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Structure-dependent activities of hydroxylated polybrominated diphenyl ethers on human estrogen receptor

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) have been shown to affect the estrogen receptor (ER) signaling pathway, and one of the proposed disruption mechanisms is direct binding of hydroxylated PBDE (OH-PBDE) to ER. In this paper, the binding affinity of 22 OH-PBDEs with different degrees of bromination to ER was assessed quantitatively using a surface plasmon resonance biosensor technique. Seven OH-PBDEs were found to bind directly with ER with K_D ranging from 1.46×10^{-7} M to 7.90×10^{-6} M, and the affinity is in the order of 6-OH-BDE-047 ≧ 4′-OH-BDE-049 > 4′-OH-BDE-017 > 6′-OH-BDE-099 ≧ 5′-OH-BDE-099 > 2′-OH-BDE-007 > 3′-OH-BDE-028. In MVLN luciferase gene reporter assays, 10 low-brominated OH-PBDEs induced luciferase activity alone, but are 10^5 to 10^7 fold less potent than E_2. Their estrogenic activity is in the order of 4′-OH-BDE-049 > 4′-OH-BDE-017 > 2′-OH-BDE-007 > 3′-OH-BDE-028 > 3-OH-BDE-047 ≧ 3′-OH-BDE-007. The good correlation between estrogenic activity and ER binding affinity of the low-brominated OH-PBDEs strongly suggest that these compounds induce ER transcriptional activity by binding directly with ER. The other 12 high-brominated OH-PBDEs inhibited luciferase activity of E_2 to various degrees, demonstrating their antagonistic activity. Molecular docking analysis of the ER/OH-PBDE complexes revealed two distinctive binding modes between low- and high-brominated OH-PBDEs which provided rationale for the difference in their ER activity.

1. Introduction

Over the past few decades, polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardant additives in a variety of industrial and consumer products. As a result, elevated levels of PBDEs have been detected in environmental media and the bodies of human and wildlife (Hites, 2004; Mazdai et al., 2003; Meironyte et al., 1999). In 2004, the European Union began to prohibit the use of two PBDE commercial mixtures, penta-BDE and octa-BDE. Later in 2006, the US manufacturer stopped manufacturing these two PBDE mixtures. But due to their persistence in the environment and bioaccumlative nature in the food web, PBDE concentrations in the environment have been increasing (Schecter et al., 2005; She et al., 2002). High levels of both PBDEs and hydroxylated PBDEs (OH-PBDEs) have been found in human serum samples (Sjodin et al., 2004; Athanasiadou et al., 2007). The sources of OH-PBDEs are either the bio-transformation of PBDEs, or the natural products of some marine invertebrates (Wiseman et al., 2011).

To date, many studies have focused on PBDEs' toxic effects through some nuclear hormone receptor (NR) mediated pathways, in particular the pathways involving thyroid hormone receptor (TR), estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) (Hamers et al., 2006; Kitamura et al., 2008; Wiseman et al., 2011). The estrogenic activity of PBDEs has been demonstrated in both experimental animals and cells. Several studies have shown that, when exposed to rats, BDE-99 disrupted the regulation of estrogen target genes (Ceccatelli et al., 2006), impaired spermatogenesis (Kuriyama et al., 2007), decreased circulating sex steroids and reduced anogenital distance in males (Lilienthal et al., 2006). Recent studies have shown that the neurotoxicity and endocrine disrupting effects of PBDEs might be enhanced by metabolizing to OH-PBDEs (Meerts et al., 2001; Hamers et al., 2006; Dingemans et al., 2008). As a result, OH-PBDEs have become the focus in toxicity studies.

The mechanisms of PBDE and OH-PBDE toxicity are complex and have not been fully resolved. In the only report on direct ER binding measurement, six OH-PBDEs (4′-OH-BDE-17, 2′-OH-BDE-28, 4-OH-BDE-42, 3-OH-BDE-47, 6-OH-BDE-47, 4′-OH-BDE-49) showed competitive binding with ERα (Mercado-Feliciano and Bigsby, 2008). In vitro estrogenic activity of OH-PBDEs has been investigated by cell transactivation assays in different cell lines. Two thyroid hormone-like OH-PBDEs were tested to be ER agonists by ER-CALUX assays (Hamers et al., 2006, 2008; Meerts et al., 2001). Using ER-CALUX assay in MCF-7 breast cancer

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cells, Kitamura et al. found that 3′-OH-BDE-7 and 4′-OH-BDE-17 exhibited estrogenic activity, but 4-OH-BDE-42, 3-OH-BDE-47, 4′-OH-BDE-49, and 4-OH-BDE-90 did not (Kitamura et al., 2008). Similarly, 4′-OH-BDE-17 and 4-OH-BDE-42 showed estrogenic activity, whereas 6-OH-BDE-47 antagonized the effect of E_2 at high concentrations (Mercado-Feliciano and Bigsby, 2008). In addition, 4′-OH-BDE-17 and 4-OH-BDE-42 showed agonistic activities in ER-CALUX assays in BG1Luc4E2 ovarian cancer cells, and 4′-OH-BDE-49 showed antagonistic activity (Kojima et al., 2009). Recently, Liu et al. studied the estrogenic potential of four OH-PBDEs by reporter gene assays. It was found that 4′-OH-BDE-17 showed strong estrogenic activity, whereas 2′-OH-BDE-28, 6-HO-BDE-47, and 4′-OH-BDE-49 exhibited anti-estrogenic potency (Liu et al., 2011).

Although the data are contradictory for some OH-PBDEs, the general trend seems to be that low-brominated OH-PBDEs act as ER agonists, whereas high-brominated ones are antagonists. However, due to the limited number of OH-PBDEs investigated in each study, the relationship between OH-PBDE structure and their estrogenic activity cannot be established convincingly. Furthermore, most of the studies employed transactivation reporter gene assays to characterize receptor-mediated estrogenic activity and elucidate the mechanisms of action without assessing the binding affinity of OH-PBDEs directly with ER. As suggested by Freyberger and Schmuck (Freyberger and Schmuck, 2005), some compounds could exhibit anti-estrogen-like activity without true binding affinity for ER. Therefore, it is advisable to investigate both direct ER binding affinity and ER-mediated transcription activity for the same compound.

In the present study, we employed a surface plasmon resonance (SPR) biosensor technique and measured the binding affinity of twenty-two OH-PBDEs with ER-LBD. These OH-PBDEs carry different numbers of bromines at different positions of the diphenyl rings. The SPR sensor technique can monitor the molecular interactions in real time, without labeling, and also provides kinetic parameters for ligand association and dissociation. By using the combination of SPR ligand binding assay, transactivation reporter gene assay and molecular docking, the estrogenic activity of these OH-PBDEs was investigated and analyzed.

2. Materials and methods

2.1. Materials

All the twenty two OH-PBDEs including 2′-hydroxy-4-monobromodiphenyl ether (2′-OH-BDE-003), 3′-hydroxy-2,4-dibromodiphenyl ether (3′-OH-BDE-007), 2′-hydroxy-2,4-dibromodiphenyl ether (2′-OH-BDE-007), 4′-hydroxy-2,2′,4-tribromodiphenyl (4′-OH-BDE-017), 3′-hydroxy-2,4,4′-tribromodiphenyl ether (3′-OH-BDE-028), 2′-hydroxy-2,4,4′-tribromodiphenyl ether (2′-OH-BDE-028), 4-hydroxy-2,2′,3,4′-tetrabromodiphenyl ether (4-OH-BDE-042), 4′-hydroxy-2,2′,4,5′-tetrabromodiphenyl ether (4′-OH-BDE-049), 3-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (3-OH-BDE-047), 5-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (5-OH-BDE-047), 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE-047), 2′-hydroxy-2,3′,4,5′-tetrabromodiphenyl ether (2′-OH-BDE-068), 4-hydroxy-2,2′,3,4′,5-pentabromodiphenyl ether (4-OH-BDE-090), 6-hydroxy-2,2′,3,4,4′-pentabromodiphenyl ether (6-OH-BDE-085), 6-hydroxy-2,2′,3,3′,4-pentabromodiphenyl ether (6-OH-BDE-082), 6′-hydroxy-2,2′,4,4′,5-pentabromodiphenyl ether (6′-OH-BDE-099), 5′-hydroxy-2,2′,4,4′,5-pentabromodiphenyl ether (5′-OH-BDE-099), 3-hydroxy-2,2′,4,4′,6-pentabromodiphenyl ether (3-OH-BDE-100), 6-hydroxy-2,3,3′,4,4′,5′-hexabromodiphenyl ether (6-OH-BDE-157), 6-hydroxy-2,2′,3,4,4′,6′-hexabromodiphenyl ether (6-OH-BDE-140), 3′-hydroxy-2,2′,4,4′,5,6′-hexabromodiphenyl ether (3′-OH-BDE-154), and 4-hydroxy-2,2′,3,4′,5,6,6′-heptabromodiphenyl ether (4-OH-BDE-188) were purchased from AccuStandard (New Haven, CT, USA) as solutions in acetonitrile with concentrations ranging from 10 μg/mL to 50 μg/mL. They were of 99% or higher purity. More concentrated stock solutions for our experiments were prepared by evaporating the solvent of the purchased solutions under a gentle stream of nitrogen and then dissolving the solid into 1 mM solutions in DMSO. 17β-Estradiol (E_2) and 4-hydroxytamoxifen (OHT) were bought from Sigma Aldrich (St. Louis, MO, USA). The chemical structures of these compounds are shown in Fig. 1. Testosterone was from Selleck Chemicals (Houston, TX, USA). N-(3-dimethylaminopropy)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), GST capture kit (with polyclonal anti-GST antibody, recombinant GST and glycine-HCl pH 2.1 as regeneration solution) and CM5 sensor chips were from GE Healthcare (Uppsala, Sweden). Recombinant human estrogen receptor alpha ligand binding domain (hERα-LBD) with GST-tag was from Invitrogen (Carlsbad, CA, USA).

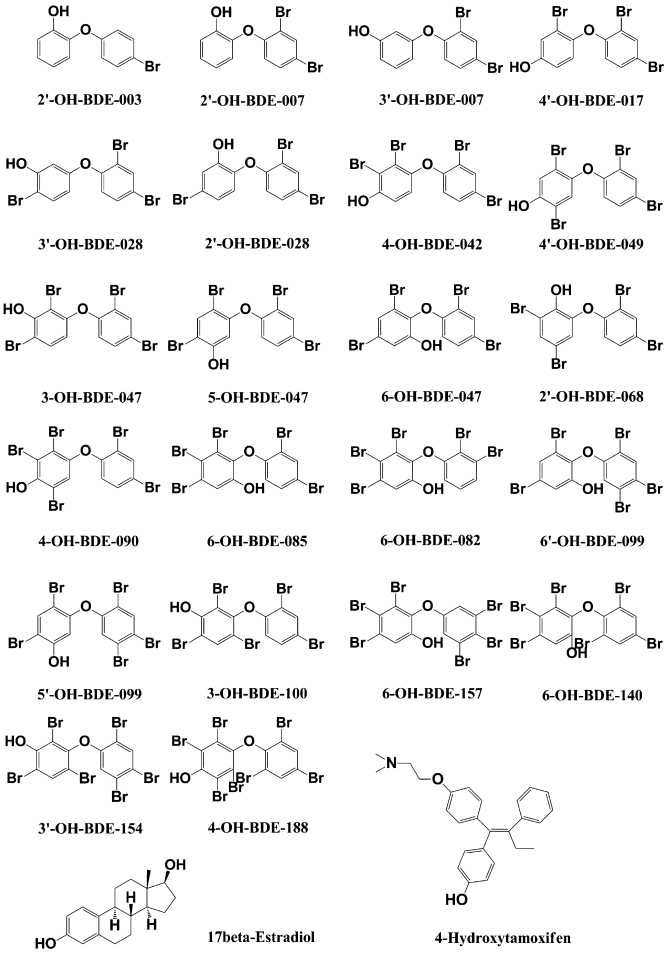

Fig. 1. Structures of the twenty-two hydroxylated polybrominated diphenyl ethers, estradiol, and 4-hydroxytamoxifen investigated in this study.

2.2. Measurement of OH-PBDEs binding with hERα-LBD

Direct binding of OH-PBDEs with hERα-LBD was measured by surface plasmon resonance (SPR) technique on a Biacore 3000 optical biosensor system (Biacore, Uppsala, Sweden). The instrument measures the SPR signal of the sensor in real time as a result of ligand binding to the receptor immobilized on the sensor surface and subsequent change of the refractive index at the sensor/liquid interface. The design of the sensor surface is illustrated in Figure S1.

Firstly, anti-GST antibody was immobilized on the CM5 sensor surface in both the sample cell and control cell, using the standard amine-coupling procedure and HBS-EP (0.01 M HEPES, 0.15 M NaCl, 0.005% v/v Surfactant P20, pH 7.4) as the running buffer (Karlsson et al., 2000). Forty microliters of a 30 μg/mL anti-GST antibody solution (in 10 mM sodium acetate, pH 5.0) was flew through the activated sensor surface for 4 min at 10 μL/min, followed by a 7 min injection of 1 M ethanolamine (pH 8.5) to block the remaining activated group on the surface. The high affinity sites of anti-GST antibody on the surface were blocked by running 2 cycles of 4-min injection of recombinant GST at a concentration of 5 μg/mL in HBS-EP buffer to make the surface regeneration possible. Secondly, GST tagged hERα-LBD (1.5 μM in 20 mM sodium phosphate, 150 mM sodium chloride, pH 7.4) was injected over the sample cell for 5 min. The receptor was captured by the anti-GST antibody on the sensor surface, resulting in an SPR signal of about 1800 RU.

Ligand binding measurement was carried out by passing a ligand solution through both the sample cell (with ER) and the control cell (without ER) at 30 μL/min, and recording the SPR signal as a function of time. Immediately before the measurement, OH-PBDEs stock solutions in DMSO were diluted into 20 mM sodium phosphate, 150 mM sodium chloride, pH 7.4 to yield 30 μM aqueous stock solutions (with 3% residual DMSO). Concentration series of a ligand were then prepared by serial dilution of the aqueous stock solution in the same buffer. The association and dissociation phase of the SPR measurement were 60 s and 180 s respectively for