PRODUCTION MECHANISM OF HYDROXYLATED POLYCHLORINATED BIPHENYLS (OH-PCBS) BY OXIDATIVE DEGRADATION OF PCBS USING TITANIUM DIOXIDE IN WATER, AND ENDOCRINE DISRUPTING EFFECTS OF THEIR OH-PCBS

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Abstract

Oxidative degradation behaviors of PCBs (CB30, CB54, CB55, CB56, CB68, CB70, CB77, CB81, CB104, CB126, CB154, and CB169) using titanium dioxide (TiO_2) in water were investigated. The main purposes were to clarify the structural relation between the original PCBs and the intermediates derived by TiO_2 oxidation and to evaluate the endocrine disrupting effects by the treated PCBs during the oxidation.

It was estimated from calculation of the MOPAC program that the high frontier electron density (FED) point of the carbon atom combined with *para*-position chlorine of PCBs has stronger reactivity than other carbon atoms. In this result, the 4-OH-PCBs by degradation of the PCBs, such as *para*-substituted OH-group with chorine on the adjacent carbons were identified.

Estrogenicity of the OH-PCBs was assessed by using a yeast two-hybrid assay. It is presumed that the chemical structures of the some 4-OH-PCBs are similar to that of 17β-estradiol (E2); these intermediates present estrogenicity. Thyroid hormone activity was not detected in oxidation of all PCBs. However, it have been reported that in the structure activity relationship data on binding affinity to transthyretin (TTR) by OH-PCBs, chlorine-substitution on the phenolic-ring adjacent to the *meta*- and *para*- OH group enhanced the binding of OH-PCBs for TTR.

Introduction

PCBs and their biological metabolites as environmental contaminants have been shown to affect the endocrine systems in humans and wildlife. Within organisms and environment, PCBs are metabolized and oxidized into OH-PCBs [1,2]. These metabolites are formed by the cytochrome P450 enzymatic system in the human body that generally involves the oxide intermediates. OH-PCBs detected in human biological media as well as in wildlife have endocrine disrupting effects[3,4]. The research regarding estrogenic and thyroid hormonal activity among toxicological researchers of OH-PCBs has been frequently reported in the past decade [3,4]. However, there is no information as to what kinds of PCBs produce OH-PCBs in environment. Therefore, understanding conversion by oxidation of PCBs into OH-PCBs can add to our fundamental knowledge for estimating the fate of PCBs in environment.

A study was conducted to clarify the production process of OH-PCBs and their relation to their original PCBs. Titanium dioxide (TiO_2) photocatalyst was employed to rapid oxidation of PCBs in water to elucidate degradation pathways in environmental water. We have already reported on the production mechanism of OH-PCBs by oxidation of PCBs using TiO_2 in water [5,6].

In this study, the identification of the OH-PCBs and oxidative pathways of the twelve kinds of PCBs (the three kinds of characteristic chemical structures, (*ortho*-PCBs: CB30, CB54, CB104, and CB154; coplanar-PCBs: CB77, CB81, CB126, and CB169; mono-*orhto*-PCBs: CB55, CB56, CB68, and CB70)) was attempted by GC/MS data and reference compounds. The oxidative degradation pathway was estimated by reasonable combinations between identified intermediates and their quantitative transition at degradation time of the PCBs.

Moreover, the endocrine disrupting effects of OH-PCBs by degradation of the PCBs was investigated. The endocrine disrupting effects of PCB intermediates were evaluated for cases of estrogenic activity and thyroid hormone activity by using a constructed yeast two-hybrid assay for human estrogen receptor α (hERα) and human thyroid receptor α (hTRα) [4].

On the basis of these results, a series of experiments were conducted to clarify the structural relation between the original PCBs and the intermediates derived by TiO_2 oxidation.

Materials and Methods

The nanostructured TiO_2 thin film was immobilized on quartz beads by an advanced sol-gel method. The photoreactor used in the study was already reported[5,6]. Each PCBs (CB30, CB54, CB55, CB56, CB68, CB70, CB77, CB81, CB104, CB126, CB154, and CB169) solution (1 mg/L) 500 mL, was introduced into the photoreactor and recirculated while being irradiated with UV light. The temperature of each sample solution was maintained at 25 °C. Portions of the sample solution (400 mL) were sampled periodically to determine variation of the intermediates concentration according to irradiation time. That sample solution was removed completely with a rotary evaporator, and the residue was applied onto a silica-gel column for separation of unchanged PCBs, OH-PCBs and other intermediates. The first fraction containing the PCBs was eluted with hexane/benzene (1:1, 10 mL), and the second fraction containing the OH-PCBs and other intermediates was collected subsequently with acetone (10 mL). The 2 mL volume of the acetone solution was taken from each of the second fractions, and these volumes were dried completely with a rotary evaporator. Every dried residue of the sample solution was derivatized by BSTFA (0.5 mL). Each 2 µL volume of trimetylsilyl derivatives solution was injected into a GC/MS instrument. The 8 mL of the second fractions were used for the yeast two-hybrid assay.

The endocrine disrupting effects (for hERα and hTRα) of identified sample solutions from PCBs treated by TiO_2 at 0, 60, 120, 180, and 240 min irradiation time were measured by a yeast two-hybrid assay [4].

The MOPAC 2003 program in this study is provided by CAChe Scientific Inc. (Fujitsu Co., Japan). The PM5 (Parametric Method 5) Hamiltonian parameter is used to optimize stable structure and FED for PCBs [5,6].

Results and Discussion

Oxidative Degradation Curves of PCBs Using TiO_2. Oxidative degradation curves of the PCBs with the three kinds of characteristic chemical structures (*ortho*-PCBs: (CB30, CB154), coplanar-PCBs: (CB81, CB169), and mono-*orhto*-PCBs: (CB56, CB70)) at irradiation time by using TiO_2 are shown in Figure 1. Approximately 10-15% of the PCBs in water were decomposed within 240 min UV irradiation without the TiO_2 photocatalyst. With the photocatalyst, approximately 90% of the initial concentrations of PCBs (coplanar-PCBs and mono-*orhto*-PCBs) were decomposed within 180 min of UV irradiation. However, approximately 50% of the initial concentrations of CB154 were decomposed within 240 min. The degradation of CB154 was lower than that of the other PCBs. Since CB154 has 2,4,6-arrangement position of chlorines, it is estimated that the chemical structures might be electrically stable.

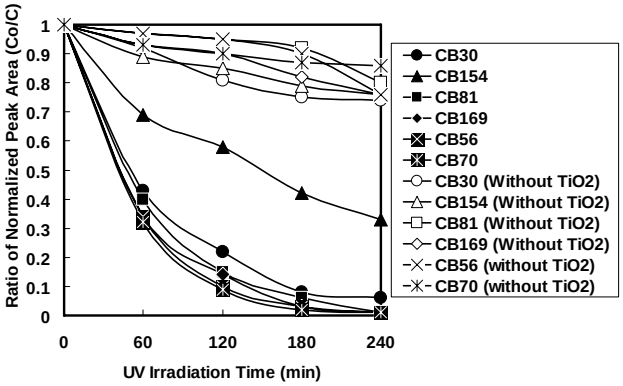

Figure 1. Oxidative degradations of the PCBs in water (1 mg/L). (Co = Internal Standard: 1 mg/L of phenanthrene-d10, T = 25 °C).
Each peak area was normalized by that of the initial PCBs peak areas (Co/C = 1)

Proposed Oxidative Degradation Pathways of PCBs. Intermediates from the degradation of the PCBs, such as OH-PCBs, carboxylic intermediates, phenolic intermediates, and other intermediates produced by the cleavage of a benzene ring were identified and quantified by using the reference compounds. Fluctuations of these intermediates according to the irradiation time reveal some possible step-flows in the degradation pathways of PCBs. Almost all the produced amount of OH-PCBs increased within 60 min of irradiation time, and gradually decreased thereafter. The produced amount of these OH-PCBs was less than about 10% of the initial PCBs at peak area on mass chromatograms before beginning of UV irradiation.

We calculated the partial electric charge distributions and FED on carbon atoms for the PCBs with the MOPAC program. The frontier electron densities shows the orbit with high chemical reactivity by the interaction that matches highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO) (Figure 2-a). These sites are those expected to be the most likely sites of attacks by neutral OH

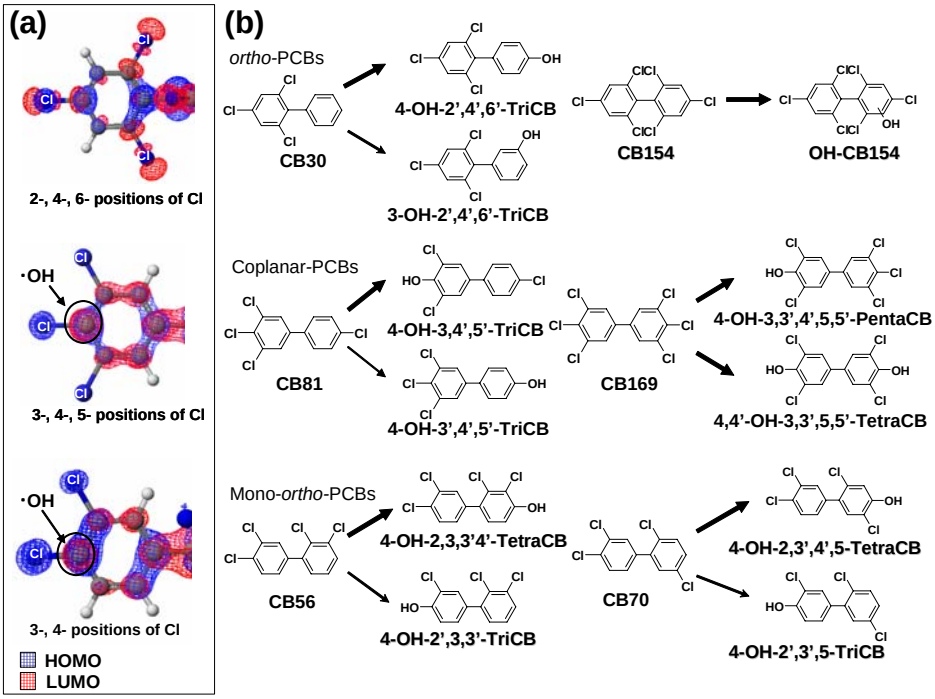

Figure 2. Proposed oxidation of PCBs in water. (a)**:** Imaging of frontier electron densities of PCBs composed by HOMO and LUMO. (b): Proposed oxidation of the three kinds of characteristic chemical structures of PCBs (*ortho*-PCBs: CB30 and CB154; coplanar-PCBs: CB81 and CB169; mono-*orhto*-PCBs: CB56 and CB70).

radicals. High frontier electron density points of carbon atoms positions were *para*- position of the coplanar-PCBs and mono-*ortho*-PCBs. The most negatively partial electric charged carbon atoms were the *ortho*- position carbon atoms of the coplanar-PCBs and mono-*ortho*-PCBs. These calculation results will serve basic information in the elucidation of the degradation mechanism of the PCBs.

The proposed oxidations of PCBs (*ortho*-PCBs: CB30 and CB154; coplanar-PCBs: CB81 and CB169; mono-*orhto*-PCBs: CB56 and CB70) in water using TiO_2 are shown in Figure 2-b. We determined the production of many 4-OH-PCBs by the PCBs oxidation. The production of the 4-OH-PCBs with dechlorination was strongly related to PCBs having 3,4- and 3,4,5-chlororine substitution. These PCBs suggests that the steric congestion of the 3,4,5-arrangement enhances the reactivity of the 4- position chlorine due to 3- and 5- chorines which are located in the vicinity of the 4- chlorine [5,6]. In short, we concluded that the high FED points of the carbon atom in 4- position chlorine have stronger reactivity than other carbon atoms. These sites are among those expected to be the most likely points of OH radical attacks. From the above result, the 4-OH-PCBs were produced by hydroxylation of PCBs with dechlorination in water. In contrast, the production of OH-PCBs with dechlorination was not determined from oxidation of the *ortho*-PCBs having 2,4,6-chlororine substitution. This result shows that PCBs having 2,4,6-chlororine substitution might be electrically stable. As a result of the MOPAC calculation, these PCBs did not have a high FED points on the carbon atoms.

Endocrine Disrupting Effects of Sample Solutions Obtained by Decomposition of PCBs Using TiO_2. To evaluate the potential endocrine disrupting effects (estrogenic activity and thyroid hormone activitiy) induced by degradation of PCBs in water environment, it is important to investigate the endocrine disrupting effects of these intermediates. The sample solutions obtained by the treatment of PCBs with TiO_2 were measured by yeast two-hybrid assay. Yeast cells were exposed to the aqueous solution from the degradation process, and the results for the samples decomposed for 0, 60, 120, 180, and 240 min revealed β-galactosidase activity. The estrogenic activity was found for decomposed CB30, CB54, and CB81 solutions at 60–120 min (Figure 3). It is presumed that this estrogenic activity originates from some CB30: (4-OH-2',4',6'-triCB and 3-OH-2',4',6'-triCB), CB54: (4,4'-DiOH-2,2',6,6'-tetraCB), and CB81: (4-OH-3,4',5-triCB and 4-OH-3',4',5'-triCB) included in solutions at

60–120 min. It is surmised that the estrogenic activity of 4-OH-PCB (The estrogenic activities of the 4-OH-2,2',4',6'-tetraCB, 4-OH-2',4',6'-triCB, and 3-OH-2',4',6'-triCB were 1.7%, 0.79%, and 0.2% for hERα of the relative activities of the E2 set to 100%) as a mimic for E2 might be due to the *para*-substituted phenol ring [4].

The thyroid hormone activity was not detected in decomposed all PCB solutions. The structural requirements of OH-PCBs for thyroid hormone activity are different from those required for estrogenic activity. The thyroid hormone receptor activity was associated with at least one *ortho*-chlorine, with two chlorines in the phenolic ring and, importantly, two chlorines in the non-phenolic ring, and with one or two chlorines *ortho*- to the hydroxyl group [4]. In this experiment, OH-PCBs with those chemical structures have not been detected. It is suggested that the OH-PCBs produced by an oxidation of the PCBs not have the thyroid hormonal receptor activity. However, it have been reported that in the structure-activity relationship (SAR) data on binding affinity to TTR by OH-PCBs, chlorine-substitution on the phenolic-ring adjacent to the *meta*- and *para*- OH group enhanced the binding of OH-PCBs for TTR [3]. It have been reported that 4,4'-diOH-3,3',5,5'-tetraCB by oxidation of CB169 selectively bind TTR in blood and inhibit amyloidogenesis [7]. The 4,4'-diOH-3,3',5,5'-tetraCB displaces T4 from TTR, rationalizing the toxicity observed in rodents, where TTR is the major T4 transporter [7].

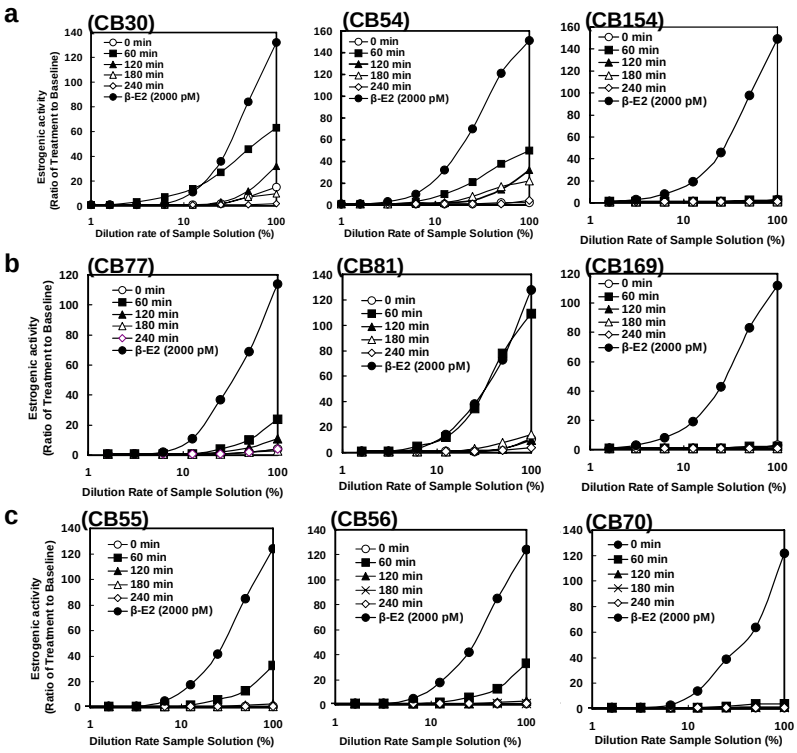

Figure 3. Dose-response curves for aqueous decomposed PCBs solutions at various irradiation times using the yeast two-hybrid assay for hERα. The values are represented as the ratio of chemiluminescence (CLN) intensity (T/B) of β-galactosidase.

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