

Comparative Study of Affinity for Thyroid Hormone and Estrogen Receptors of Hydroxylated Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers

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Introduction

Polychlorinated biphenyls (PCBs) have been widely used in industry as heat transfer and electrical insulation agents, and in non-carbon copying paper. However, because of their lipophilic character, they accumulate in the environment. PCBs are metabolized *in vivo* to hydroxylated PCBs (OH-PCBs), which have been identified in human serum (Sandau et al., 2000). On the other hand, polybrominated diphenyl ethers (PBDEs) are widely used as flame-retardant additives in electronic circuit boards and other electronic equipment. PBDEs and their hydroxylated metabolites (OH-PBDEs) have been detected in human tissue samples, such as blood and breast milk (Inoue et al., 2006).

PCBs and PBDEs disrupt the thyroid hormone system. They have high binding affinity for thyroid receptor (TR) and the serum thyroid hormone-binding transport protein transthyretin, from which they consequently displace endogenous thyroid hormone (Cheek et al., 1999; Lans et al., 1994; Meerts et al., 2000). Thus, exposure to PCBs and PBDEs may influence the growth of fetuses and children. OH-PCBs also show high binding affinity for TR and transthyretin. However, the mechanisms through which these compounds disrupt thyroid hormonal action are not understood in detail.

In addition, some OH-PCBs and OH-PBDEs showed agonistic or antagonistic action at the estrogen receptor (ER) and exhibit estrogenic activity in the mouse uterus (Connor et al., 1997; Kojima et al., 2009; Korach et al., 1988). However, few studies have addressed the binding ability of these compounds with both TR and ER.

In this study, we examined the thyroid hormone and estrogenic activities of OH-PCBs and OH-PBDEs by means of TR-binding assay, as well as two thyroid hormone-dependent cell culture assays (Fig.1). Furthermore, the results were compared with the estrogenic activity observed in ERE-luciferase reporter assay.

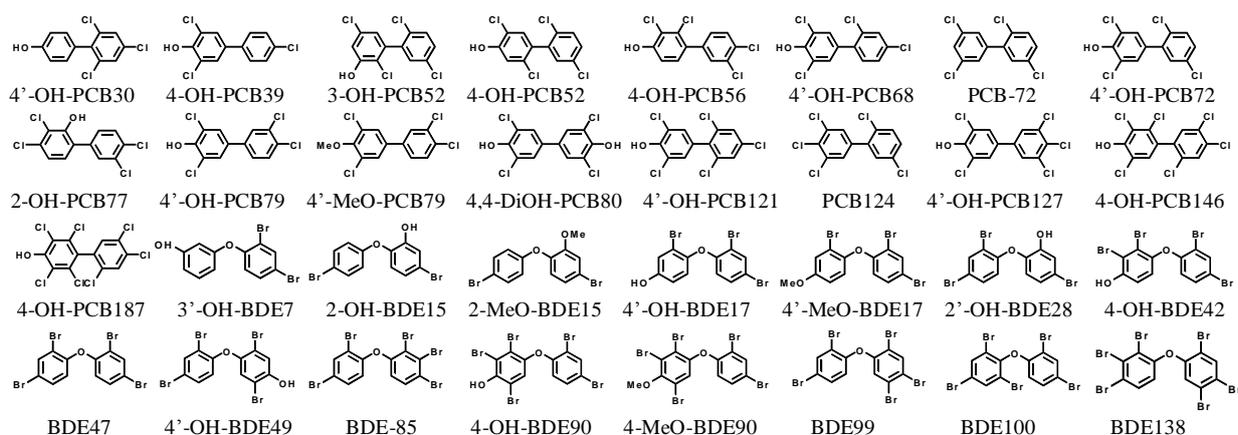


Fig.1 Chemical structures of compounds used in this study

We found that OH-PCBs and OH-PBDEs exhibited significant thyroid hormone-disrupting activity, as well as estrogenic activity. We also clarified the structure-activity relationship for thyroid hormone and estrogenic activity of these compounds.

Materials and Experiments

OH-PCBs were synthesized by the reported method (Bergman et al., 1994). OH-PBDEs were synthesized or obtained from Accu Standard (New Haven, CT, USA). The rat pituitary cell line, MtT/E-2 was constructed by our laboratory. (Fujimoto et al., 1999)

Competitive binding assay to thyroid hormone receptor - Nuclear extracts of MtT/E-2 were used for the assay, since this cell line expresses large amounts of thyroid hormone receptor. Test chemicals and $^{125}\text{I-T}_3$ were incubated with the nuclear suspension. After incubation, the radioactivity of the pellets was counted with a gamma counter.

Growth hormone (GH) production assay in GH3 cells - Test compounds were added to cultured GH3 cells. After incubation for two days, growth hormone in the culture medium was measured by radioimmunoassay. (Kitamura et al., 2002)

Thyroid hormone-dependent reporter gene assay - We transfected MtT/E-2 cells with reporter plasmid, pTRE-TK-Luc, a gift from Dr. T. Nagaya at Nagoya University (Nagaya et al., 1992) and expression plasmid, pSG5-rTR β . After transfection, cells were treated with various concentrations of test chemicals. After incubation, the assay was performed with a Dual Luciferase assay kitTM.

Assay of estrogenic activity - We transfected MCF-7 cells with reporter plasmid (pERE3-SV40-Luc). After transfection, cells were treated with various concentrations of test chemicals.

Results

Competitive binding assay for thyroid hormone-like compounds

The inhibitory effects of OH-PCBs and OH-PBDEs on binding of triiodothyronine (T₃) to TR were examined. T₃ competitively inhibited the binding of $^{125}\text{I-T}_3$ (1×10^{-10} M) to TR in the concentration range of 1×10^{-7} - 1×10^{-4} M.

(1) **Competitive binding assay of OH-PCBs** 4-OH-PCB39, 4'-OH-PCB68, 4'-OH-PCB72, 4'-OH-PCB79, 4,4'-diOH-PCB80, 4'-OH-PCB121, 4'-OH-PCB127, 4-OH-PCB146 and 4-OH-PCB187 markedly inhibited the binding of $^{125}\text{I-T}_3$ to TR. 4'-OH-PCB30, 3-OH-PCB52, 4-OH-PCB52, 4-OH-PCB56, PCB72, 2-OH-PCB77 and PCB124 showed little or no affinity for TR.

(2) **Competitive binding assay of OH-PBDEs** 2'-OH-BDE28, 4-OH-BDE42 and 4-OH-BDE90 markedly inhibited the binding of $^{125}\text{I-T}_3$ to TR. 2-OH-BDE15, 4'-OH-BDE17 and 4'-OH-BDE49 exhibited weak TR binding activity. BDE-47, BDE-85, BDE-99, BDE-100 and three methoxy-PBDEs showed little or no affinity for TR.

Elevated binding affinity was observed for OH-PCBs with halogen substitution adjacent to the hydroxyl group on the phenyl group. In contrast, PCBs and PBDEs without the hydroxyl group and methoxy-PBDEs showed little or no affinity.

Thyroid-hormonal activity of OH-PCBs and OH-PBDEs in cell culture

(1) **GH release assay of OH-PCBs** The thyroid hormone activity of OH-PCBs were examined by measuring the ability of these compounds to induce the production of GH in GH3 cells. 4'-OH-PCB79, 4,4'-diOH-PCB80 and 4-OH-PCB146 induced GH release. However, 3-OH-PCB52, 4-OH-PCB52 and PCB72 did not. No compound showed antagonistic action towards GH production induced by T₃.

(2) **Reporter gene assay of OH-PBDEs** - We found that 4'-OH-BDE17 showed agonistic activity in the thyroid hormone-responsive reporter assay in MtT/E-2 cells. 4-OH-BDE42, BDE-47 and BDE-100 showed weak activity. However, 2-OH-BDE15, 2-MeO-BDE15, 2'-OH-BDE28, 4'-OH-BDE49,

BDE-85, BDE-99 and 4-OH-BDE90 exhibited little or no activity. None of the compounds tested had a significant inhibitory effect on the antagonistic activity induced by T₃.

Agonistic activities of BDE47, PCB72 and BDE100 might be attributable to metabolic activation by hydroxylation in cells.

Estrogen-dependent reporter gene assay of OH-PCBs and OH-PBDEs

(1) Reporter gene assay of OH-PCBs 4'-OH-PCB30 exhibited a significant estrogenic activity in the estrogen-responsive reporter assay in MCF-7 cells. 3-OH-PCB52, 4-OH-PCB52, 4-OH-PCB56, 2-OH-PCB77 and 4'-OH-PCB79 exhibited low activity. However, PCB72, 4,4'-diOH-PCB80, 4'-OH-PCB146 and PCB124 showed no estrogenic activity.

(2) Reporter gene assay of OH-PBDEs 3'-OH-BDE7 and 4'-OH-BDE17 exhibited estrogenic activity. However, 4-OH-BDE42, 3-OH-BDE47, 4'-OH-BDE49, 4-OH-BDE90, 4-MeO-BDE90 and BDE-138 showed little or no activity.

These experiments indicate that the hydroxyl group of OH-PCBs and OH-PBDEs is essential for estrogenic activity, but hydroxylated PCBs and PBDEs with chlorine or bromine substituent show decreased estrogenic activity.

Discussion

We present evidence that OH-PCBs and OH-PBDEs exhibit thyroid hormone and estrogenic activity through interaction with TR and ER, respectively. However, PCBs and PBDEs without the hydroxyl group showed no thyroid hormone activity. OH-PCBs and OH-PBDEs substituted with halogen adjacent to the hydroxyl group on phenyl ring showed enhanced TR-binding activity. The chemical structures of these compounds resemble that of thyroid hormone, generally being more similar to T₄ than to T₃. Because the atomic size of chlorine and bromine is smaller than that of iodine, two or three chloro or bromo substituents may be favorable for binding to TR. The results of our GH release assay in GH3 cells and TR reporter assay in MtT/E-2 cells indicated that some OH-PCBs and OH-PBDEs show agonistic activity at TR. In contrast, it was reported that synthesized hydroxylated PCB acts as an antagonist by suppressing the interaction between TR and a coactivator (Iwasaki et al., 2002; Kojima et al., 2009). This apparent discrepancy between the two experiments may be attributed to the use of different cell lines. The inhibitory potencies of OH-PCBs and OH-PBDEs in the TR binding assay were generally lower than the agonistic activities observed in TR reporter assay. This may be due to factors such as the cell permeability of the test compound and binding to serum proteins, such as transthyretin. This result suggests that the thyroid hormone-disrupting action of OH-PCBs and OH-PBDEs may be mediated through interaction not only with TR, but also with transthyretin.

In contrast, we found that OH-PCBs and OH-PBDEs without halogen atoms adjacent to the hydroxyl group showed estrogenic activity in an estrogen-responsive reporter assay using MCF-7 cells. However, PCBs and PBDEs substituted with halogen adjacent to the hydroxyl group on phenyl ring exhibited little estrogenic activity. These results are consistent with the estrogenic activity of OH-PCBs reported elsewhere. (Korach et al., 1988; Ramamoorthy et al., 1997)

It is interesting that the thyroid hormonal activity of OH-PCBs and OH-PBDEs which have halogen substitution adjacent to the hydroxyl group are markedly increased, whereas their estrogenic activity is not. The results suggest that OH-PCBs and OH-PBDEs exhibit endocrine-disrupting action via effects on at least two different hormonal activities *in vivo*. (Kitamura et al., 2005a, 2005b, 2008)

Conclusion

Some OH-PCBs and OH-PBDEs showed thyroid hormonal activity and estrogenic activity. The structural requirement of OH-PCBs and OH-PBDEs for thyroid hormonal activity is halogen

substitution adjacent to the hydroxyl group. In contrast, the requirement for estrogenic activity is a hydroxyl group, but halogen substitution reduces the estrogenic activity (Fig.2). It is important to understand the key structural requirements for thyroid hormone and estrogenic activity in xenobiotics. Furthermore, the possibility of multiple hormone-like actions *in vivo* should be taken into consideration in risk assessment of environmental chemicals.

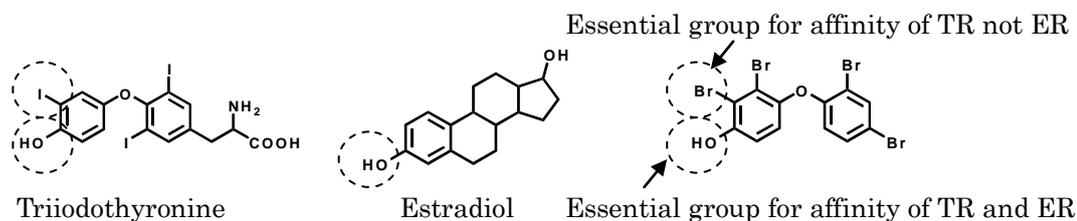


Fig.2 Structural Requirements for TR and ER

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Acknowledgments

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