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Covalent molecular imprinting of bisphenol A using its diesters followed by the reductive cleavage with $LiAlH_4$

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Abstract

Bisphenol A (BPA) recognition materials were synthesized by a covalent imprinting technique using BPA–dimethacrylate or BPA–diacrylate as the template monomer. Binding sites in the polymers consisted of two hydroxyl groups that are generated by reducing the ester bonds of the template monomer with lithium aluminum hydride. The polymers strongly adsorbed BPA and structurally related compounds, however, other endocrine disruptors were hardly adsorbed. The BPA–dimethacrylate-based polymer interacted with the samples more strongly than the BPA–diacrylate-based polymer.

Keywords: Molecular imprinting; Reductive cleavage; Bisphenol A

1. Introduction

Bisphenol A (BPA) is suspected as one of endocrine disruptors, and it is known that BPA is adsorbed on estrogen receptors [1,2]. The binding of BPA to the receptors is weak compared to 17β-estradiol binding (about 0.33% affinity of 17β-estradiol to estrogen receptor β). However, since BPA is known to leak from common epoxy resins and polycarbonates and act as an endocrine disruptor, people have been concerned about it. Therefore, materials that can recognize and adsorb BPA have been investigated from the environmental point of view.

Molecular imprinting has been known as a preparation technique for tailor-made molecular recognition materials [3–5]. The binding sites generated have suitable sizes and shapes complementary to a target molecule (template), resulting in the strong adsorption of the template. Moreover, molecularly imprinted polymer can easily be prepared and the resulting materials are generally chemical- and heat-resistant. Recently, BPA or BPA analogues-imprinted polymers have been reported by non-covalent imprinting techniques [6–9].

In this work, in order to develop the recognition materials that interact with BPA, we employed a covalent imprinting technique [10–16], in which more homogeneous binding sites would be yielded in comparison with non-covalently imprinted polymers [17]. In general, covalently imprinting techniques involve a step of template cleavage by hydrolysis, however, we employed reductive cleavage for it, which was first reported by Byström et al. [18]. The imprinting technique proposed here involves several steps described in Scheme 1. A polymerizable BPA diester (Fig. 1), a cross-linking agent and an initiator are mixed and polymerized. After BPA was removed from the polymer network by reduction using lithium aluminum hydride (LAH), two hydroxyl groups could be generated in the binding sites that re-bind BPA and related compounds by hydrogen bonding. Two BPA-imprinted polymers are prepared using two different template monomers, BPA–dimethacrylate and BPA–diacrylate, and the binding characteristics are investigated.

2. Experiments

2.1. Materials

Bisphenol A–dimethacrylate (BPA–dimethacrylate) was obtained from Aldrich Chemical Co. (Milwaukee, WI).

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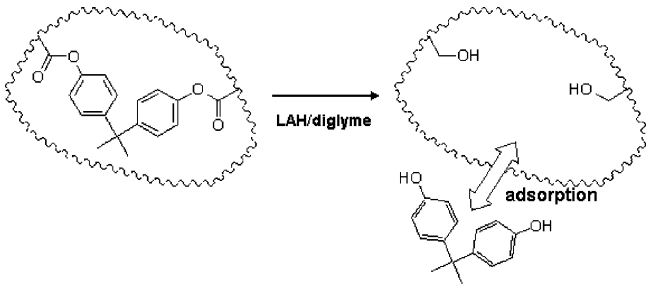

Scheme 1. Schematic illustration of the binding site generation in the proposed BPA-imprinted polymers.

Divinyl benzene (DVB), styrene and chloroform were obtained from Wako Pure Chemical Industry (Osaka, Japan). Diethylene glycol dimethyl ether (diglyme) was obtained from Tokyo Chemical Industry (Tokyo, Japan). DVB, styrene, chloroform and diglyme were purified by distillation prior to use. Other solvents were used without further purification.

2.2. Preparation of BPA–diacrylate

To a cooled solution (in ice-bath) containing BPA (8.00 g, 35 mmol) and triethylamine (8.57 g, 84 mmol) in dry THF (168 ml) was added dropwise a solution containing acryloyl chloride (6.86 g, 75.8 mmol) in dry THF (85.0 ml), and the resulting suspension was stirred for 24 h at room temperature. The obtained white precipitate was removed by filtration, and the filtrate was dried in vacuo to yield brown oil. This residue was dissolved in ethyl acetate, and the solution was washed with a saturated aqueous copper(II) sulfate solution, water and a saturated aqueous sodium chloride solution. After evaporation of the solvent, the residue was purified by a silica gel column (ethyl acetate:hexane, 1:3). Removal of the solvent in vacuo gave 1.00 g (8.5%) of white solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm) = 7.01–7.26 (m, aromatic, 8H), 6.62–5.96 (m, acryl, 6H), 1.67 (s, $-CH_3$, 6H).

2.3. Preparation of BPA–dimethacrylate-based polymer P(M)

BPA–dimethacrylate (0.608 g, 1.67 mmol), DVB (4.61 g, 35.4 mmol), styrene (1.23 g, 11.8 mmol) and 2,2′-azobis(isobutyronitrile) (0.334 g) were added to a glass tube, and were dissolved in dry chloroform (7.00 ml). After the mixture was sparged with nitrogen gas for 3 min, the glass tube was closed by a screw cap and placed under UV light (XX-15L, UVP, Upland, CA) for 18 h at 5 °C. The obtained polymer was dried in vacuo and taken out from the tube. The polymer was crushed roughly, washed with methanol and dried in vacuo again.

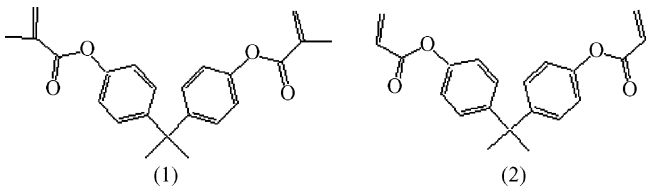

Fig. 1. Structures of the template monomers used: (1) bisphenol A–dimethacrylate and (2) bisphenol A–diacrylate.

2.4. Preparation of BPA–diacrylate-based polymer P(A)

BPA–diacrylate (0.422 g, 1.25 mmol), DVB (3.46 g, 26.6 mmol), styrene (0.922 g, 8.85 mmol) and 2,2′-azobis(isobutyronitrile) (0.250 g) were added to a glass tube and were dissolved in dry chloroform (5.25 ml). The polymerization procedure was the same as P(M).

2.5. Determination of BPA removed from P(M) and P(A) under various reduction conditions

P(M) (193 mg, containing BPA 0.05 mmol), LAH (380 mg, 10.0 mmol) and a dry solvent (THF or diglyme, each 10 ml) were placed in a round-bottom flask (50 ml). The suspension was stirred and allowed to react under nitrogen atmosphere. The reaction was carried out in THF (70 °C) or diglyme (120 °C) for 5–90 h. After cooling the suspension in ice bath, cold water was added slowly until the reaction was completely finished, and acidified with 6 M HCl then filtered. The polymer was washed with methanol (50 ml) in triplicate. All washings were collected and the amount of BPA contained was determined by a Waters HPLC system (Milford, MA, USA) equipped with a 626 pump, a 717 auto-sampler and a 996 photodiode array detector. A reversed phase column (SUPELCOSIL LC-8-DB, 5 μm, 150 mm × 4.6 mm i.d.) was used with a mobile phase of water/acetonitrile (60:40 (v/v), 1.0 ml min^{-1}). The sample volume was 10 μl and the effluent was monitored at 260 nm. P(A) (192 mg, containing BPA 0.05 mmol) was also treated and evaluated with the same procedure.

2.6. Preparation of BPA–dimethacrylate-based imprinted polymer (P(M)R) and BPA–diacrylate-based imprinted polymer (P(A)R) for chromatographic tests

P(M) (2.89 g, BPA containing 0.75 mmol) and LAH (5.70 g, 150 mmol) were added to dry diglyme (150 ml), and the suspension was stirred at 120 °C for 90 h under nitrogen atmosphere. After cooling the suspension in ice bath, cold water (200 ml) was added to this suspension very slowly until the reaction was completely finished. The polymer suspension was acidified with 6 M HCl, stirred for 1 h and filtered. The polymer was washed with 1 M HCl, water and methanol successively. After the polymer was dried in vacuo, the reduction was again carried out with LAH (5.70 g, 150 mmol) in diglyme (150 ml) at 120 °C for 20 h. The same treatment as the first reduction was followed. All washings were collected and the amount of BPA recovered was determined by the same HPLC as described in Section 2.5. The

polymer was ground, sieved (32–63 μm) in methanol, and packed in a stainless steel column (50 mm × 4.6 mm i.d.).

P(A)R was prepared as follows: P(A) (3.84 g, BPA containing 1.00 mmol) and LAH (7.60 g, 200 mmol) were added to dry diglyme (200 ml), the suspension was stirred at 120 °C for 48 h under nitrogen atmosphere. The following procedure was the same as that of P(M)R except for the re-reduction conditions (7.60 g (200 mmol) of LAH in 200 ml diglyme at 120 °C for 20 h).

2.7. IR spectra

IR spectra of the polymers were measured on FT–IR spectrometer Excalibur FTS3000MX (DIGILAB, Randolph, MA). The tablet was prepared from 1.5 mg of polymer particles and 80 mg of KBr and measured from 400 to 4000 cm^{-1}.

2.8. Chromatographic tests

Retention factors of BPA, structurally related compounds and other endocrine disruptors on the imprinted polymer-packed columns (P(M)R or P(A)R) were investigated with various eluents. The measurement was carried out by the same HPLC as described in Section 2.5. The concentrations of all samples were adjusted with the eluent to 1 mM and injected independently in triplicate.

A reference polymer for P(M)R was prepared with 2-hydroxyethyl methacrylate and BPA–dimethacrylate. BPA–dimethacrylate (0.109 g, 0.3 mmol, 18% of the original amount), HEMA (0.357 g, 2.74 mmol, 82% of the theoretical amount of hydroxyl group after the reduction), DVB (4.61 g, 35.4 mmol), styrene (1.23 g, 11.8 mmol) and 2,2′-azobis(isobutyronitrile) (0.334 g) were added to a glass tube and were dissolved in dry chloroform (7.0 ml). The polymerization procedure was the same as P(M). The polymer was ground, sieved (32–63 μm) in methanol, and packed in a stainless steel column (50 mm × 4.6 mm i.d.) for chromatographic tests.

3. Results and discussion

3.1. Conditions of the reductive cleavage of BPA from the polymers

We employed LAH reduction for the removal of BPA from the polymer networks of P(M) and P(A). At first, the reduction conditions were investigated with small amounts of P(M) and P(A). The solvents selected were THF or diglyme, and the reaction time and temperature were examined (Table 1). Higher recoveries of BPA from P(M) and P(A) were obtained in diglyme than in THF because of the higher reaction temperature. BPA was more easily removed from P(A) than P(M). It may be due to less steric hindrance of acrylate than methacrylate, and as a result, LAH could more easily reduce BPA–diacrylate in P(A) than BPA–dimethacrylate in P(M). After 90 h reduction, 72% of BPA was recovered from P(M), while 80% was recovered from P(A) in diglyme after 48 h reduction. In order to improve the recovery, the reduction was again carried out for another 20 h in each case. After the re-reduction process, the recovery from P(M) was increased to be 80%, however, only a few BPA was released from P(A) (81%).

Table 1
BPA recovery from P(M) and P(A) after the LAH reduction

Polymer	BPA recovery (%)			
	5 h	20 h	48 h	90 h
P(M) in THF	–	30	–	–
P(M) in diglyme	48	57	–	72 (77[a])
P(A) in THF	31	67	–	–
P(A) in diglyme	67	–	80 (81[a])	–

Reaction temperatures in THF and diglyme are 70 and 120 °C, respectively.
[a] This is the recovery obtained by re-reduction of the polymer after first reduction processing.

According to the above results using small amount of polymers, the reduction procedures to yield P(M)R and P(A)R for the chromatographic tests were determined as described in Section 2.6. More than 10 times increased amounts of P(M) and P(A) were used for the preparation of P(M)R and P(A)R and the amount of BPA recovered was 82% in P(M), and 83% in P(A), respectively.

IR spectra of obtained imprinted polymers were measured (Fig. 2). P(M) and P(A) showed carbonyl peaks at 1748 and 1751 cm^{-1}, respectively. However, the carbonyl peaks of P(M)R and P(A)R were reduced and the broadening OH peaks were newly shown (3438 cm^{-1} of P(M)R and 3442 cm^{-1} of P(A)R). This may prove that ester bonds of the template monomers were cleaved by the reduction and hydroxyl groups were generated in the binding sites.

3.2. Chromatographic tests

BPA and toluene were injected into the P(M)R-packed column using various eluents, and retention factors of BPA were evaluated (Table 2). When chloroform was used as the eluent, BPA was strongly bound to the polymer ($k' = 60.8$).

Table 2
BPA adsorption properties of P(M)R using various eluents

Sample	Retention time (min) and k'			
	$CHCl_3$	$CHCl_3$/MeCN (9:1)	$CHCl_3$/MeOH (9:1)	AcOEt
Toluene	0.58	0.59	0.61	0.60
BPA	35.9	5.80	0.64	2.12
k'[a]	60.8	8.83	0.05	2.06

[a] Retention factors (k') were calculated by the equation $k' = (t_R - t_0)/t_0$, where t_R is the retention time of the sample and t_0 the retention time of toluene as a marker.

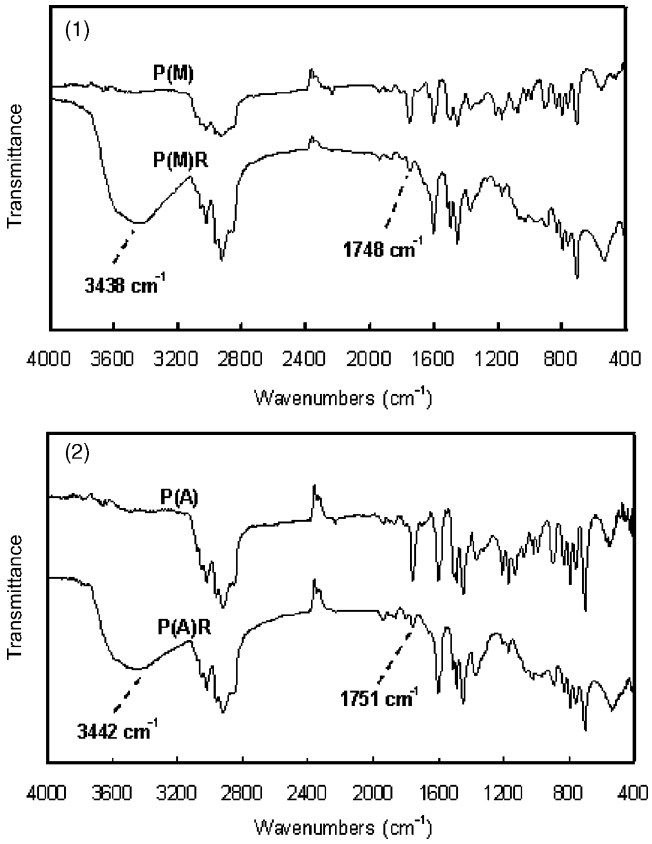

Fig. 2. IR spectra (KBr): (1) P(M) and P(M)R and (2) P(A) and P(A)R.

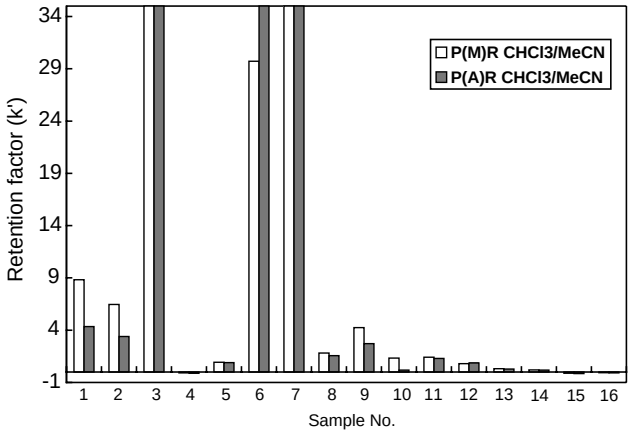

Fig. 4. Retention factors (k') of the samples on P(M)R and P(A)R. Acetonitrile or chloroform/acetonitrile (9:1 (v/v)) was used as the eluent. The k'-values were calculated using toluene as a marker.

However, the retention factors rapidly decreased when acetonitrile or methanol was added to the eluent. Polar solvents interfered with the BPA binding, meaning that the binding mechanism would be due to the formation of hydrogen bonding between BPA and hydroxyl groups in the binding sites.

Selectivity of the imprinted polymers for BPA and other related compounds (Fig. 3) was examined using chloroform/acetonitrile (9:1 (v/v)) as eluent (Fig. 4). P(M)R exhibited high affinity for BPA, **1**, and other compounds having two hydroxyl groups at 4,4′-positions, namely, bisphenol B

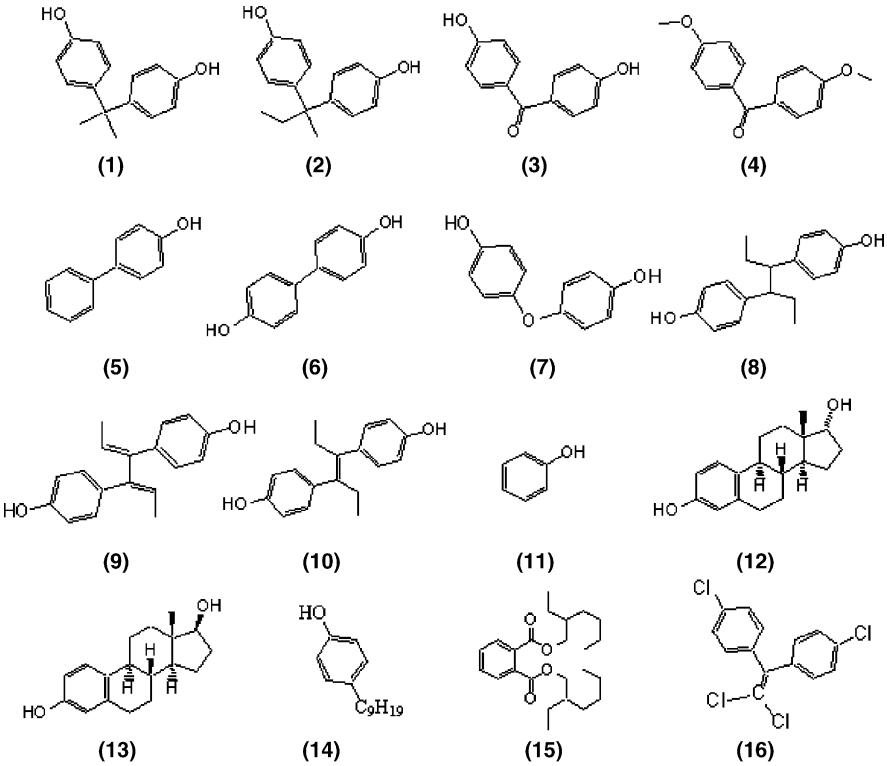

Fig. 3. Structures of the samples tested: **1**, BPA; **2**, bisphenol B; **3**, 4,4′-dihydroxybenzophenone; **4**, 4,4′-dimethoxybenzophenone; **5**, *p*-hydroxybiphenyl; **6**, 4,4′-dihydroxybiphenyl; **7,** 4,4′-dihydroxydiphenyl ether; **8**, hexestrol; **9**, dienestrol; **10**, diethylstilbestrol; **11**, phenol; **12**, 17α-estradiol; **13**, 17β-estradiol; **14**, 4-nonylphenol; **15**, bis(2-ethylhexyl)phthalate; and **16**, *p,p′*-DDE.

2, 4,4′-dihydroxybenzophenone **3**, 4,4′-dihydroxybiphenyl **6**, and 4,4′-dihydroxydiphenyl ether **7**. When **3** and **7** were injected, no clear peak was obtained during 90 min run. The two compounds seemed to be strongly adsorbed to P(M)R. The strong affinity could be explained that the two compounds have rigid structure and/or small size as compared with BPA, therefore, the hydroxyl groups of the two compounds would easily interact with the functional groups, which were arranged in suitable positions for BPA in the binding sites. The compounds bearing only one phenolic hydroxyl group such as *p*-hydroxybiphenyl **5** and phenol **11** showed low affinity to the polymer. Estradiols and other endocrine disruptors, namely, 17α-estradiol **12**, 17β-estradiol **13**, 4-nonylphenol **14** and bis(2-ethylhexyl)phthalate **15**, had different structures from the template and was hardly retained. In addition, 4,4′-dimethoxybenzophenone **4** and *p*,*p*′-DDE **16** did not interact with the polymer due to the lack of strong hydrogen bond formation. Hexestrol **8**, dienestrol **9** and diethylstilbestrol **10** are bigger and/or the structures are more flexible than BPA, thus they were slightly adsorbed on the polymer.

In order to verify the imprinting effects, we prepared a reference polymer using BPA–dimethacrylate (18% of the initial amount of BPA–dimethacrylate) and 2-hydroxyethyl methacrylate (82% of the theoretical amount of hydroxyl group after the reduction). The amounts used are adopted according to the results of the BPA recovery after the reductive cleavage of BPA moiety from the polymer. The polymer was used without the reduction treatment, therefore, the polymer may contain almost same amount of hydroxyl groups that were generated by the reduction treatment and BPA residues that were still uncleaved after the reduction step in P(M)R. All compounds showed k'-values of <0.1, suggesting that randomly located hydroxyl groups may not give high binding ability and selectivity. It is clearly confirmed that the imprinting process assembles the binding sites and develops the selectivity. Also low non-specific binding would be expected in P(M)R because of such low binding ability of the reference polymer.

Chromatographic tests for P(A)R were also carried out. P(A)R showed almost the same selectivity as P(M)R for tested samples (data not shown). Lower retention factors were observed for all samples in P(A)R compared to those in P(M)R. The reason may be that the binding sites in P(M)R would be more rigid due to the β-methyl group than those in P(A).

4. Conclusion

The BPA recognition materials were synthesized using the BPA–dimethacrylate and BPA–diacrylate as the template molecule. They adsorbed BPA and the structurally related compounds having rigid structures and a similar size to BPA. Covalently imprinted polymer prepared in this work may apply to adsorb and remove the compounds having estrogenic actions. P(M)R resembled P(A)R in the selectivity, and the affinity was higher than P(A)R, however, P(M)R took longer time to prepare. According to these characteristics, the two polymers can be suitably selected for the situations given.

In both, P(M)R and P(A)R, the template molecule BPA was not the best binder. Binding sites generated after the removal of BPA by reduction may not be suitable sizes enough to fit BPA. This lead us to an idea of new template molecule design such that the insertion of a sacrificial spacer between BPA and methacrylic acid moiety to generate optimal spacing of the hydroxyls for the binding of BPA.

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