DEGRADATION PRODUCTS OF BISPHENOL A IN WATER BY ULTRAVIOLET IRRADIATION AND THEIR AGONISTIC ACTIVITY FOR HUMAN ESTROGEN RECEPTOR

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Abstract

In this study, we investigated the degraded products of 2,2-bis(4-hydroxyphenyl)propane (BPA) by UV photolysis using a reversed-phase high-performance liquid chromatography (HPLC), and assessed estrogenic effects of BPA or degraded products for the human estrogen receptor α (hERα) using a yeast two-hybrid assay. Concentration of BPA in the sample after UV photolysis decreased by about 15% (362.1 mg/l) for 24 h when compared the control sample (422.5 mg/l). On the other hand, the estrogenic activity of BPA was enhanced by UV photolysis more than 40% compared with that of the control. To investigate the estrogenic activity of each fraction using a yeast two-hybrid assay, we attempted to separate and collect the degraded products of BPA by UV irradiation using a HPLC system. As a result, the estrogenic activities in the fraction of peak 1 and 2 were observed, indicating the fraction of peak 1 (retention time: 15.6 min) might be identified as 4-metyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), which is an active metabolite of BPA produced after incubation with the rat liver S9 fraction. The estrogenic activities of MBP (peak 1) and unknown compounds (peak 2) imply the enhancement of estrogenic activity observed by UV photolysis of BPA. Further studies are required to evaluate the mechanism of photolysis activation of BPA by UV irradiation, as well as the structural elucidation of the active photolysis products.

Introduction

Various endocrine-disrupting chemicals (EDCs) are known to interact with the development and function endocrine systems in wildlife and humans.[1, 2] Bisphenol A (BPA; 2,2-bis(4-hydroxyphenyl)propane), which is used mainly in the production of polycarbonate for manufacturing a wide variety of plastics and epoxy resins, is a possible xenoestrogen *in vitro* [3] and *in vivo*[4], and binds competitively to sex steroid-binding proteins in humans and rainbow trout.[5] Furthermore, Yoshihara et al. found that the estrogenic activity of 4-metyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), which is an active metabolite of BPA produced after incubation with the rat liver S9 fraction, much more than that of the parent BPA.[6, 7] A recent study demonstrated that certain BPA strongly binds to human estrogen-related receptor γ (ERRγ), an orphan receptor and one of 48 human nuclear receptors, but further research is required to clarify the effects of BPA on ERRγ signaling pathway.[8]

In the water treatment technique for removing the potential EDCs including BPA, chemical oxidation by ozone, UV photolysis, UV/H_2O_2, photo-Fenton process and TiO_2 photocatalysis are useful and advanced oxidation processes. However, survey on the chlorination of BPA has demonstrated that the produced products of chlorination exhibit greater estrogenic activity than the parent compound.[9] Thus, this finding indicates the occurrence of unintentionally generated compounds by water treatment processes. However, little information is available about the potential biological effects and the risks of these compounds on human health. Therefore, in this study, we investigated the degraded products of BPA by UV photolysis using a reversed-phase high-performance liquid chromatography (HPLC). In addition, the present study assessed the estrogenic effects of BPA or their degraded products for the human estrogen receptor α (hERα) using a yeast two-hybrid assay.

Materials and Methods

UV Irradiation procedure. The BPA solution (20 ppm, 50 ml) in milli Q water was poured into a 100-ml vial, and irradiated with an UV lamp (GL-15, Toshiba) with a 254 nm (51 μW/cm^2). After UV irradiation for 24 h, 10 ml of dichloromethane (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) were added. After mixing for 15 min, the dichloromethane was collected and this operation was repeated three-times. The collected dichloromethane solution was mixed with anhydrous sodium sulfate for dehydration. The solution was accurately measure two halves and the solution was dried under nitrogen at room temperature. The residue was dissolved in 1 ml dimethylsulfoxide (DMSO, Wako Pure Chemical Industries, Ltd., Tokyo, Japan) for

measurement of estrogenic activity and 1 ml acetonitrile (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) for HPLC analysis.

HPLC analysis. For an analytical HPLC, 20 µl of the sample solution prepared as above was applied to a PU-2080 HPLC system. (JASCO, Tokyo, Japan) equipped with a UV detector and an analytical reversed-phase column (Supelcosil ABZ+ Plus; 4.6 x 150 mm, 5 µm; Supelco, Bellefonte, PA). The separation of UV photolysis product was performed with a liner gradient of 0-70% acetonitrile in 0.06% acetic acid over 15 min, then held for 10 min at a flow rate of 2 ml/min; the chromatogram was monitored at 275 nm. The eluate was collected five times at 1-min intervals, and after dilution of each fraction with 90 ml water. The sample solution was passed through a Sep-Pak Vac C18 cartridge (Waters Associates Inc., Milford, MA) preconditioned with 10 ml acetonitrile and 10 ml water for solid-phase extraction. The cartridge was washed with 10 ml water and the remaining water was centrifuged at 3,000 rpm for 5 min. The adsorbed substances were eluted with 5 ml acetonitrile, and the eluate was dried under nitrogen at room temperature. The residue was dissolved in 200 µl DMSO for measurement of estrogenic activity.

Yeast two-hybrid assay. The assay for determining the estrogenic activity of test solutions was performed as previously described.[10] Yeast cells (*Saccharomyces cervisiae* Y190) were modified by incorporation of hERα, an expression plasmid of the coactivator TIF2, and a ß-galactosidase expression reporter in a yeast two-hybrid assay.[3] This assay system employs the interaction between the hERα ligand binding domain and TIF2.

Results and Discussion

To investigate the degradation products of BPA by UV irradiation, we measured the BPA concentrations in water before or after UV photolysis using a HPLC analysis. Concentration of BPA in the sample after UV photolysis decreased by about 15% (362.1 mg/l) for 24 h when compared the control sample (422.5 mg/l) (Table 1). On the other hand, the estrogenic activity of BPA in the yeast two-hybrid assay was enhanced by UV photolysis more than 40% compared with that of the control (Table 1, Fig. 1). These results indicate the production of potent estrogenic compounds from BPA by UV irradiation. Previous study revealed that the BPA concentration and estrogenic activity decreased by UV photolysis of BPA.[11] In contrast, Nomiyama et al. (2007) demonstrated that the estrogenic activity for medaka ERα was increased to 110% compared with the initial activity of BPA at 60 min of UV irradiation using a TiO_2 technique, and the increase in the estrogenic activity for medaka ERα might suggest the contribution of OH-BPA by the oxidation of BPA.[12] Our results suggest that the enhancement of estrogenicity of BPA may be due to the formation of active photolysis products by UV irradiation.

Table 1. Estrogenic activity for human estrogen receptor α (ERα) and concentration of BPA in the degradation products of BPA by UV irradiation.

	Estrogenic activity for human ERα		HPLC analysis
	EC_{x10} (mg/l)	E2 equivalent (µg/g)	BPA (mg/l)
Control	1.028	27.0	422.5
UV photolysis	0.714	38.8	362.1

The estrogenic activity of UV photolysis and control sample fo hERα was recorded as the EC_{x10} which was defined as the concentration of test solution producing a chemiluminescent signal 10x that of the blank control.

Figure 2a shows the HPLC chromatogram obtained by UV photolysis of BPA. Several unknown peaks, except for BPA, were found by UV photolysis of BPA. To investigate the estrogenic activity of each fraction using a yeast two-hybrid assay, we attempted to separate and collect the degraded products of BPA by UV irradiation using a HPLC system. As a result, the estrogenic activities in the fraction of peak 1 and 2 were observed (Fig. 2b). On the basis of retention time, the fraction of peak 1 (retention time: 15.6 min) might be identified as MBP.[7] Previous studies demonstrated that the estimated estrogenic activity of MBP was more than about 200-fold higher than that of BPA *in vitro*[7] and *in vivo*.[13] The estrogenic activities of MBP (peak 1) and unknown compounds (peak 2) imply the enhancement of estrogenic activity observed by UV photolysis of BPA (Fig. 1). These results suggest that the possibility that the impact on the environment of BPA increases by UV photolysis. Therefore, further studies on the mechanism of photolysis activation of BPA by UV irradiation, as

well as the structural elucidation of the active photolysis products, are in progress.

Survey on the hormonally action of BPA and their related compounds revealed that these compounds showed the anti-androgenic[14] and thyroid hormone activities.[15] Interestingly, a recent study demonstrated that certain BPA strongly binds to ERRγ.[8] Thus, further research is required to clarify the effects of degraded products of BPA by UV photolysis such as MBP and unknown compound (peak 2) on various nuclear receptors such as ERRγ, androgen and thyroid hormone receptors. Furthermore, we will investigate the occurrence and behavior of these degraded products of BPA by UV photolysis in the aquatic environment.

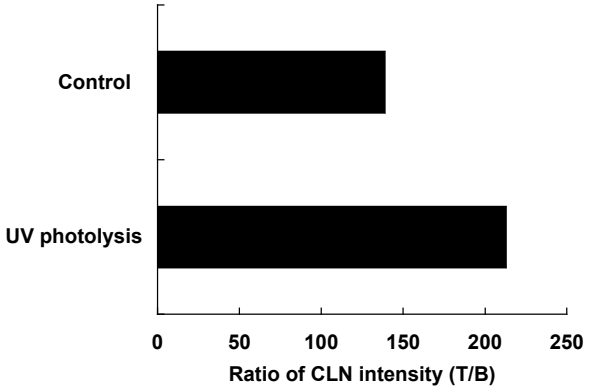

Fig. 1. Photolysis activation of estrogenic activity of BPA by UV irradiation for hERα using a yeast two-hybrid assay.

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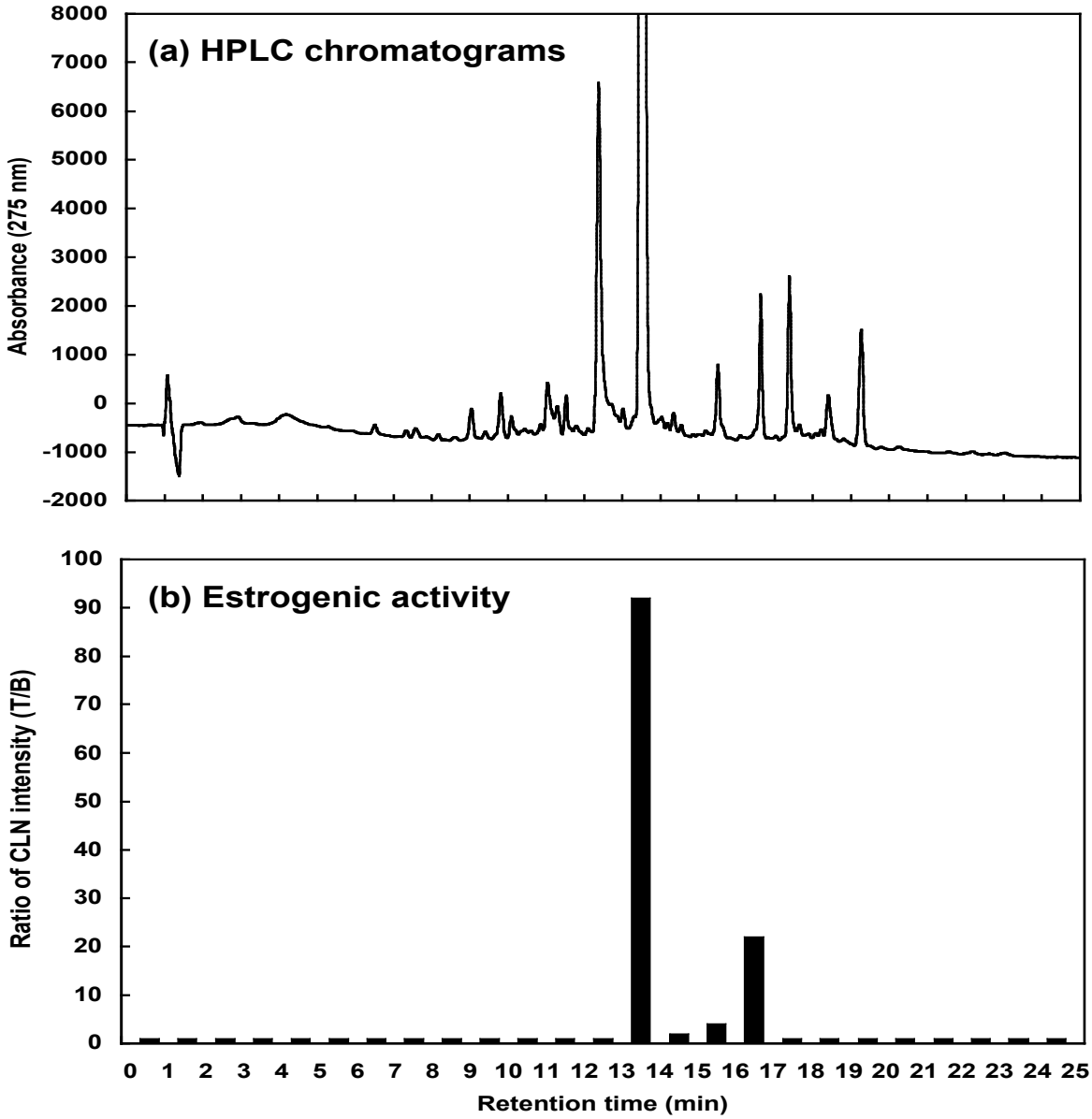

Fig. 2. Preparative HPLC of BPA photolysis by UV irradiation and estrogenic activity of separated fractions for hERα using a yeast two-hybrid assay. BPA (20 ppm) was photolyzed with the UV irradiation for 24h as described in the Materials and Methods section. Preparative HPLC was performed using a reversed-phase column with monitoring at 275 nm. The estrogenicity of the extract from each eluate collected at every 1-min interval was assayed for hERα using a yeast two-hybrid assay as described in the Materials and Methods section.