APPLICATION OF *SOLIDAGO CANADENSIS* EXTRACT TO PHYTOMONITORING OF POLYCHLORINATED BIPHENYL CONGENERS IN THE TRANSGENIC *ARABIDOPSIS* PLANTS CARRYING THE RECOMBINANT GUINEA PIG ARYL HYDROCARBON RECEPTOR-MEDIATED β-GLUCURONIDASE REPORTER GENE EXPRESSION SYSTEM

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Introduction

Persistent organic pollutants (POPs) including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are widely contaminated in the environment and highly bioaccumulated at the top of food chains in aquatic ecosystems[1], of which PCB residues are most widely contaminated in Japan. PCBs consist of 209 congeners with different numbers and sites of chlorines on aromatic rings. Among them, 12 congeners have their own toxic equivalency factors (TEFs), which are evaluated by the World Health Organization in 2005[2], and are designated as dioxin-like PCBs (polychlorinated non-ortho and mono-ortho biphenyls).

The amounts of PCDDs, PCDFs and dioxin-like PCBs in the environmental sample are usually quantified by a high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Data are gennerally represented by the toxic equivalent (TEQ), which is the sum of the concentration of each of congener multiplied by the corresponding TEFs[2]. The HRGC/HRMS method is highly sensitive and accurate. However, processes for the extraction and purification of these congeners from the environmental sample are imperative with a high cost of analysis. In contrast, various biochemical assays have been established on the bases of the aryl hydrocarbon receptor (AhR) activation and transformation in mammalian cells and tissue extract, because a correlation has been reported between the binding affinity to AhR and toxicity of the AhR ligands in mammals[3]. Gene modification of AhR was developed for recombinant AhR-mediated β-glucuronidase (GUS) reporter assay for a rapid assay of AhR ligands including PCB congeners.

It was reported that the recombinant AhR gene was constructed and transformed into tobacco[4, 5] and *Arabidopsis*[6, 7] plants together with GUS reporter gene for assays of the environmental AhR ligands. These constructions are consisting of the ligand binding domain of AhR, the DNA-binding domain of *Escherichia coli* LexA, and the transactivation domain of *herpes simplex* virus regulatory protein VP16[4, 5]. These transgenic plants were applicable to a large number of soil samples without extraction and purification processes[6-8]. Since the PCB residues are highly lipophilic and persistent in the environment, the residues may not be easily uptaken by passive diffusion processes in plants. However, zucchini (*Cucurbita pepo*) and goldenrod (*Solidago canadensis*) uptook these residues[10-13] and specifically transported them into aerial parts. It appears that theseplants has unique mechanisms of PCB residues uptake and translocation. In this study, to clarify the molecular mechanisms of the efficient uptake and high accumulation to PCB residues by *S. canadensis*, it was attempted to use lipid extraction and root exudate of *S. canadensis* for assays of PCB congeners in the transgenic *Arabidopsis* plants carrying a reconbinant guinea pig (g) AhR-mediated GUS reporter gene expression system.

Materials and methods

Preparation of *S. Canadensis* lipid fractions

Goldenrod (*S. canadensis*) was collected from wild-grown plants on the campass of the Kobe University. Leafs, stems and roots of *S. canadensis* were subjected to extraction of the total lipid fraction in a chloroform-methanol (2:1, v/v). Each of the total lipid extracts was evaporated to dryness and then subjected to silica gel column chromatography. Neutral lipids, glycolipids and phospholipids were eluted by chloroform-methanol (98:2, v/v), acetone-methanol (9:1, v/v) and methanol, respectively. The glycolipids fraction was further separated by silica gel column chromatography (chloroform-methanol 98:2, 96:4, 9:1, 4:1 and 1:1 to methanol)

to yield 6 additional subfractions. The subfractions were further purified by silica gel column chromatography. Following the separation of subfractions, the presence of glycolipids in each fraction was analyzed by TLC using HPTLC with the mobile phase containing a mixture of chloroform:methanol:water in the ratio of 65:25:4 (v/v/v).

Preparation of *S. Canadensis* root exudates

Sterile seedlings of *S. canadensis* were culture on the Murashige & Skoog (MS) medium containing 0.1% sucrose (23°C, light). Then, root exudates adsorbed to the surface of activated charcoal was eluted with acetone.

Culture of *Arabidopsis* plants and treated with chemicals

The transgenic *Arabidopsis* plant XgD2V11-6 carrying the recombinant gAhR-mediated GUS reporter gene expression system was used according to the previous report[6]. Briefly, seeds were cultured for 2 weeks (23°C, light) on MS medium containing 0.1% sucrose, 0.08% agar containing 100 μg/mL lipid extract and 10 ng/mL PCB126. Then, whole plants were subjected to the measurement of GUS activity.

GUS assay

GUS activity was measured according to the previous report[6]. Briefly, a whole *Arabidopsis* plant was added to 150 μL of extraction buffer (50 mM sodium phosphate buffer (pH 7.0), 10 mM EDTA, 0.1% (v/v) TritonX-100, 0.1% (v/v) N-lauroylsarcosine sodium salt, 10 mM 2-mercaptoethanol) and homogenized in a power homogenizer (S-303, AS ONE Corporation, Osaka, Japan). Then, the homogenate was centrifuged at 17,000 g for 5 min. GUS reaction was started by adding 2 μL of the supernatant to 18 μL of 10 mM 4-methylumbeliferyl-β-D-glucuronide and incubated at 37°C for 30 min. The reaction was terminated by adding 180 μL of 0.2M of Na_2CO_3. Fluorescence of 4-methylumbeliferone (4MU) formed was measured by excitation wavelength of 355 nm and emission one of 460 nm with the Wallac multilabel counter (ARVO SX-1420, Perkin-Elmer Japan, Kanagawa, Japan). Protein concentrations in the supernatants were determined by protein assay with a CBB solution. The specific GUS activity was calculated as nmol 4MU/min /mg protein.

Results and discussion

The transgenic *Arabidopsis* plant XgD2V11-6 carrying the recombinant gAhR-mediated GUS reporter gene expression system was useful for assays of PCB126. It was poreviously reported that both uptake of PCB126 and PCB126-induced GUS activity in the plants increased in a dose-dependent similar manner[6, 14] (Figure 1). A detection limit, which defined as a concentration showing the GUS activity over 2-fold of background level, PCB126 was roughly 10 nM.

The GUS activity in the transgenic *Arabidopsis* plants was assayed after exposure to PCB126 with the lipid fractions prepared from aerial part of *S. canadensis*. As shown in Figure 2, the glycolipid fraction increased PCB126-induced GUS activity, although total lipid did

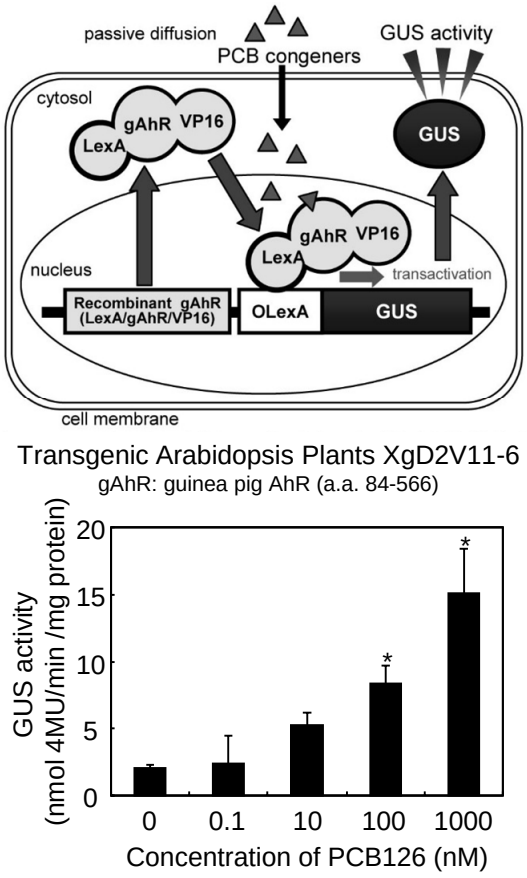

Figure 1. Phytomonitoring of PCB congeners in transgenic *Arabidopsis* plant cultivated on medium[9,14].
Seeds of the transgenic *Arabidopsis* plant XgD2V11-6 were cultured for 2 weeks on MS medium containing PCB126. Whole plants were subjected to the measurement of GUS activity. Results are represented as the mean ± SDs (n=4-6). Asterisks indicate a significant difference at $p<0.01$ (Tukey-Kramer multi-comparison test).

not show any effect. Both neutral lipid and phospholipid fractions also did not affect PCB126-induced GUS activity. Then, the glycolipid fraction was further separated by the silica gel columun chromatography and measured their GUS activity. As the results, the subfractions eluted with chloroform-methanol 98:2 and 96:4 from the glycolipids fraction from the leaves and those of chloroform-methanol 98:2, 9:1, 4:1 and 1:1 fractions from the roots, the PCB126-induced GUS activity was increased significantly. Among these subfractions, chloroform-methanol 96:4 subfraction of the glycolipids fraction from the leaves showed the strongest GUS activity. In order to identify the active component, this chloroform-methanol 96:4 subfraction was analyzed by TLC using sterylglucoside (SG) and acylsterylglucoside (aSG) as the standard compounds. We found that this subfraction contained SG as an active component. This subfraction and authentic SG was increased the PCB126-induced GUS activity to 2.4-fold and 4.8-fold, respectively. The amount of SG contained in the *S. canadensis* was higher than *Arabidopsis*. These results suggest that the glycolipid SG possible to increase the sensitivity of the monitoring of PCB congeners. In addition, the root exudate tended to increase the PCB126-induced GUS activity. Therefore, glycolipid SG and the root exudate appeared to increase the uptake of PCB126 or improve PCB126-induced GUS activity. When *S. canadensis* extract was applid to the assay of PCB126, the detection limit of PCB 126 was 10 ng/mL of medium/plant, which is corresponding to 1,000 pg-TEQ/mL.

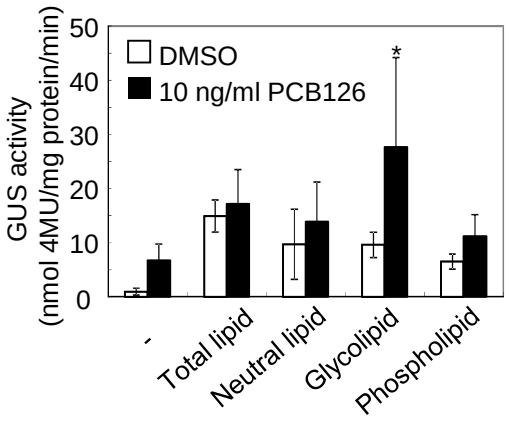

Figure 2. Effect of lipid fraction on the GUS activity induced by PCB126 in the transgenic Arabidopsis plant.
The transgenic *Arabidopsis* plant XgD2V11-6was seeded and cultured for 2 weeks on MS medium containing PCB126 in the presence lipid fraction from aerial part of *S. canadensis*. Open columns indicate solvent control (DMSO alone), while closed ones indicate PCB126. Whole plants were subjected to the measurement of GUS activity. Results are represented as the mean ± SDs (n =3-4). Asterisk indicate a significant difference at $p<0.05$ (Tukey-Kramer multi-comparison test).

In the present study, it was demonstrated that both glycolipid SG and root exudate incresed the PCB126-induced GUS activity. PCB congeners are thought to be uptaken by passive diffusion processes[15]. Previous study demonstrated that glycolipid MEL-B, produced in the culture of yeast isolated from plants, may form micelle with PCB congeners[6, 14]. The micelle may be facilitate uptake of PCB congeners from the roots by passive diffusion[10]. In addition, the protein-like material that binds to 2,3,7,8-TCDD was found in the leaf blades and root exudates of *C. pepo* and *Cucumis melo* L.[12]. A protein-like materials in the xylen sap of *C. pepo* play an important role in the translocation of dieldrin from the roots to the shoots[16]. The translocation of a variety of hydrophobic compounds by Cucurbitaceae, suggesting that these protein-like materials would have a nonspecific hydrophobic interaction with the hydrophobic compounds[16]. These results indicate that hydorophilic compounds including SG incrased the sensitivity of GUS activity induced by PCB congenes. In conclusion, the transgenic *Arabidopsis* plants carrying the recombinant gAhR-mediated GUS reporter gene expression system appeared to be practically useful for rapid assays of environmental PCB congeners even in a large number of samples without extraction and cleanup processes. Moreover, the present study suggested that possible to increase the sensitivity of the monitoring by adding a glycolipid SG. Further improvement and modification of assay conditions may be needed for sensitivity and accuracy of assay data.

Acknowledgements

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