Regulated gene expression in response to the exposure to dioxins in Arabidopsis thaliana

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Introduction

Dioxins such as PCDDs, PCDFs and dioxin-like coplanar PCBs (Co-PCBs) are hard to be decomposed due to their stability and hydrophobic nature, leading to the world-wide contamination. The precise quantitative analysis of pollution levels of dioxins has been performed using a gas chromatograph equipped with the high-resolution mass spectrometry; however, this technique has the disadvantage of a high cost or a highly educated skill. In recent years it has become evident that the expression of several genes in animals was changed in response to dioxins treatment, and then this makes these genes potential candidates for use as the biomarker of exposure to dioxins.[1-3] This biomarker-monitoring system will be expected to be a good substitution for the instrumental analysis as the first step analysis of dioxins in the environment. Since, in contrast to mobile animals, plants cannot move, they possess an original inheritance gained in the long process of evolution. This inheritance consists especially of characters adapting plants for sub-optimal environmental conditions involving the chemical pollutant stress. This leads us to suspect the existence of special gene(s) in the genome of plant, particularly responding to the chemical stress of dioxins. In this study, several dioxins-response genes in the genome of *Arabidopsis thaliana (A. thaliana)* were reported to investigate their efficiency for the biomarker in the environmental risk assessment of dioxins contamination.

Materials and Methods

Chemicals

3,3',4,4',5-Pentachlorobiphenyl (PCB126) in toluene was purchased from Wellington Labs (Ontario, Canada) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in dimethyl sulfoxide (DMSO) was obtained from AccuStandard Inc. (New Haven, USA), and each standard solution was prepared in DMSO to a concentration of 5 µg/ml. All solvents were pesticide free reagent grade. All other chemicals used were of analytical grade.

Plant material and exposure to chemicals

A. thaliana ecotype Columbia (The Sendai Arabidopsis Seed Stock Center, Japan) was grown on Murashige and Skoog (MS) solid medium at 26°C under a 16hr light / 8hr dark cycle. Seven-day-old seedlings were transferred to MS liquid medium and grown for another 9 days with shaking at 100 rpm. After growth, seedlings were exposed to PCB 126 or TCDD at a concentration of 5 ng/ml for 2 or 48 hours. Biphenyl and DMSO were used for control chemicals in the exposure test.

Screening of the genes responding to the chemical stress

The cDNA microarray assay system with the *Arabidopsis* 9.2K cDNA microarray (W. M. Keck Facility of Yale University, New Haven, USA) was used for screening comprehensively the up- or down-regulated genes to the chemical stress of PCB 126 or TCDD, respectively.

Results and Discussion

Two kinds of fluorescent cDNA probe for hybridization of the cDNA microarray chip were constructed from mRNAs prepared from PCB 126 and DMSO exposed plants, respectively. The hybridization of a cDNA microarray chip was carried out with the mixture of probes from PCB 126 and DMSO treated plants at 42 ºC for 16 hours. DMSO was a solvent of PCB 126 and used as a control chemical for PCB 126 hybridization. The up- or down-regulated genes by PCB 126 exposure were defined as the genes that had over 2.0 or under 0.5 of the ratio of fluorescent hybridization signal with PCB 126 probe to that with DMSO probe, respectively. As a result, some regulated genes were detected in approximately 9,200 cDNAs, as shown in Table 1.

Table 1. The summary of up- or down-regulated genes by the exposure to PCB 126

Regulation	Exposure time(hours)	Number of genes

Up	2	80
	48	31
Down	2	281
	48	71

Overall the down-regulated genes were more than up-regulated genes in both exposure time of 2 and 48 hours, and the number of genes in the exposure time of 2 hours was found to be more than that in the exposure time of 48 hours in both up- and down-regulation. Besides the co-hybridization assay using two fluorescently labeled probes prepared from mRNAs from PCB 126 and DMSO treated plants, two more co-hybridization assays were performed with the mixed probe from PCB 126 and Biphenyl treated plants, and with that from PCB 126 and TCDD treated plants. Among the up- or down-regulated genes by the exposure to PCB 126 shown in Table 1, some stress response genes and functionally unknown genes were found to be characteristic of PCB 126 and TCDD treatment (Table 2). The exposure to TCDD or Co-PCBs has been shown to cause oxidative stress in a variety of model animals[4,5], and that several genes in rat liver were reported that the expression levels were changed in response to PCB 126 application.[1] Also in *A. thaliana*, oxidative stress response genes such as At1g78380, At4g23680 and At4g34710 were found to be up-regulated by the exposure to PCB 126 and TCDD. Among these three genes, only At1g78380 gene was expressed at the ratio of over 2.0 in response to PCB 126 treatment to the expression in the treatment of DMSO, Biphenyl and TCDD;

Table 2. The summary of characteristic genes responding to PCB 126 and TCDD stress

Signal ratio			Gene locus	Description
PCB 126 / DMSO	PCB 126 / Biphenyl	PCB 126 / TCDD		
2.37 (48h)	2.25 (48h)	2.32 (48h)	At1g78380	oxidative stress response
2.29 (48h)	2.70 (48h)	0.39 (48h)	At4g23680	oxidative stress response
2.25 (2h) 2.31(48h)	2.55 (48h)	0.31 (48h)	At4g34710	oxidative stress response
2.49 (48h) 0.37 (2h)	2.36 (48h)	unchanged	At2g05380	salt tolerance
2.88 (2h)	unchanged	2.27 (48h)	At1g03220	unknown
2.28 (48h)	2.32 (48h)	0.37 (48h)	At5g14920	unknown
0.46 (48h)	0.46 (48h)	2.17 (48h)	At2g34420	light stress response
0.44 (48h)	0.48 (48h)	2.10 (48h)	At2g34430	light stress response
0.44 (48h)	0.39 (48h)	3.11 (48h)	At2g40000	nematode resistance
0.36 (48h)	0.40 (48h)	4.18 (48h)	At2g45990	unknown
0.46 (48h)	0.46 (48h)	3.27 (48h)	At3g07180	unknown
3.61 (2h) 0.39 (48h)	0.40 (48h)	4.34 (48h)	At5g36710	unknown

however, the expression rates of At4g23680 and At4g34710 were under 0.5 in response to PCB 126 compared to the response in TCDD exposure. These results suggest that only PCB 126 up regulated the expression of At1g78380 gene, on the other hand, the expression of At4g23680 and At4g34710 genes was up-regulated by both PCB 126 and TCDD, and TCDD had the ability twice or more as much as that of PCB 126 to enhance the gene expression. The At1g78380 gene encodes one of 47 members of the glutathione S-transferase (GST) super-family identified in the *Arabidopsis* genome, and that GST has been well investigated in response to biotic and abiotic stress including the oxidative stress.[6] The At4g23680 and At4g34710 genes encoding the putative major latex protein and arginine decarboxylase, respectively have been also known to be up-regulated by a wide variety of oxidative stresses such as hydrogen peroxide, paraquat, ozone and nitric oxide.[7,8] The At2g34420 and At2g34430 genes, both of which encode the photosystem II type I chlorophyll a/b binding protein involving the oxygenic photosynthesis that produce active oxygen species, seem to be down-regulated by both PCB 126 and TCDD treatment.[9] In addition to the oxidative stress response genes other kinds of stress response genes (i.e. At2g05380 or At2g40000) and functionally unknown genes were found to be up- or down-regulated by both PCB 126 and TCDD treatment.

We reported here the existence of some genes in the genome of *A. thaliana* that responded to the stress of PCB 126

and/or TCDD exposure using the comprehensive cDNA microarray assay system, and then the dose or exposure time effects of dioxins, or congener specificity needs to be elucidated using the precise measurement system like a real-time quantitative PCR method.

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