DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE PRE-SCREENING OF COPLANAR POLYCHLORINATED BIPHENYLS

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Introduction

A class of coplanar polychlorinated biphenyls (co-PCBs) is known as dioxins. Conventional methods for the analysis of co-PCBs using high resolution gas chromatography/mass spectrometry (GC/MS), are expensive and time consuming and require specialized equipments. Therefore, alternative simple and cost-effective methods for the determination of these compounds are highly desired. One of the methods that may satisfy these requirements and be an efficient screening tool is an immunoassay method based on poly- or monoclonal antibodies. Although several antibodies and ELISA systems for the detection of dioxins have been developed for the last decade, only a few trials have been done for the co-PCBs[1, 2]. We have developed plural monoclonal antibodies against co-PCBs.[3] One of these targets was the most common congener among co-PCBs; 2,3',4,4',5-pentachlorobiphenyl (PCB 118). Although PCB 118 has low toxic equivalency factor (TEF) value, the amount of this congener is more ubiquitous than those of other co-PCB congeners[4]. The total amount of toxicity of PCB 118 is, therefore, not negligible and should never be ignored. In this study, we report the development of enzyme-linked immunosorbent assay (ELISA) system for the quantification of co-PCB utilizing monoclonal antibody against PCB 118.

Materials and Methods

Competitive Enzyme-linked immunosorbent assay (ELISA) and assay validation

Each well of 96-well microtiterplates (Nunc; maxysoup) was coated with 50 µL (4 µg/mL) of purified monoclonal antibody (MAb) against PCB 118 in TBS (20mM Tris, 150 mM NaCl; pH 7.5) at 4 °C overnight. After washing three times with TBS, the wells were blocked with 150 µL of 1 % BSA with sucrose in TBS at 4 °C overnight. After washing with TBST (TBS with 0.05 % Tween 20), 25 µL of PCB standards in TBS-50 % DMSO were added to each well. Then, 25 µL of PCB competitor-HRP conjugates diluted with TBSTB (TBST with 1 % BSA) were added and incubated for 30 min at room temperature with shake. The wells were washed three times and 50 µL of TMB solution (Amarsham) were added and incubated for 20 min. The color development was stopped with 1N H_2SO_4 and measured at 450 nm with a microplate reader. Results were expressed as the ratio of B (OD_{450} for the sample) to B_0 (OD_{450} with no PCB). The dose-response curves (B/B_0 vs. logarithmic concentration of the analyte) were fitted by the four-parameter logistic model using the computer software (Microplate Manager Ver. 5.1; Bio-Rad Lab.). The cross-reactivities of the MAb were described as ratios between the concentration exhibiting 50 % inhibition (IC_{50}) by each compound and the IC_{50} value of PCB 118.

Table 1. Cross-reactivity of various related compounds for the ELISA.

Co-PCB congeners	IUPAC #	Cross-reactivity	PAHs	Cross-reactivity	Other related compounds	Cross-reactivity
3,4,3',4'-TeCB	77	70.1	Naphthalene	< 0.5	Biphenyl	< 0.5
3,4,4',5-TeCB	81	23.1	Acenaphthyrene	< 0.5	1,2-dichlorobenzene	< 0.5
3,3',4,4',5-PeCB	126	3.3	Acenaphthene	< 0.5	3,4-dichloroaniline	< 0.5
3,3',4,4',5,5'-HexCB	169	< 0.5	Fluorene	< 0.5	3,4-dichloroanisole	< 0.5
2,3,3',4,4'-PeCB	105	10.4	Phenanthrene	< 0.5	3,4-dichloronitro-benzene	< 0.5
2,3,4,4',5-PeCB	114	6.7	Fluoranthene	< 0.5	3,4-dichlorophenol	< 0.5
2,3',4,4',5-PeCB	118	**100**	Pyrene	< 0.5	3,4-dichlorotoluene	< 0.5
2',3,4,4',5-PeCB	123	< 0.5	Benzo(a)anthracene	< 0.5	1,2,3-trichlorobenzene	< 0.5
2,3,3',4,4',5-HexCB	156	14.3	Chrysene	< 0.5	3,4,5-trichloroaniline	< 0.5
2,3,3',4,4',5'-HexCB	157	< 0.5	Benzo(b)fluoranthene	< 0.5	3,4,5-trichlorophenol	< 0.5
2,3',4,4',5,5'-HexCB	167	< 0.5	Benzo(k)fluoranthene	< 0.5		
2,3,3',4,4',5,5'-HepCB	189	< 0.5	Benzo(a)pyrene	< 0.5		
2,2',3,3',4,4',5-HepCB	170	< 0.5	Indeno(123cd)pyrene	< 0.5		
2,2',3,4,4',5,5'-HepCB	180	< 0.5	Dibenzo(ah)anthracene	< 0.5		
			Benzo(ghi)perylene	< 0.5		

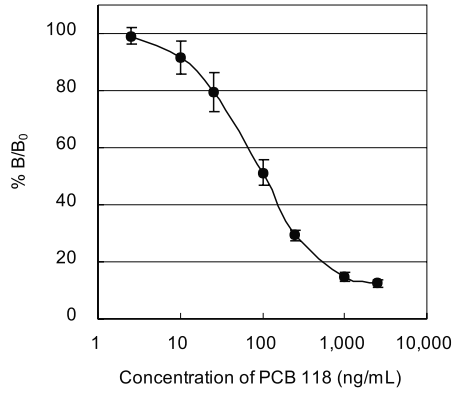

Figure 1. Typical standard curve of PCB 118 for the ELISA.

Results and Discussion

Assay specificity

The cross-reactivity of the MAb for each of co-PCB congeners, polyaromatic hydrocarbons (PAHs) and other related compounds was summarized in Table 1. The MAb showed the strongest recognition of PCB 118, followed by PCB 77, and showed weak responses to PCBs 81, 156, 105, 114 and 126. All of the tested PAHs, biphenyl and chlorobenzene derivatives were not recognized in amounts up to 10 ppm. These data suggested that the developed ELISA system was highly selective for co-PCB

congeners. Especially, coplanar congeners that had 3,4-chlorinated pattern seemed to be highly recognized.

Assay validation

Figure 1 shows the typical standard curve for PCB 118. Each point was described as the mean ± SD calculated from eight separate analyses. Since the average CV of the individual points on the standard curve was 8.0% , the good reproducibility was demonstrated. The IC_{50} value and the limit of detection (LOD; calculated as the IC_{15} value) for PCB 118 were 114 ng/mL (5.7ng/well) and 34.6 ng/mL (1.7ng/well), respectively.

Application for the pre-screening of TEQ of co-PCBs and the estimation of total amounts of PCBs

Even PCB 118 has low TEF value, due to the congener exists in high concentration in sample matrix, it is possible to use ELISA system for a pre-screening of PCB 118 without complicated clean-up procedure. Recently, Glynn *et al.* reported that the concentrations of PCB 118 were highly correlated to the toxic equivalents (TEQ) derived from co-PCBs in breast milk[5]. This indicates that the ELISA system developed in this study would be suitable for the pre-screening of TEQ of co-PCBs.

From the other point of view, the presented ELISA system could also be applied for the estimation of the total amounts of PCB because PCB 118 is one of the predominant congeners in the commercial PCB preparations (*e.g.*, Kanechlor in Japan[6]). Actually, good correlations between the ELISA measurements and the total amounts of PCBs were observed in a wide range of PCB-containing mineral oil (presented by Takigami *et al.* at this symposium).

In conclusion, the ELISA system developed here is possibly useful for the pre-screening both of the TEQ of co-PCBs and the total amounts of PCBs. More studies which investigate the correlations between the ELISA and the instrumental analyses with various sample matrices would be necessary to ascertain the usefulness of the ELISA.

Acknowledgments

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