Direct Measurement of Dioxins and Endocrine Disrupting Chemicals in Degradation Enzyme Mixture with Contaminated Soil by Immunoassay

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Introduction

Environmental endocrine disrupting chemicals (EEDs) include synthetic and naturally occurring chemicals such as polychlorinated dibenzo-p-dioxins that influences on the balance of normal hormonal functions in humans as well as in animals. Quantitation of EEDs in biological and environmental materials is of absolute necessity to understand the mechanisms and effects of EEDs and assess their environmental impact. Microquantitative analysis of the EEDs has mainly been carried out by a combination of gas chromatography (GC) and mass spectrometry (MS). Despite the sensitivity of the MS-based methodology, it requires complicated extraction procedures and laborious cleaning processes prior to measurement. In addition, low efficiency and high cost of the methodology and limited availability of the instrument are drawbacks to routine practical quantitation of the EEDs in a soil specimen. The present study developed a simple, sensitive and rapid enzyme-linked immunosorbent assay (ELISA) method for the dioxins with novel type of labeled antigen. The assay system was proved to recognize the tetra- and trichlorinated dibenzo-p-dioxins to the same extent and quantitate those structures without cleaning processes from contamitated soil as a fly ash soil model.

Materials and Methods

1. Enzyme sample used for the tests were prepared by the methods to collect thermophile cell lysate by ultra sonication and purify the cell membrane of Geobacillus midousuji ATCC No. 202050 by ultracentrifuge. This dioxin biodegradation enzyme was dried out at low temperature with vacuum centrifugation method and mixed with yeast extract powder (DIFCO).

2. Dioxin degradation was performed in silicon lining seal test tube with 0.03g of flyash, 0.18g of model soil suspended in 1mL of corn steep liquor (50x dilution, Sanwa Starch) and tripticase soy broth (3%, BBL) at pH8 condition. The enzyme(2.5mg) and yeast extract(17.5mg) was added in this solution and stands up at 20C, 6 days and 65C, 4 days afterward. Control reaction was tested as same contents without enzyme powder.

Methods for ELISA with the antibody to 2,3,7-trichlorodibenzo-p-dioxin

The antibody to 2,3,7-TCDD has cross reactivity to the other dioxin isomers listed in Table 1. Principle of the ELISA methods was shown in Figure 1. based on the competitive analysis with biotinilated 2,3,7-TCDD, horse radish peroxidase, and its substrate of 3,3', 5, 5'-tetramethyle benzidine. ELISA methods were performed at 4C , 24 hours of 1st reaction and 20C, 20 hours of 2nd reaction in citrate buffer.

Results and Discussion

1. Evaluation of immunoassay

The crossreactions of the assay system with various dioxin-related compounds are shown in Table 1, which demonstrated high specificity of the present system to the tetra- and trichlorinated dibenzo-p-dioxins. Since a moderate degree of crossreactivity was observed with the 2,3-dichlorinated dioxin, the assay system is likely to recognize more strongly the 2,3-dichlorinated benzene ring of compound antigen.

The system was proved to detect standard antigen in a range of 100pg to 1,000ng/mL. The values in soil samples preliminarily measured by the present ELISA were compared with those measured by GC/MS method. There was thus found high correlation (r=0.978) between the two results obtained. However, immunoassay in general measures collectively all the derivatives having the same structures as those of the epitopes of the antibody used. In this context, further analysis by GC/MS may be necessary in order to assign each component of the dioxins collectively measured by the ELISA. The present ELISA system, therefore, is useful for routine measurement of the dioxins in a frequent measurement to compare the time course which ought to be further examined by other methodology such as GC/MS in terms of individual components of the dioxins contained.

2. Degradation of dioxin in contaminated soil

Dioxin degradation was performed with 0.03g of flyash, 0.18g of model soil suspended in 1mL of corn steep liquor (50x dilution, Sanwa Starch) and tripticase soy broth (3%, BBL) at pH8 condition. The enzyme(2.5mg) and yeast extract(17.5mg) was added in this solution and stands up at 20C, 6 days and 65C, 4 days afterward. Results are shown in Table 2 and Figure 2, in which relaive amount of labeled antigen bound to antibody is represented by optical density (OD492nm) of the product of enzymatic reaction between HRP on labeled antigen bound and o-phenylendiamine. The table indicates the labeled antigen-binding activities of the BLANK (nothing reaction mixture except ELISA reaction solution) is 1.661, enzyme degraded dioxins in contaminated soil is 0.838. Despite the control mixture showed OD as 0.483. We calculated a OD measurement to dioxin concentration through a typical standard curves for the present dioxins ELISA. This result confirmed that dioxin degradation was performed the level of 218.2ng/mL to 57.4ng/mL after 6 days incubation at 20C combined with 4 days incubation at 65C. However, concentration of dioxins in contaminated soil

(control sample) was 2ng/g (0.4ng/0.21g contaminated soil/mL mixture) by analysis of GC/MS method. So, we could estimate the flyash contained a lot of dioxins (218.2ng/mL-0.4ng/mL =217.8ng/mL) with less than three chlorinated molecules as endocrine disrupting chemicals.

Acknowledgements

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References

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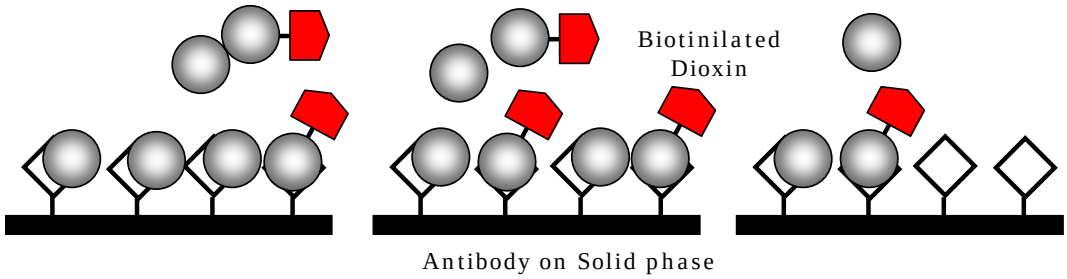
2. Sadayori Hoshina, et.al, Direct measurement of dioxins in degradation enzyme mixture using immunoassay. Organo Halogen Compound 2005.

Competitive analysis of ELISA method with chlorinated dioxin and biotinilated dioxin



Cross reactivity of the antibody to 2,3,7-trichloro-Dioxin

		TEF	Cross Reactivity
2,3,7-TriCDD		0	1
2,3,7,8-TCDD		1	2.4
1,2,3,7,8-PeCDD		1	0.25
1,2,3,4,7,8,-HxCDD		0.1	0.003
1,2,3,6,7,8,-HxCDD		0.1	<0.001
1,2,3,7,8,9,-Hx-CDD		0.1	<0.001
1,2,3,4,6,7,8,-HpCDD		0.01	<0.001
1,2,3,4,6,7,8,9,-OCDD		0.0001	0.001
2,3,7,8-TCDF		0.1	0.15
1,2,3,7,8-PeCDF		0.05	0.01
2,3,4,7,8-PeCDF		0.5	0.39
1,2,3,4,7,8-HxCDF		0.1	0.007
1,2,3,6,7,8-HxCDF		0.1	0.003
1,2,3,7,8,9-HxCDF		0.1	<0.001
2,3,4,6,7,8,-HxCDF		0.1	0.001
1,2,3,4,6,7,8-HpCDF		0.01	<0.001
1,2,3,4,7,8,9-HpCDF		0.01	<0.001
1,2,3,4,6,7,8,9-OCDF		0.0001	<0.001
3,4,4',5-TeCB	#81	0.0001	0.02
3,3',4,4'-TeCB	#77	0.0001	0.03
3,3',4,4',5-PeCB	#126	0.1	0.003
3,3',4,4',5,5'-HxCB	#169	0.01	<0.001
2',3,4,4',5-PeCB	#123	0.0001	<0.001
2,3',4,4',5-PeCB	#118	0.0001	0.003
2,3,3',4,4'-PeCB	#105	0.0001	0.005
2,3,4,4',5-PeCB	#114	0.0005	0.001
2,3',4,4',5,5'-HxCB	#167	0.00001	<0.001
2,3,3',4,4',5-HxCB	#156	0.0005	0.001
2,3,3',4,4',5'-HxCB	#157	0.0005	<0.001
2,3,3',4,4',5,5'-HpCB	#189	0.0001	<0.001
2,3DiCDD			0.04
2,7/2,8DiCDD			0.07

Degradation Results measured by Immunoassay

	O.D.	Concentration	Degradation Rate
BLANK	1.661	—	—
Enzyme	0.838	57.4	73.7
Control	0.483	218.2	—

(ng/mL)