaching to sheep plasma (Sajiki et al., 1999) and seawater (Sajiki and Yonekubo, 2003) from PCP at body temperature (37 °C), and the higher the concentration of phosphate and the pH, the greater the amount of leached BPA. These phenomena indicate that phosphate contained in

* Corresponding author. Fax: +81-43-265-5544.
E-mail address: j.sjk@ma.pref.chiba.jp (J. Sajiki).

0045-6535/$ - see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/j.chemosphere.2003.11.065

those samples could influence the leaching of BPA from PCP. However, the concentration of BPA leached to water containing a phosphate concentration similar to that of plasma was much lower than that leached to plasma or seawater. Therefore, other substances contained in those samples might be influencing BPA leaching from PCP. We were interested in whether amino acids and proteins, important nutrients in both plasma and seawater, are the factors that accelerate BPA leaching.

On the other hand, BPA is easily degraded in the presence of reactive oxygen species (ROS) (Sajiki, 2001; Sajiki and Yonekubo, 2002). Methionine, a potent scavenger of hypochlorous acid (HClO), protected BPA in NaCl solution from degradation by ROS (Sajiki and Yonekubo, 2002). However, it has not been clarified whether methionine could protect BPA from degradation by ROS other than HClO.

In this study, in order to investigate how BPA is leached to plasma or seawater, BPA concentrations leached from PCP to water with specified amino acids and proteins added at various pH values were determined. Furthermore, the effect of amino acids on the degradation of BPA in the presence of ROS was elucidated.

2. Materials and methods

2.1. Materials

PCP centrifuge tubes (110 mm×16 mm I.D.) were purchased from Nalgenunc International (Tokyo, Japan). BPA (>95%) and other chemicals (albumin from human serum, glycine, methionine, phosphate and barbiturate) of special grade were purchased from Wako Pure Chemical Ind. Ltd. (Tokyo, Japan). HPLC-grade acetonitrile and distilled water were purchased from Kanto Chemical (Tokyo, Japan). Throughout the experiment, BPA-free water was prepared using ODS-silica Sep-pak cartridges (Waters, MA, USA), after deionization of tap water using a Milli-RX12 α water purification system, which uses a combination of reverse osmosis and electrodeionization (Nihon Millipore, Tokyo, Japan).

To avoid contamination of BPA, glass tubes were rinsed with 99% ethanol (EtOH) before use.

2.2. Preparation of samples for leaching of BPA from PCP tubes

Solutions containing 50 mM glycine or 50 mM methionine were adjusted by addition of 1 N-HCl or 1 N-NaOH to various pH values (pH 3–11 for glycine, pH 11 for methionine). Fifty millimolar phosphate buffers (pH 6.5–8.2) and 50 mM barbital buffers (pH 7–9.9) were prepared by mixing 50 mM K_2HPO_4 with 50 mM NaH_2PO_4, and by adjusting 50 mM sodium barbital with N-HCl, respectively. Ten millilitres of each water sample was put into individual PCP tubes and allowed to stand without stirring at 37 for a specified period. The samples were mixed by shaking the tubes at every sampling time. Every week, 0.1 ml of sample was taken from each tube for BPA assay.

2.3. In vitro degradation by ROS of BPA leached into amino acid solution from PCP tubes

Superoxide anions (O_2^-) and hydroxyl radicals (HO·) were produced by the reaction of H_2O_2 with $FeCl_3 \cdot 6H_2O$ (Fenton-like reaction). BPA degradation was carried out by adding 100 μl of 0.28 M H_2O_2 and 100 μl of 1.11 mM $FeCl_3 \cdot 6H_2O$ to 50 μl of a 50 mM amino acid leaching solution (pH 11) that had leached BPA at 37 °C for about 100 days. The BPA concentration was adjusted to 5 μg/ml. A model experiment using authentic BPA was also carried out. Fifty microlitres of either a 7.5 mM glycine solution or a 5 mM methionine solution with 5 μg/ml BPA was added to 100 μl of 0.28 M H_2O_2 and 1.11 mM $FeCl_3 \cdot 6H_2O$. The mixtures were allowed to react at 37 °C.

2.4. Extraction and determination of BPA and BPA–o-quinone

BPA was purified according to the method of Sajiki et al. (1999). Oasis HLB extraction cartridges (Waters, MA, USA) used for the solid phase extraction of BPA were pre-washed with 3.5 ml EtOH and 3.5 ml water. Before use, an Oasis HLB extraction cartridge was eluted with ethyl acetate, which was then evaporated and checked for contamination. The samples were applied to Oasis HLB extraction cartridges, and polar lipids were removed from the column with 3.5 ml 15% EtOH. Next, 3.5 ml petroleum ether was used to remove nonpolar lipids, after washing with 3.5 ml water. Finally, BPA was eluted with 3.5 ml ethyl acetate. The solvent was evaporated under N_2. The residue was dissolved in 1 ml acetonitrile–water (40:60) solution. BPA analysis was performed by using an HPLC (Model LC-10 AD, Shimadzu, Kyoto, Japan) equipped with a Shim-Pack VP-ODS column (150 mm×4.6 mm I.D., Shimadzu, Kyoto, Japan), and an electrochemical detector (ECD, Coulochem II 5200A, ESA, MA, USA). The mobile phase was composed of acetonitrile (40%) and water (60%) and the pH was adjusted to 3 by using phosphate buffer. The flow-rate and column temperature were 1.0 ml/min and 40, respectively, and the injection volume was 50 μl. ECD conditions were as follows: guard cell potential, E 600 mV; analytical cell potentials, E1 300 mV and E2 550 mV; sensitivity, 1 μA. BPA identification was carried out by comparing the HPLC

retention times of BPA peaks to those of the authentic standards. For complete identification, BPA peaks eluted by HPLC were confirmed by using GC-MS (QP-5000, Shimadzu, Kyoto, Japan) after silylation with bis(trimethylsilyl)-trifluoroacetamide and co-chromatography using authentic standards. Blank test runs using water and solvents were carried out in each analysis, to make sure there was no contamination.

For BPA–*o*-quinone determination, an HPLC (Alliance 2690 model, Waters, MA, USA) equipped with a Symmetry C18 column (3.5 μm, 150 mm × 2.1 mm I.D.) and a Waters 996 Photodiode array UV/VIS detector and a ZMD Z-spray mass spectrometer with an ESI interface system (Waters, MA, USA) was used. An acetonitrile-water (40:60) solvent was used. Flow-rate and column temperature were 0.25 ml/min and 40 °C, respectively. Injection volume was 10 μl. The analytical conditions, in the negative ion scanning modes of ESI, were as follows: capillary voltage, 3.1 kV; cone voltage, 33 V; source block temperature, 90 °C; desolvation temperature, 175 °C. The concentration of BPA–*o*-quinone was expressed as peak area monitored at ($m/z = 241$), a pseudomolecular ion (M-H)- of BPA–*o*-quinone.

2.5. Statistical analysis

All values reported are the means of duplicates or triplicates. Data were analyzed according to one-way analyses of variance, and differences between treatments were tested at the 1% level of least significant difference.

3. Results

3.1. Leaching of BPA from PCP tube

As the amount of BPA leached from PCP tubes in all samples tested in this study showed a linear dependence on time over 10 weeks, BPA leaching velocity (BPA-LV), the amount of BPA leached per day from a PCP tube, was obtained from the slope of the plot of BPA concentration in the leaching solution vs. sampling time. BPA-LV from PCP tubes to 50 mg/ml albumin (purified from human serum at 95% purity) at pH 7.2 was 3 ng/ml/day, which is six times higher than that to water with the same pH (0.5 ng/ml/day). The change in the BPA-LV as a function of pH in the 50 mM glycine buffer is illustrated in Fig. 1, and the corresponding changes in the 50 mM phosphate buffer and the 50 mM barbital buffer are shown in Fig. 2. In the 50 mM glycine buffers, BPA-LV at pH 6 and 7 corresponded to twice that of water, although there was no difference in the BPA-LV between water and glycine buffers below pH 4. Above pH 7, the BPA-LV was high and the value increased greatly at pH 11 (311.6 ng/ml/day). In the 50 mM barbital buffers, the BPA-LVs at pH 7, 8, 9 and 9.9 were 2.9, 3.6, 19.9 and 79.6 ng/ml/day, respectively (Fig. 2). In the phosphate buffers, the BPA-LVs at pH 6.5, 7, 7.5, 8 and 8.2 were 0.7, 2.1, 3.0, 16.9 and 52.4 ng/ml/day, respectively (Fig. 2). The pH values that corresponded to 50 ng/ml/day BPA-LVs in 50 mM glycine, phosphate and barbital buffers were 9.8, 8.2 and 9.5, respectively. When PCP tube was filled with both 5 ml 50 mM glycine buffer and 5 ml 50 mM phosphate buffer at pH 7, the BPA-LV (1.41 ng/ml/day) was similar to the sum of the BPA-LVs (1.55 ng/ml/day) from PCP tubes with 25 mM glycine buffer (0.63 ng/ml/day) and 25 mM phosphate buffer (0.92 ng/ml/day) at pH 7.

Changes in the BPA concentration leached from PCP tubes to 50 mM NaOH, 50 mM glycine and 50 mM methionine solutions at pH 11 are shown in Fig. 3. BPA-LVs in the 50 mM NaOH, 50 mM glycine and 50 mM

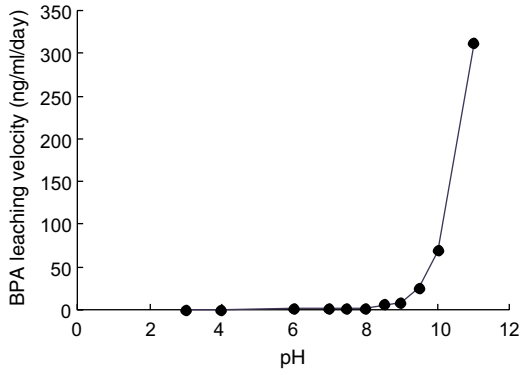

Fig. 1. Change in BPA leaching velocity (BPA-LV) from PCP tube to glycine solutions as a function of pH. Samples were allowed to stand at 37 °C for 10 weeks.

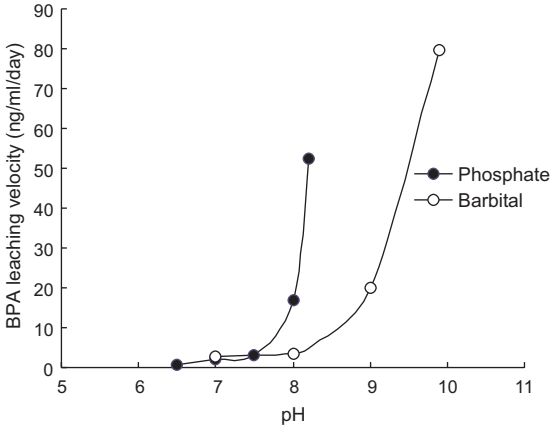

Fig. 2. Change in BPA leaching velocity (BPA-LV) from PCP tube to phosphate and barbital buffers as a function of pH. Samples were allowed to stand at 37 °C for 10 weeks.

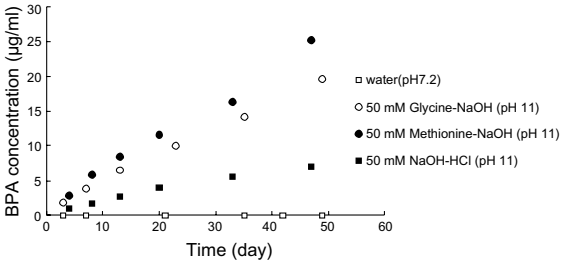

Fig. 3. Change in BPA concentration leached from PCP tube to various kinds of 50 mM amino acid solutions adjusted to pH 11, as a function of time. Samples were allowed to stand at 37 °C.

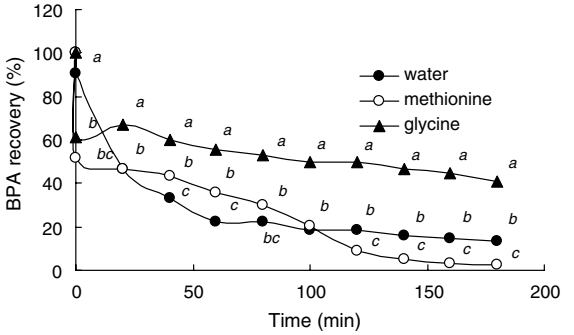

Fig. 4. Change in the concentration of BPA leached into 50 mM amino acid solutions at pH 11, as a function of time after reaction with ROS. Reaction was carried out at 37 °C. Amino acid solutions were adjusted to 5 μg/ml BPA with water. Water sample was prepared by addition of 5 μg/ml BPA. ROS was produced by mixing 0.1 M H_2O_2 with 0.44 mM $FeCl_3 \cdot 6H_2O$. BPA concentrations with different superscript letters among three samples in the same experimental time are significantly different ($p < 0.01$).

methionine solutions at pH 11 were 139.3 ± 8.2, 380.7 ± 20.4 and 490.2 ± 28.3 ng/ml, respectively.

3.2. Degradation of BPA leached from PCP tubes with amino acid solutions

Concentrations of BPA leached from PCP tubes over a period of around 100 days at 37 °C into 50 mM glycine and 50 mM methionine at pH 11 were 26.1 and 42.7 μg/ml, respectively. Sample BPA concentrations were diluted with water to around 5 μg/ml, and 5 μg/ml BPA in water was used as a control. The final BPA concentration in the reaction mixture was 1 μg/ml. Although concentrations of amino acids could not be determined in this study, the final concentrations of glycine and methionine in the reaction mixtures with ROS were estimated as 1.7 and 0.9 mM respectively, according to calculations based on their initial concentrations. The final pH values of the reaction mixtures with glycine, methionine and the control were 6, 4 and 3, respectively. The degradation curves of the BPA leached into these solutions as a function of time in the presence of ROS are shown in Fig. 4. BPA was rapidly degraded in the early stages (within 20 min) after ROS addition, in all samples. In particular, BPA in the two solutions containing glycine and methionine was degraded drastically, within 0.1 min of the start of the reaction. In the control water, BPA recovery decreased logarithmically until 60 min, and the level recovered at 60 min (22.6%) was maintained up to 180 min. In glycine solution, BPA recovery was maintained at 61.2% from 0.1 min until 60 min, and then gradually decreased to 40.9% at 180 min. In methionine solution, BPA recovery was maintained at 52% from 0.1 min until 40 min, and it then decreased linearly, to 2.3% at 180 min. BPA–*o*-quinone, a metabolite of BPA formed in Fenton reaction, was observed immediately (0.1 min) after the start of the reaction, and its concentration increased at 20 min, and then later decreased gradually until 180 min in control water, as reported in previous paper (Sajiki and Yonekubo, 2003). No BPA–*o*-quinone peak was detected in either glycine or methionine solution at any time during the experimental period. In the additional experiment, when 8 mM methionine was allowed to stand at room temperature for 100 days in a glass tube, followed by addition of 1 μg/ml BPA in the presence of ROS for 3 h, BPA recovery ($5.6 \pm 0.3\%$) was significantly lower than for the experiment without methionine ($21.7 \pm 1.9\%$). In contrast, when freshly prepared methionine at the same concentration was reacted with BPA, BPA recovery was $69.5 \pm 4.3\%$.

In a model experiment where freshly prepared amino acids at similar concentrations to those in the experiment mentioned above were added to 1 μg/ml authentic BPA in the presence of ROS, BPA recovery decreased as a function of time in a similar manner to the control (which had water added instead of amino acid), as shown in Fig. 5. Throughout the experimental period, BPA recovery in both 1.5 mM glycine and 1 mM methionine was higher than in the control water. Changes in BPA–*o*-quinone formation as a function of time in the BPA samples with added amino acids are shown in Fig. 6. In the two different freshly prepared amino acid solutions, BPA–*o*-quinone was newly formed, as in the control water. The formation of BPA–*o*-quinone in glycine solution was synchronous to that in control water. In the methionine sample, however, BPA–*o*-quinone formation was inhibited until 60 min after the start of the reaction with ROS, and then increased after 80 min, which resulted in the highest amount of BPA–*o*-quinone found among the three

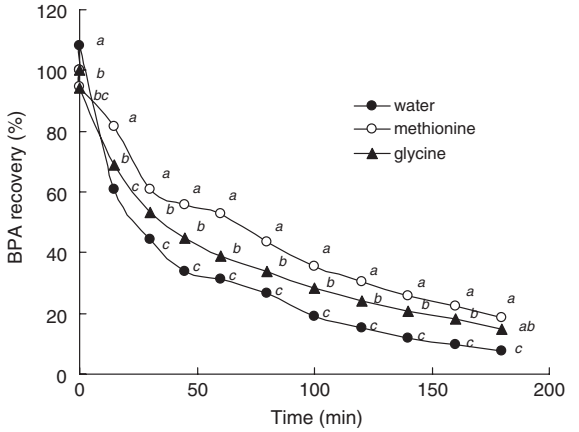

Fig. 5. Change in BPA recovery in freshly prepared glycine and methionine solutions with added BPA, as a function of time after reaction with ROS. Reaction was carried out at 37 °C. ROS was produced by mixing 0.1 M H_2O_2 with 0.44 mM $FeCl_3 \cdot 6H_2O$. Final concentrations reacted with ROS were 1.7 mM for glycine, 0.9 mM for methionine and 1.0 μg/ml for BPA. BPA recoveries with different superscript letters among three samples in the same experimental time are significantly different ($p < 0.01$).

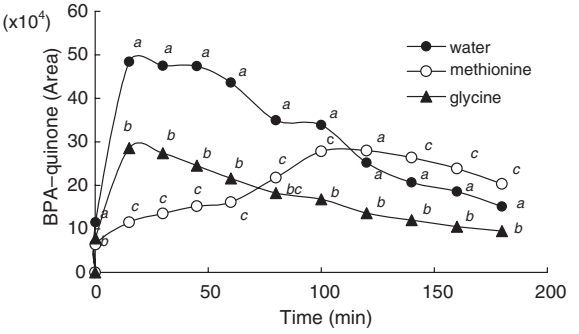

Fig. 6. Change in BPA–*o*-quinone concentration in freshly prepared amino acid solutions as a function of time after reaction with ROS. Reaction was carried out at 37 °C. ROS was produced by mixing 0.1 M H_2O_2 with 0.44 mM $FeCl_3 \cdot 6H_2O$. Final concentrations reacted with ROS were 1.7 mM for glycine, 0.9 mM for methionine and 1.0 μg/ml for BPA. BPA recoveries with different superscript letters among three samples in the same experimental time are significantly different ($p < 0.01$).

samples, from 120 to 180 min. In both the water and the glycine solution, the maximum BPA–*o*-quinone peak area was observed at 20 min. In contrast, the maximum BPA–*o*-quinone peak area was observed at 100 min in the methionine solution. The maximum peak area of BPA–*o*-quinone in glycine solution was around half that of the control water.

4. Discussion

We found that BPA was easily leached from PCP tube, a BPA polymer, with more being leached to seawater than to river water and control water. The velocity of BPA leaching from PCP tubes to seawater depends on the temperature. Why BPA was easily leached from PCP tubes into seawater has, however, not been clarified.

In our previous paper, the amount of BPA leached from PCP tubes to water increased with increases in pH and phosphate concentration (Sajiki and Yonekubo, 2003). In the present study, the amount of BPA leached from PCP tubes increased significantly above pH 8.0 in phosphate, barbital and glycine buffers, and pH values that showed 50 ng/ml/day of BPA-LV in phosphate buffer had different BPA-LV results in the two other buffer solutions, indicating that environmental pH and some elements dissolved in water could be important factors for BPA leaching into water. Considering that the pH of seawater (around 8) is higher than the pH of river or control water, phosphorous and amino acids in seawater are considered to be key elements that accelerate BPA leaching from PCP.

In our previous paper, we also reported that plasma facilitated BPA leaching from PCP tubes at 37 °C (body temperature), that abundant BPA was detected in commercial sera stored in plastic containers, and that the BPA-LV to plasma was 4 times higher than that to seawater (Sajiki and Yonekubo, 2003). Other researchers have also reported abundant BPA leaching to biosamples such as blood (Yamasaki et al., 2001) and saliva (Olea et al., 1996). In the present study, the BPA-LV in water containing amino acids at pH 7 was also faster than that for control water, and the BPA-LV from PCP to 50 mg/ml albumin at pH 7.2, the physiological concentration in plasma, was six times higher (3 ng/ml/day) than that to water possessing the same pH. These results indicate that amino acids and proteins could play an important role in the leaching of BPA from PCP tube to plasma.

The concentration of BPA leached from PCP tube in the mixed solution of 50 mM glycine buffer with the same volume of 50 mM phosphate buffer at pH 7 (giving a solution with final glycine and phosphate concentrations of 25 mM) was almost the same as the sum of BPA leached in 25 mM glycine buffer and 25 mM phosphate buffer at pH 7. These results suggest that the BPA-LV could be represented as a cumulative BPA concentration leached from PCP tube to various substances contained in plasma, and that it could be enhanced, depending on increases in the concentrations of such substances.

In our previous paper, we reported that the BPA-LV in 1 mM Na_2HPO_4 at pH 8 from PCP tube was approximately 0.7 ng/ml/day (Sajiki and Yonekubo, 2003). In the present study, BPA-LV in Na_2HPO_4 was lower at pH 7 than at pH 8. As the phosphorous

concentration in plasma was considered to be 1.15 mM at pH 7 (Bronner, 1964), it was presumed that BPA-LV from PCP owing to phosphorous would be below 0.7 ng/ml/day. The BPA-LV due to amino acids was also estimated to be below 1.0 ng/ml/day, which is the value of BPA-LV in 50 mM glycine buffer at pH 7, since the BPA-LV was dose dependent and the amino acid concentration in plasma was reported to be 4 mM (Harper et al., 1952). The BPA-LV due to albumin, amino acids and phosphorous totaled 4.7 ng/ml/day, which was much lower than the actual BPA-LV in plasma (46.2 ng/ml/day), suggesting that there are factors other than the three elements mentioned above that might be responsible for the enhancement of BPA leaching from PCP.

It is well known that BPA is more soluble at alkaline pH values (Boscolo Boscoletto et al., 1994). Some researchers reported that 100% bisphenol A dimethacrylate (Bis-DMA) was converted to BPA at a strongly alkaline pH (Schmalz et al., 1999; Kadoma and Tanaka, 2000), which indicates that the ester bond of Bis-DMA is easily hydrolyzed under alkaline conditions. BPA was leached rapidly from PCP tubes under alkaline conditions, especially pH 11, which also indicates that BPA polymer is easily dissociated to BPA under alkaline conditions. In contrast, the amount of BPA leached under acidic conditions (pH 2–4) was almost the same as for the control water in this study, suggesting that PCP tubes are stable under acidic conditions. It has been reported that Bis-DMA was also hydrolyzed to BPA in a time-dependent manner in strong acid (0.05 N HCl) at 37 °C (Kadoma and Tanaka, 2000). In the present study, even 0.05 N HCl did not accelerate BPA leaching from PCP tube at 37 °C, which suggests that the chemical properties of BPA polymer are different from those of BPA-related monomers.

At pH 11, the BPA-LV from PCP tube in water containing amino acids was significantly faster than that in water without amino acids, which suggests that amino acids could promote BPA leaching from PCP tube under alkaline conditions. Considering that the pK_a values of the amino acids glycine and methionine are 9.78 and 9.21 respectively, anion type as well as pH might be associated with the BPA leaching in solution.

BPA is considered to be a degradable compound in the environment. We observed in vitro that BPA was degraded rapidly in the presence of ROS at 20 °C (Sajiki, 2001). Amino acids depressed the degradation of fortified BPA in freshly prepared solutions of both 1 mM methionine and 1.5 mM glycine. As it is well known that amino acids are easily oxidized by ROS (Griffiths, 2000), amino acids could act as antioxidants, thus preventing oxidative BPA degradation. The degradation rate of BPA leached over 100 days from PCP in the sample containing methionine at pH 11 was significantly faster than that for BPA leached from control water in the presence of ROS, though the degradation rate of BPA added to freshly prepared methionine solution was lower than that in control water. These results indicate that the mechanism of BPA degradation in methionine-containing samples that have stood for a long time is different from that in samples containing freshly prepared methionine. Methionine is considered to be one of the important thiol-containing amino acids, and is easily oxidized by ROS to form methionine sulfoxide (Schoneich et al., 1993), resulting in protein oxidation (Hensley et al., 1995), lipid oxidation (Garner et al., 1998) and DNA and RNA oxidation (Pagano et al., 1997) in various biological systems. Using LC-MS, we observed dose-dependent formation of methionine sulfoxide and methionine sulfone in freshly prepared methionine solution reacted with Fenton reagents (data not shown). These newly formed radicals in samples allowed to stand for 100 days at 37 °C might cause further degradation of BPA in methionine solutions. The fact that BPA recovery was significantly higher in freshly prepared methionine solution than in methionine solution that had stood at room temperature for 100 days and in control water in the presence of ROS indicates that methionine was altered when it was allowed to stand for a period of time, which supports the assumption mentioned above.

BPA is considered to be degraded to BPA–*o*-quinone by reaction with Fremy's salt (Atkinson and Roy, 1995) and Fenton reagents (Sajiki and Yonekubo, 2003) in aquatic samples. BPA–*o*-quinone formation differed between the glycine and methionine solutions during the course of BPA degradation in the presence of ROS. The formation of BPA–*o*-quinone in glycine solution was synchronized to that in the control water, where BPA–*o*-quinone formation increased at 20 min and then decreased gradually until 180 min. In contrast, after the reaction with ROS in methionine, the formation of BPA–*o*-quinone was inhibited until 60 min, and it then increased after 80 min, which resulted in the highest amount of BPA–*o*-quinone formed among the three samples, after 120–180 min. This finding for the methionine solution suggests the suppression of further metabolism of BPA–*o*-quinone. Methionine is considered to be one of the first biologically relevant products of oxidative attack (Griffiths, 2000) and is oxidized to sulfoxide, as mentioned above. Thus, the BPA oxidation mechanism in the Fenton reaction might be different in the presence of methionine than in the presence of glycine because there is a difference between the oxidative mechanisms of the two kinds of amino acids.

Glycine simply inhibits BPA oxidation, but methionine complicates BPA oxidation due to its methylthiol group.

In this study, although the concentration of BPA leached at pH 11 in both methionine and glycine decreased in a time-dependent manner, no BPA–*o*-quinone was detected in either sample throughout the reaction

period with ROS. It is considered that BPA–*o*-quinone is easy to decompose at alkaline pH. However, as the pH in the reaction mixtures with ROS is adjusted to neutral, decomposition of the metabolite which occurred under alkaline conditions might become negligible.

Further studies are needed to conclusively determine the fate of BPA metabolites in the presence of amino acids in aquatic samples.

5. Conclusion

BPA leached from PCP more easily in amino acid-containing solutions than in water. BPA-LV was faster at basic pH than at acidic and neutral pH. BPA degradation in the presence of ROS was inhibited by addition of freshly prepared methionine or glycine solution. However, BPA degradation by ROS in 100-day leaching solution with methionine was accelerated, while it was slowed down in the 100-day leaching solution with glycine. It is concluded that BPA leaching from PCP was influenced by pH and the presence of amino acids, and BPA degradation by ROS was influenced differently by the different kinds of amino acids.

References

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

Boscolo Boscoletto, A., Gottardi, F., Milan, L., Pannocchia, P., Tartari, V., Tavan, M., 1994. Electrochemical treatment of Bisphenol-A containing wastewaters. J. Appl. Electrochem. 24, 1052–1058.

Bronner, F., 1964. In: Comar, A.C.L., Bronner, F. (Eds.), Mineral Metabolism II. Academic Press, New York, p. 342.

Garner, B., Witting, P.K., Waldeck, A.R., Christison, J.K., Raferty, M., Stocker, R., 1998. Oxidation of high density lipoproteins. I. Formation of methionine sulfoxide in apolipoproteins AI and AII is an early event that accompanies lipid peroxidation and can be enhanced by alpha-tocopherol. J. Biol. Chem. 273, 6080–6087.

Gonzalez-Casado, A., Navas, N., del Olmo, M., Vilchez, J.L., 1998. Determination of bisphenol A in water by micro liquid–liquid extraction followed by silylation and gas chromatography–mass spectrometry analysis. J. Chromatogr. Sci. 36, 565–569.

Griffiths, H.R., 2000. Antioxidants and protein oxidation. Free Rad. Res. 33, S47–S58.

Harper, H.A., Hutchin, M.E., Kimmel, J.R., 1952. Concentrations of nineteen amino acids in plasma and urine of fasting normal males. Proc. Soc. Exp. Biol. Med. 80, 768–769.

Hensley, K., Hall, N., Subramaniam, R., Cole, P., Harris, M., Aksenov, M., Aksenova, M., Gabbita, S.P., Wu, J.F., Carney, J.M., Butterfield, D.A., 1995. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J. Neurochem. 65, 2146–2156.

Kadoma, Y., Tanaka, M., 2000. Kinetic evaluation of reactivity of bisphenol A derivatives as radical scavengers for methacrylate polymerization. Dent. Mater. J. 19, 139–152.

Miyakoda, H., Tabata, M., Onodera, S., Takeda, K., 1999. Passage of bisphenol A into the fetus of the pregnant rat. J. Health Sci. 45, 318–323.

Mountfort, K.A., Kelly, J., Jickells, S.M., Castle, L., 1997. Investigations into the potential degradation of polycarbonate baby bottles during sterilization with consequent release of bisphenol A. Food Addit. Contam. 14, 737–740.

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

Pagano, G., Bonassi, S., De Biase, A., Degan, P., Deeva, I.B., Doronin, Y.K., Iaccarino, M., Oral, R., Warnau, M., Korkina, L.G., 1997. L-methionine induces stage-dependent changes of differentiation and oxidative activity in sea urchin embryogenesis. Pharmacol. Toxicol. 81, 134–143.

Sajiki, J., 2001. Decomposition of bisphenol A (BPA) by radical oxygen. Environ. Int. 27, 315–320.

Sajiki, J., Takahashi, K., Yonekubo, J., 1999. Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. J. Chromatogr. B 736, 255–261.

Sajiki, J., Yonekubo, J., 2002. Degradation of bisphenol-A in the presence of reactive oxygen species and its acceleration by lipids and sodium chloride. Chemosphere 46, 345–354.

Sajiki, J., Yonekubo, J., 2003. Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species. Chemosphere 51, 55–62.

Schmalz, G., Preiss, A., Arenholt-Bindslev, D., 1999. Bisphenol A content of resin monomers and related degradation products. Clin. Oral Invest. 3, 114–119.

Schoneich, C., Zhao, F., Wilson, G.S., Borchardt, R.T., 1993. Iron-thiolate induced oxidation of methionine to methionine sulfoxide in small model peptides. Intramolecular catalysis by histidine. Biochim. Biophys. Acta 1158, 307–322.

Soderholm, K.J., Mariotti, A., 1999. BIS-GMA-based resins in dentistry: are they safe? J. Am. Dent. Assoc. 130, 201–209.

Staples, C.A., Dom, P.B., Klecka, G.M., O'Block, S.T., Harris, L.R., 1998. A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 36, 2149–2173.

Yamamoto, T., Yasuhara, A., 1998. Quantities of bisphenol A leached from plastic waste samples. Chemosphere 38, 2569–2576.

Yamasaki, H., Nagake, Y., Makino, H., 2001. Determination of bisphenol A in effluents of hemodialyzers. Nephron 88, 376–378.