UNDERSTANDING THE ESTROGENIC AND ANTIESTROGENIC ACTIVITIES OF SELECTED HYDROXYLATED POLYBROMINATED DIPHENYL ETHERS USING MOLECULAR SIMULATION

Zhang A Q, Mu YS, Lin Y, Wang L S

State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, 210093, China

Abstract
Six OH-PBDEs, which have been reported as weak ERα ligands, were selected for mechanism exploration in this work, and their binding interactions with ERα were explored through molecular simulation. The results indicated that both mode of interaction and intensity of interaction may decide the estrogenic and anti-estrogenic activities of the chemicals. Three para-hydroxylated PBDEs display higher binding affinity for ERα, while their different binding sites and orientation in the pocket lead to different estrogenic effect. A strong hydrogen bond net is formed between 4'-OH-BDE-17 with Arg394 and Glu353, while the phenolic hydroxyl of 4'-OH-BDE-49 and 4-OH-BDE-42 only can interact with His524. However, all present comparatively similar alignment as E_2 in the pocket, thereby help H12 closing the narrow entrance to the pocket, and allow coactivators to bind. On the contrary, one phenyl ring of the two ortho-hydroxylated PBDEs, 2'-OH-BDE-28 and 6-OH-BDE-47, stretches out and points to the pocket entrance. Such may push away H12, induce a different orientation of H12, and finally adapt the protein conformation into its antagonism status. In addition, steric effect of Br substitution adjacent to the phenolic hydroxyl may result in intensity decrease of the related hydrogen bonds, which alters the activity of OH-PBDEs.

Introduction
Polychlorinated diphenyl ethers (PBDEs) belong to a kind of additive brominated flame retardants which are widely used in many commercial products. As stable compounds, PBDEs have become ubiquitous in the environment[1,2]. Moreover, PBDEs are suspected to initiate estrogen receptor-mediated endocrine disrupting effects because of their structure similarity to polychlorinated biphenyls[3,4]. Recent studies reveal that the hydroxylated metabolites (OH-PBDEs) may cause more severe cytotoxicity and higher endocrine disrupting potency[5-8]. Due to its strong hydrophobicity and environmental persistence, high bioaccumulation potential and close linkage with possible human health impacts, some PBDEs has just been put in the control list of the Stockholm Convention on Persistent Organic Pollutants in May, 2009.

DE-71 is a commercial mixture of mostly tetra- and penta-PBDEs. Mercado-Feliciano and Bigsby found it can not only be metabolized in the mouse to produce OH-PBDEs but also exhibit mild estrogenic activity[9,10]. Further estrogenicity test of DE-71 and 6-OH-PBDEs metabolites adopted both recombinant ERα binding assay and estrogen response element-luciferase assays. They confirmed that OH-PBDEs metabolites of DE-71 are weak ERα ligands (Table 1). Specifically, para-hydroxylated PBDEs exhibited 10- to 30-fold higher ERα affinity compared with ortho-hydroxylated ones, while two ortho-hydroxylated PBDEs were antiestrogenic in the stable reporter assay[11]. In this study, molecular simulation methods were applied to elucidate and differentiate the estrogenic and antiestrogenic activities of six OH-PBDEs.

Materials and Methods
Considering hydrophobic property of OH-PBDEs, the crystal structure of LBD in human ERα (*h*ERα) with 2-[5-hydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl] ethanenitrile (W244) (PDB ID: 1X7E) was selected for constructing the receptor model of ER. All molecular simulation studies were performed on DELL Precision 370 work station and SYBYL7.3 software package (Tripos Inc. Co., 1699 South Hanley Road, St. Louis, MO 63144, USA). The relevant energy optimization of ligands was conducted using Tripos Force Field with atom charge calculated by Gasteiger-Hückel method, and the criterion for energy gradient convergency was 0.05kcal·mol^{-1}. SiteID module was used for searching the active binding pocket in the receptor. The molecular docking process was performed by Surflex method in SYBYL7.3. The total score (TS) was then calculated, and

the contributions of different interaction forces such as crash (CE) and polar effect (PE) were recorded. Besides, the flexibility of key residues in the active binding pocket was also taken into consideration by small-scale molecular dynamics simulations[12].

Result and Discussion

E_2 and all studied OH-PBDEs can be docked into the active site of *h*ERα. Table 1 listed the relevant data. Figure 1 showed that the key residues involved in hydrogen bonding process were Glu353, Arg394, and His524. Simulation result shown in Figure 1A is confirmed by the reported conformation of E_2-*h*ERα, and ligand recognition is successfully obtained through a combination of specific hydrogen bonds with the above resides and the complementarities of the binding cavity to ligand non-polar character. The phenolic hydroxyl on the A-ring builds strong direct hydrogen bonds to the oxygen atom on carboxylate of Glu353 and NH_2 on guanidinium group of Arg394. The OH on D-ring only makes a single hydrogen bond with His524[13]. The strong hydrogen bond net also consist with the highest PE value in Table 1, which decided by hydrogen bonding aspects of the complex to a certain extent.

Table 1 Simulation data of E_2 and OH-PBDEs

Compound	Relative Affinity (%)[a]	Relative effect (ratio)[b]	Cotreatment Effect[c]	TS	CE	PE
E_2	1.00	1.00	-	6.56	-2.36	3.79
4'-OH-BDE-17	0.03	1.30	estrogenic	4.53	-1.39	3.07
4'-OH-BDE-49	0.03	0.36	estrogenic	3.03	-2.52	2.01
4-OH-BDE-42	0.005	NA	NS	2.92	-2.66	1.99
6-OH-BDE-47	0.001	-	antiestrogenic	2.87	-3.08	1.84
2'-OH-BDE-28	0.001	-	antiestrogenic	0.27	-4.26	1.71
3-OH-BDE-47	0.001	-	estrogenic	0.69	-2.75	0

Abbreviation : NA, not available due to insufficient data; NS, not statistically significant.

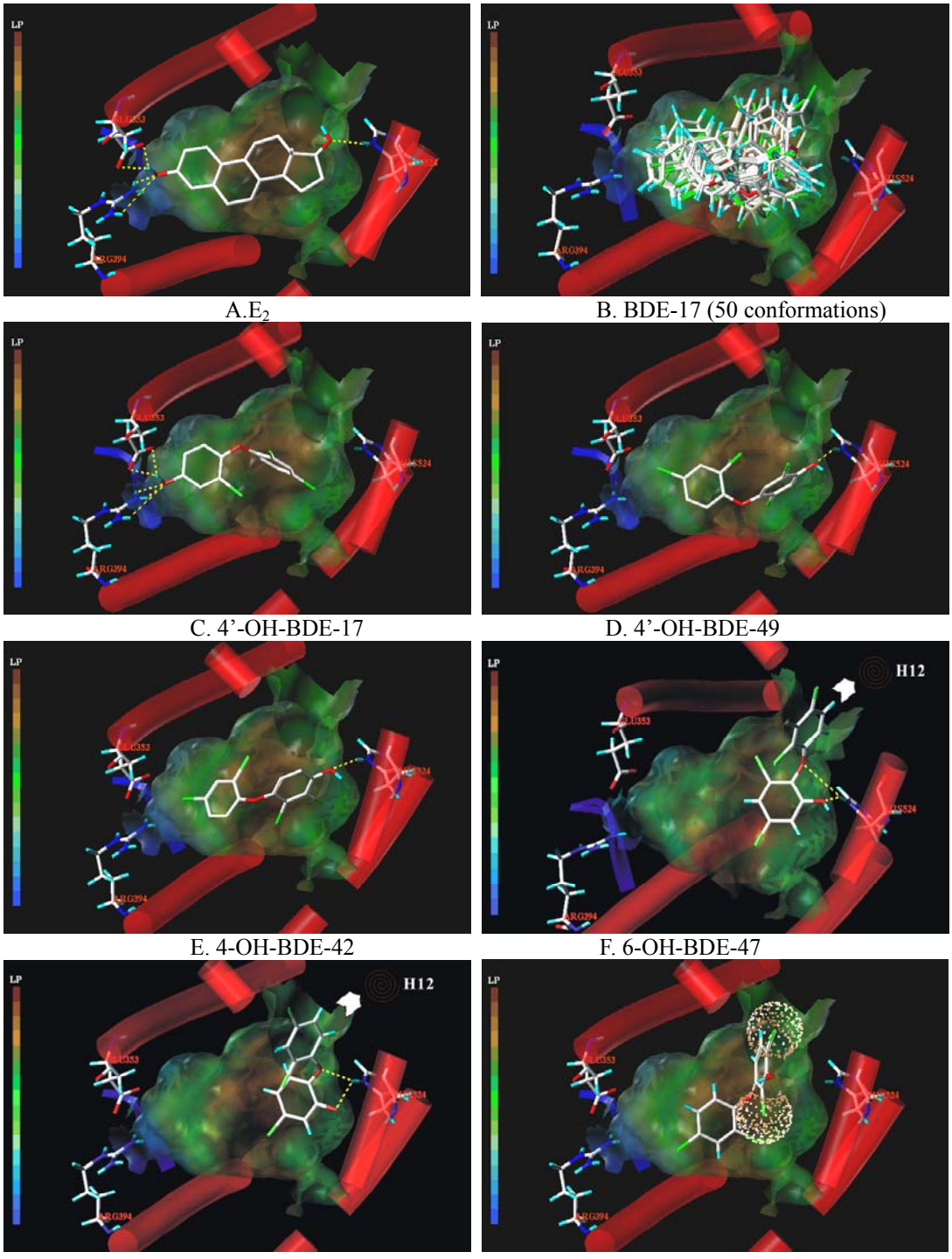
[a] ERα relative binding affinity, cited from literature 11.

[b] Relative effect in ERE-luciferase induction, cited from literature 11.

[c] Effect in ERE-luciferase induction in BG1luc4E2 cells after cotreatment with E_2, cited from literature 11.

In fact, the parent PBDEs also enter the binding pocket of modeled *h*ERα although there is no typical hydrogen bond formed in the receptor-ligand complex. The hydrophobic contact and van der Waals' force play an important role in BDE17-*h*ERα interaction, and Figure 1B provides clear view of such interaction. 50 possible conformations of BDE17 were listed in Figure 1B, which gives an example of relatively free positioning of BDE17 within the hydrophobic pocket since no hydrogen bonds make it fix. The extremely low binding affinity of BDE17 to the receptor explains the phenomenon that DE-71 can not replace E_2 from ERα. As for the estrogenicity shown in the estrogen response element-luciferase assays, it might involve other possible binding site on the surface of ER[14,15].

Three para-hydroxylated PBDEs display higher binding affinity for ERα compared with other OH-PBDEs, and their hydrogen bond formation with ERα mimics the E_2-*h*ERα interaction. Each para-OH-PBDEs can insert deeply into the pocket, and hereby help helix 12 (H12) covering the narrow entrance to the pocket, stabilize the alignment of H12 over the cavity, and allow coactivators to bind and invoke following estrogenic activity[13,16]. Nevertheless, their different binding sites and orientation in the pocket lead to different estrogenic effect. Figure 1C exhibited a strong hydrogen bond net between 4'-OH-BDE-17 with several key residues including Arg394 and Glu353, while the phenolic hydroxyl and connected phenyl ring of 4'-OH-BDE-17 were fastened into the crack formed by Leu387, Arg394, and Glu353, which functioned similarly to the binding mode of estrogen in ERα. Thus leads to both high binding affinity and intensive estrogenic effect. As for 4'-OH-BDE-49 and 4-OH-BDE 42, the mode of interaction is quite different (Figure 1D and 1E). The hydrogen atom of their phenolic hydroxyl only formed a hydrogen bond with His524 in the active pocket, and the steric effect of adjacent bromine would alter the orientation of the phenolic hydroxyl as well as the hydrogen binding intensity,



A. E_2 B. BDE-17 (50 conformations)

C. 4'-OH-BDE-17 D. 4'-OH-BDE-49

E. 4-OH-BDE-42 F. 6-OH-BDE-47

G. 2'-OH-BDE-28 H. 3-OH-BDE-47

Figure 1. Molecular Simulations of ligand-ERα interaction. Constructed *h*ERα binding site is displayed as folds and helix, and the binding cavity is shown by lipophilic potential (LP) surface surrounding the residues. Dashed yellow lines indicate the hydrogen bonds, and the key residues and ligands are labeled and displayed as stick modes. The steric effect of bromine atom is depicted by van der Waals' surface as dotted area. White arrow presents the leaving trend of H12.

which interprets relative strong hydrogen bond in 4'-OH-BDE-49-ER complex.

On the contrary, the position of two ortho-hydroxylated PBDEs is quite abnormal. They may present in the conformation shown in Figure 1F and 1G, in which 2'-OH-BDE-28 and 6-OH-BDE-47 lie near the surface of pocket. Such binding mode will push H12 away induce a different orientation of H12. The agonism and antagonism of ERα are closely related processes. Normally, H12 sits snugly over the binding cavity, forms the lid of the cavity, and its inner hydrophobic surface points towards the bound ligands[13,17]. Figure 1 indicates that one phenyl ring of the ortho-hydroxylated PBDEs stretches out and projects to the H12. Consequently, the alignment of H12 over the cavity is prevented by 2'-OH-BDE-28 and 6'-OH-BDE-47, and the competent transcriptional activation function (AF-2) that is capable of interacting with coactivators can not be generated due to the repositioning of H12. As for the only meta-OH-PBDE tested, steric effect of Br substitution adjacent to phenolic hydroxyl may result in difficulty in forming hydrogen bond with the key residue (Figure 1H). However, the rotation of the key residue to reinforce ligand-receptor complexes can successfully be applied in receptor preparation step, and the resulted ligand binding mode changes dramatically to a type that can stabilize the position of H12 over the binding cavity.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (No. 20777035, No. 20737001) and the National High Technology Research and Development Program of China (863 Program) (No. 2007AA06Z416 and 2006AA06Z424).

References

1. Eriksson P, Jakobsson E, and Fredriksson A. *Environ Health Perspect* 2001; 109:903.
2. de Wit C.A. *Chemosphere* 2002; 46: 583.
3. Pijnenburg A.M., Everts J.W., De Boer J., and Boon J.P. *Rev Environ Contam Toxicol* 1995; 141:1.
4. Dang V.H., Choi K.C., and Jeung E.B. *Toxicol Sci* 2007; 97:504.
5. Haglud P.S., Zook D.R., Buser H-R, and Hu J.W. *Environ Sci Technol* 1997; 31:3281.
6. Valters K., Li H.X., Alaee M., D'Sa I., Marsh G., Bergman A., and Letcher R.J. *Environ Sci Technol* 2005; 39:5612.
7. Ueno D., Darling C., Alaee M., Pacepavicius G., Teixeira C., Campbell L., and Letcher R.J. *Environ Sci Technol* 2008; 42:1657.
8. Meerts I.A., Letcher R.J., Hoving S., Marsh G., Bergman A., and Lemmen J.G. *Environ Health Perspect* 2001; 109:399.
9. Qiu X., Mercado-Feliciano M., Bigsby R.M., and Hites R.A. *Environ Health Perspect* 2007; 115:1052.
10. Mercado-Feliciano M. and Bigsby R.M. *Environ Health Perspect* 2008; 116:605.
11. Mercado-Feliciano M. and Bigsby R.M. *Environ Health Perspect* 2008; 116:1315.
12. van Lipzig M.M.H., ter Laak A.M., Jongejan A, Vermeulen N.P.E., Wamelink M., Geerke D., and Meerman J.H.N. *J Med Chem* 2004; 47: 1018.
13. Brzozowski A.M., Pike A.C.W., Dauter Z., Hubbard R.E., Bonn T., Engström O., Öhman A., Greene G.L., Gustafsson J-A, and Carlquist M. *Nature* 1997, 389: 753.
14. Martin P.M., Berthois Y., Jensen E.V. *Proc Natl Acad SciUSA* 1988; 85:2533.
15. Wang Y., Chirgadze N.Y., Briggs S.L., Khan S., Jensen E.V., and Burris T.P. *Proc Natl Acad Sci USA* 2006; 103:9908.
16. Skafar D.F., Koide S. *Mol Cell Endocrinol* 2006; 246:83.
17. Peräkylä M., Molnár F., and Carlberg C. *Chem Bio* 2004; 11: 1147.