Organohalogen Compounds, Volume 60, Pages 287-290 (2003)

DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAY SPECIFIC FOR NON-ORTHO COPLANAR-POLYCHLORINATED BIPHENYLS WITH USE OF NOBEL TYPE OF LABELED ANTIGEN

Yoshifumi Ohno[1], Yasuteru Usuki[1], Sakiko Iida[1], Ikuo Kato[2], Kazuyuki Kitamura[2], Shingo Nagasawa[2] and Chizuko Yanaihara[2]

1 EDC Analysis Center, Otsuka Life Science Initiative, Otsuka Pharmaceutical Co, Ltd. 224-18, Ebisuno Hiraishi Kawauchi-cho Tokushima 771-0195, Japan

2 Yanaihara Institute, Inc. 2480-1, Awakura, Fujinomiya-shi Shizuoka 418-0011, Japan

Introduction

Polychlorinated biphenyls (PCBs) are a group of well-known widespread highly toxic envirronmental pollutants, among which most of higher toxic compounds belong to non-ortho coplanar-PCBs (Co-PCBs)[1,2]. This paper describes development of ELISA highly specific for non-ortho Co-PCBs with use of nobel type of labeled antigen, which provided us a rapid and cost-effective mass screening method for Co-PCBs contaminated environmental matrixes.

Methods and Materials

Production of antisera

Polyclonal antibodies were raised in 3 rabbits by injecting 4-[(3,4,5-trichlorobiphenyl-4`-yl) carbamoyl] butanoyl-porcine thyroglobulin 6 times at two weeks interval. One of them, RY934 that showed the highest titer was used for ELISA.

Preparation of labeled antigen

Used as labeled antigen in ELISA was 6-(3,3`,4`,5`-tetrachlorobiphenyl-4-yloxy) hexanoyl-Arg-Arg-NHNH-biotin (Biotin-PCB). The compound was prepared by coupling 6-(3,3`,4`,5`-tetrachlorobiphenyl-4-yloxy)hexanoic acid with H-Arg(Pbf)-Arg(Pbf)-NHNH-biotin using HOBt/WSCD, followed by removal of the Pbf groups with TFA and purification by reverse phase HPLC.

ELISA development

IgG fraction from anti-PCB antiserum RY934 was purified by protein A column chromatography. Wells of microplate were coated with the anti-PCB IgG fraction. Standard antigen 3,4,5-trichlorobiphenyl (3,4,5-TriCB) or sample (50µL) and Biotin-PCB (50µL) were added to each well and mixed. The plate was then incubated for 2h at room temperature and washed 3

times. Peroxidase-labeled streptoavidin (100µL) was added to each well and incubated for 1h at room temperature. After washing, 3,3`,5,5`-tetramethylbenzidine (TMB) (100µL) was added and allowed to react for 10 min at room temperature. The enzyme reaction was terminated by adding 2N sulfonic acid (100µL) and the color was determined at 450nm (ref. 650nm) using a microplatereader.

Preparation of sample

The extraction and cleanup procedures from soil for sample are shown in Fig.2. The extracts dried up with N_2 gas were dissolved in DMSO and diluted ELISA buffer (1:9).

Results and Discussion

PCBs are highly hydrophobic compounds, which is the most difficult problem against development of practical immunoassay system for PCBs. To solve the problem, we synthesized novel type of PCB derivatives for labeled antigen possessing favorable solubility in aqueous medium. In practice, two Arg residues were introduced in tandem in between PCB moiety and biotin. Improvement in solubility in aqueous medium of the reagent facilitated PCBs immunoassay development. In fact, the newly developed ELISA for PCBs was proved to be sensitive enough for practical use in environment assessment.

Fig.1 indicates a typical standard curve of the assay system, which recognized such a wide range as that from 0.15 to 150 ng/well of 3,4,5-TrCB. The intra- and inter-assay coefficients of variation were 8.3 14.0 % and 4.4 9.7 %, respectively. The crossreactivities of the ELISA system against various PCB-related compounds examined are summarized in Table 1, in which the IC_{50} of 3,4,5-TrCB (#38) was regarded as 1. Three (#81, #126 and #169) of 4 non-ortho-chlorinated coplanar PCBs (non-ortho Co-PCBs) showed appreciably high crossreactivities, which are all chlorinated at 3,4 and 5 positions as is the standard 3,4,5-TrCB (#38). Other 24 related compounds examined showed sustantially no crossreactivities in the assay system. The result confirmed the remarkably high specificity of the present ELISA system for non-ortho Co-PCBs chlorinated at 3,4 and 5 positions. Chlorination at position 5 seems to be essential for recognition by the assay. In additoin, compounds #126 and #169 are known to have higher TEF as compared other compounds examined. We also examined Kanechlor (KC), commercial products of PCBs, in the present assay and found some crossreactivities with KC-300 and 400 and little with KC-500 and 600 (data not shown), indicating possible existence of non-ortho Co-PCBs, chlorinated at least at positions 3,4 and 5, in KC-300 and 400. We further measured Co-PCB in soil samples (n=33) by the present ELISA. The range of Co-PCBs contents was 0 9828 pg/g (Mean±S.D.= 1158±1687 pg/g) .

The results confirmed the usefulness of the present Co-PCBs specific ELISA for rapid and cost-effective mass screening of environmental specimens containing higher concentration of highly toxic non-ortho Co-PCBs which need to be further examined for its individual components by other methodology such as GC/MS.

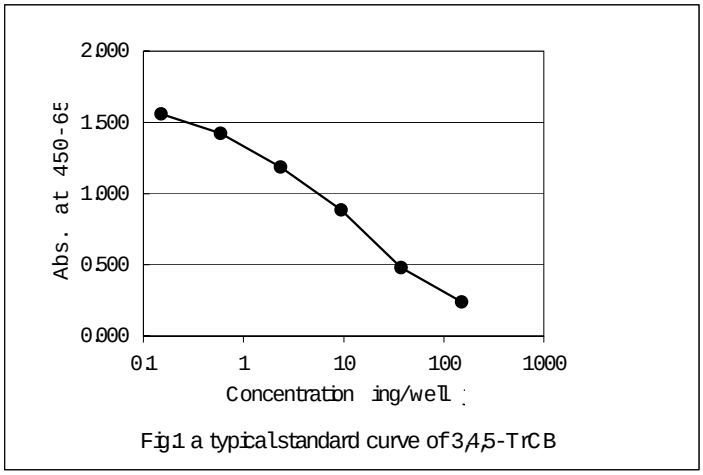
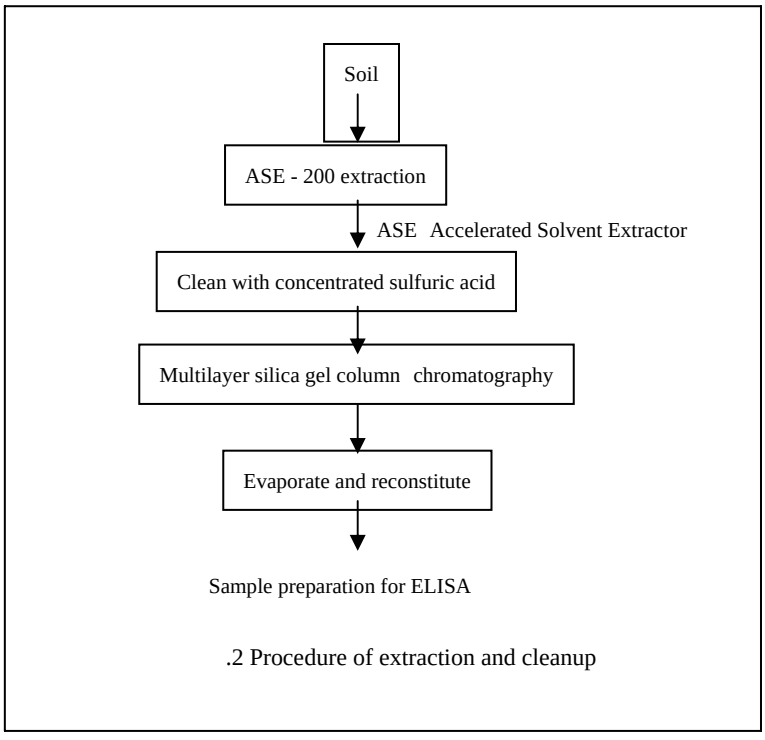


Fig.1 a typical standard curve of 3,4,5-TrCB



.2 Procedure of extraction and cleanup

Table 1 Crossreactivities of PCBs, PCDDs, PCDFs and other related compounds

	Type of Co-PCB		TEF	Crossreactivity
		3,4,5-TrCB (#38)	-	1
Co-PCBs	Non-ortho	3,4,4',5-TeCB (#81)	0.0001	0.84
		3,3',4,4'-TeCB (#77)	0.0001	0.02
		3,3',4,4',5-PeCB (#126)	0.1	0.52
		3,3',4,4',5,5'-HxCB (#169)	0.01	0.63
	Mono-ortho	2',3,4,4',5-PeCB (#123)	0.0001	0.004
		2,3',4,4',5-PeCB (#118)	0.0001	<0.0001
		2,3,3',4,4'-PeCB (#105)	0.0001	<0.0001
		2,3,4,4',5-PeCB (#114)	0.0005	<0.0001
		2,3',4,4',5,5'-HxCB (#167)	0.00001	<0.0001
		2,3,3',4,4',5-HxCB (#156)	0.0005	<0.0001
		2,3,3',4,4',5'-HxCB (#157)	0.0005	<0.0001
		2,3,3',4,4',5,5'-HpCB (#189)	0.0001	0.002
Some PCBs		2,3-DiCB (#5)	-	<0.0001
		2,4'-DiCB (#8)	-	<0.0001
		2,2',5-TriCB (#18)	-	<0.0001
		2,4,4'-TriCB (#28)	-	<0.0001
		2,4',5-TriCB (#31)	-	<0.0001
		3,4,4'-TriCB (#37)	-	0.09
		2,2',3,5'-TeCB (#44)	-	<0.0001
		2,2',3,3',6,6'-HxCB (#136)	-	<0.0001
		2,2',4,4',5,5'-HxCB (#153)	-	<0.0001
		2,2',3,3',4,4',5-HpCB (#170)	-	<0.0001
		2,2',3,4,4',5,5'-HpCB (#180)	-	<0.0001
PCDDs		2,3,7,8-TeCDD	1	<0.0001
		1,2,3,7,8-PeCDD	1	0.005
		1,2,3,4,7,8-HxCDD	0.1	<0.0001
		1,2,3,6,7,8-HxCDD	0.1	<0.0001
		1,2,3,7,8,9-HxCDD	0.1	<0.0001
		1,2,3,4,6,7,8-HpCDD	0.01	<0.0001
		1,2,3,4,6,7,8,9-OCDD	0.0001	0.01
PCDFs		2,3,7,8-TeCDF	0.1	0.006
		1,2,3,7,8-PeCDF	0.05	<0.0001
		2,3,4,7,8-PeCDF	0.5	0.03
		1,2,3,4,7,8-HxCDF	0.1	<0.0001
		1,2,3,6,7,8-HxCDF	0.1	0.005
		1,2,3,7,8,9-HxCDF	0.1	<0.0001
		2,3,4,6,7,8-HxCDF	0.1	0.006
		1,2,3,4,6,7,8-HpCDF	0.01	<0.0001
		1,2,3,4,7,8,9-HpCDF	0.01	<0.0001
		1,2,3,4,6,7,8,9-OCDF	0.0001	<0.0001
KC		KC-300	-	0.0012
		KC-400	-	0.0008
		KC-500	-	<0.0001
		KC-600	-	<0.0001
Other related compounds		Biphenyl	-	<0.0001
		Chlorobenzene	-	<0.0001
		o-Dichlorobenzene	-	<0.0001
		m-Dichlorobenzene	-	<0.0001
		p-Dichlorobenzene	-	<0.0001
		1,2,3-Trichlorobenzene	-	<0.0001
		1,2,4-Trichlorobenzene	-	<0.0001

TEF : 2,3,7,8-TeCDD toxicity equivalency factor

References

1. Ahlborg U.G., Becking G.C., Birnbaum L.S., Brouwer A., Derks H.J.G.M., Feeley M., Golor G., Hanberg A., Larsen J.C., Liem A.K.D., Safe S.H., Schlatter C., Warn F., Younes M. and Yrjanheikki E. (1994) Toxic equivalency factors for dioxin-like PCBs. Chemosphere, 28, 1049-1067.
2. Hack A. and Selenka F. (1996) Mobilization of PAH and PCB from contamined soil using a digestive tract model. Toxicol. Lett. 88, 199-210.