ASSESSMENT OF HYDROXYLATED METABOLITES OF PCBs, PCDFs AND CHLORODIPHENYL ETHERS AS POTENTIAL *ESTROGENS BY YEAST TWO-HYBRID SYSTEM*

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Introduction

Polychlorinated biphenyls (PCBs) and dibenzofurans (PCDFs) are in general biotransformed mainly to hydroxylated metabolites. Several hydroxylated metabolites of PCBs have been shown to be estrogenic or anti-estrogenic, depending on their structures. For instance, 4-OH-2',5'-dichlorobiphenyl (diCB), 4-OH-2',4',6'-triCB and 4-OH-2',3',4',5'-tetraCB were revealed to be estrogenic by E-screen assay based on MCF-7 cell proliferation, receptor binding assay and/or uterotrophic assay [1, 2, 3]. 4-OH-2',4',6'-triCB is the most potent estrogenic congener among the OH-PCBs that have been ever tested. On the other hand, certain hydroxylated PCB metabolites such as 4-OH-2,2',3,4',5,5',6-heptaCB, which were identified in human blood, have been found to exhibit anti-estrogenic activity in an *in vitro* assay [4, 5]. However, other hydroxylated PCB metabolites identified in animal experiments have not yet been elucidated for their estrogenic or anti-estrogenic activity. Also little information is available on the biological activities of hydroxylated metabolites of PCDFs and chlorodiphenyl ethers.

Yeast two-hybrid assay is a very simple and rapid method to detect suspected endocrine disruptors [6]. This method is based on the ligand-dependent interaction of hormone receptors with coactivators, and hormonal activity is detected by β-galactosidase activity. In this study, we examined the estrogenic activity of some hydroxylated PCBs including the PCB metabolites identified in animal experiments, hydroxylated PCDFs and hydroxylated monochlorodiphenyl ethers by yeast two-hybrid system.

Methods and Materials

Yeast cells introduced with the estrogen receptor (ER α) and the coactivator (TIF2), which carry a β-galactosidase reporter gene, were used. Yeast two-hybrid assay was carried out according to the method previously reported [6]. In short, the yeast cells were preincubated overnight at 30℃ in SD medium free from tryptophan and leucine. The 250 μl of culture was then added to a DMSO solution (2.5 μl) containing test chemicals (final concentration: 10^{-9}~10^{-5} or 10^{-4} M) and incubated for 4 hr at 30℃. After collecting the cells by centrifugation, the cells were treated enzymatically by incubation with Zymolyase for 15 min at 37℃ and mixed with 40 μl of 2-nitrophenyl-β-D-galactoside (4mg/ml) to start enzymatic reaction. After the development of a yellow color

(30℃, 30 min), 100 μl of 1 M Na_2CO_3 were added to stop the reaction. Absorbances were read on a microplate reader to estimate estrogenic activity.

Results and Discussion

In yeast two-hybrid system, 10^{-9} M 17β-estradiol (E2) caused an induction of β-galactosidase activity and this induction increased with E2 concentration (10^{-9} ~10^{-5} M). The induced β-galactosidase activity was almost saturated at 10^{-7} M of E2.

1. Hydroxylated PCBs

Among the hydroxylated PCBs tested (Table 1), 4-OH-2',4',6'-triCB, 4-OH-4'-monoCB, 4-OH-biphenyl and 4,4'-$(OH)_2$-biphenyl showed a dose-dependent estrogenic activity. The activities were evaluated by the concentrations of test compounds showing 10% activity of 10^{-7} M E2, REC10 (10% relative effective concentration). The values of REC10 are also included in Table 1. 4-OH-2',4',6'-triCB was also the most potent estrogenic in yeast two-hybrid assay and the estrogenic activity was two times higher than that of 4-nonylphenol (REC10 : 4×10^{-7} M, mixture of compounds with branched sidechain). The estrogenic activities of 4-OH-4'-monoCB, 4-OH-biphenyl and 4,4'-$(OH)_2$-biphenyl were about 1/10 of 4-OH-2',4',6'-triCB, but these compounds demonstrated 10 times higher estrogenicity than Bisphenol A (REC10 : 2×10^{-5}M). On the other hand, 2-OH-/3-OH-biphenyl, 3-OH-/4-OH-2,2',5,5'-tetraCB (metabolites of 2,2',5,5'-tetraCB), 5-OH-3,3',4,4'-/4-OH-3,3',4',5-tetraCB (metabolites of 3,3',4,4'-tetraCB), 3-OH-/5-OH-2,3',4,4'-tetraCB (metabolites of 2,3',4,4'-tetraCB) and 4-OH-2',3,5,5'-tetraCB (metabolite of 2,3',4',5-tetraCB) were inactive at the highest concentrations we tested (10^{-5} M), indicating that these hydroxylated metabolites of PCBs do not possess the estrogenic effects through the interaction with estrogen receptor.

For the expression of estrogenicity of OH-PCBs, a hydroxy group in the 4-position with no chlorine substituents on the adjacent carbons to the hydroxy group was required.

Table 1 Estrogenic activity of hydroxylated PCBs by yeast two-hybrid assay

Compound	REC10 (M)[1]	Relative potency[2]	Compound	REC10 (M)[1]	Relative potency[2]
17β-estradiol(E2)	3×10^{-10} [3]	100	3-OH-2,2',5,5'-tetraCB[4]	$>1\times10^{-5}$	—
2-OH-biphenyl	$>1\times10^{-5}$	—	4-OH-2,2',5,5'-tetraCB[4]	$>1\times10^{-5}$	—
3-OH-biphenyl	$>1\times10^{-5}$	—	5-OH-3,3',4,4'-tetraCB[5]	$>1\times10^{-5}$	—
4-OH-biphenyl	3×10^{-6}	0.01	4-OH-3,3',4',5-tetraCB[5]	$>1\times10^{-5}$	—
4,4'-$(OH)_2$-biphenyl	3×10^{-6}	0.01	3-OH-2,3',4,4'-tetraCB[6]	$>1\times10^{-5}$	—
4-OH-4'-monoCB	2×10^{-6}	0.015	5-OH-2,3',4,4'-tetraCB[6]	$>1\times10^{-5}$	—
4-OH-2',4',6'-triCB	2×10^{-7}	0.15	4-OH-2',3,5,5'-tetraCB[7]	$>1\times10^{-5}$	—

1) concentration of compounds showing 10% activity of 10^{-7}M E2

2) relative potency was calculated using the REC 10 of test compounds and E2

3) REC 10 of E2 was quoted from the reference 6

4) metabolite of 2,2',5,5'-tetraCB 5) metabolite of 3,3',4,4'-tetraCB 6) metabolite of 2,3',4,4'-tetraCB

7) metabolite of 2,3',4',5-tetraCB

2. Hydroxylated PCDFs

Table 2 shows the hydroxylated PCDFs tested and their REC10. 8-OH-2-monochlorodibenzofuran (monoCDF), 7-OH-/8-OH-3,4-diCDF, 8-OH-3,4,6-triCDF and 3,8-$(OH)_2$-2-monoCDF exhibited a dose-dependent estrogenic activity (REC10 : 8×10^{-7} M ~ 1×10^{-5} M). 3,8-$(OH)_2$-2-monoCDF showed the most highest estrogenicity among these congeners and the activity was revealed to be comparable to that of 4-nonylphenol (mixture). The estrogenic activities of other OH-PCDFs above were 2 ~ 4 times higher than that of Bisphenol A. However, no estrogenic activities were observed for 2-OH-dibenzofuran, 3-OH-2,8-diCDF, 6-OH-3,4-diCDF and 7-OH-1,2,3,6,8-pentaCDF at concentrations as high as 10^{-4} M.

A hydroxy group in the 2(8)- or 3(7)-position was necessary for the estrogenic activity of OH-PCDFs, but one chlorine substituent on the adjacent carbons to the hydroxy group inhibited completely the estrogenic activity.

Table 2 Estrogenic activity of hydroxylated PCDFs by yeast two-hybrid assay

Compound	REC10 (M)[1]	Relative potency[2]	Compound	REC10 (M)[1]	Relative potency[2]
17 β -estradiol(E2)	3×10^{-10} [3]	100	7-OH-3,4-diCDF	6×10^{-6}	0.005
2-OH-dibenzofuran	$>1\times10^{-4}$	—	8-OH-3,4-diCDF	5×10^{-6}	0.006
8-OH-2-monoCDF	1×10^{-5}	0.003	8-OH-3,4,6-triCDF	1×10^{-5}	0.003
3-OH-2,8-diCDF	$>1\times10^{-4}$	—	7-OH-1,2,3,6,8-pentaCDF	$>1\times10^{-4}$	—
6-OH-3,4-diCDF	$>1\times10^{-4}$	—	3,8-$(OH)_2$-2-monoCDF	8×10^{-7}	0.04

1) concentration of compounds showing 10% activity of 10^{-7}M E2

2) relative potency was calculated using the REC 10 of test compounds and E2

3) REC 10 of E2 was quoted from the reference 6

3. Hydroxylated chlorodiphenyl ethers

4-OH-3'-, 4-OH-4'-chlorodiphenyl ether and 4,4'-$(OH)_2$-diphenyl ether showed a dose-dependent estrogenic activities. The estrogenic activities of the two hydroxylated chlorodiphenyl ethers (REC10: 1×10^{-6} M and 2×10^{-6} M; relative potency: 0.015 and 0.03, respectively) were almost as same as that of 4-OH-4'-monoCB. However, the activity of 4,4'-$(OH)_2$-diphenyl ether (REC10: 7×10^{-6} M, relative potency: 0.004) was about 1/8 of these two congeners.

In conclusion, the order of estrogenic activities of these hydroxylated compounds based on REC10 was as follows; 4-OH-2',4',6'-triCB, 4-nonylphenol (mixture) > 3,8-$(OH)_2$-2-monoCDF, 4-OH-3'-chlorodiphenyl ether, 4-OH-4'-monoCB $\gg$ 8-OH-3,4-diCDF, 4,4'-$(OH)_2$-diphenyl ether , Bisphenol A.

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