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Molecular docking and molecular dynamics studies on the interactions of hydroxylated polybrominated diphenyl ethers to estrogen receptor alpha

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ABSTRACT

Environmental estrogens have attracted great concerns. Recent studies have indicated that some hydroxylated polybrominated diphenyl ethers (HO-PBDEs) can interact with estrogen receptor (ER), and exhibit estrogenic activity. However, interactions between HO-PBDEs and ER are not well understood. In this work, molecular docking and molecular dynamics (MD) simulations were performed to characterize interactions of two HO-PBDEs (4′-HO-BDE30 and 4′-HO-BDE121) with ERα. Surflex-Dock was employed to reveal the probable binding conformations of the compounds at the active site of ERα; MD simulation was used to determine the detailed binding process. The driving forces of the binding between HO-PBDEs and ERα were van der Waals and electrostatic interactions. The decomposition of the binding free energy indicated that the hydrogen bonds between the residues Glu353, Gly521 and ligands were crucial for anchoring the ligands into the active site of ERα and stabilizing their conformations. The results showed that different interaction modes and different specific interactions with some residues were responsible for the different estrogenic activities of the two HO-PBDEs.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used as flame-retardant additives in electronic circuit boards and household consumer products. As a kind of derivatives of PBDEs, hydroxylated PBDEs (HO-PBDEs) have elicited increasing attention because they have been detected in the wild animals and human tissue samples (Qiu et al., 2009; Zota et al., 2011). Six PBDE congeners 28, 47, 99, 100, 153, and 154 were the most abundant observed in human blood and their mean concentrations ranged from 2.3 to 70 ng/g and from 0.5 to 17 ng/g lipid in the fetal and maternal samples, respectively (Qiu et al., 2009). Over the last few years, scientific studies have suggested that HO-PBDEs might have endocrine disrupting effects (Kitamura et al., 2008; Li et al., 2010; Meerts et al., 2001, 2000). Furthermore, some recent toxicological studies reported that HO-PBDEs exhibited stronger endocrine disrupting effects than either their parent compounds or their derivatives with other substituents (Hamers et al., 2008; Li et al., 2010). In Hamers et al.'s (2008) work, hydroxylated metabolites of BDE-47 exhibited 160–1600 and 2.2–220 times transthyretin (TTR)-binding and estradiol-sulfotransferase (E2SULT)-inhibiting potencies than BDE-47 itself.

The results of in vitro test have suggested that HO-PBDEs of similar molecular structures may display different estrogenic activities, and some structure–activity relationships studies have been performed to explain this phenomenon (Kitamura et al., 2008; Mercado-Feliciano and Bigsby, 2008). Furthermore, competitive binding with estrogen receptor (ER) and blocking endogenous estrogen access were found the major mechanism for the estrogenic activity of HO-PBDEs (Hong et al., 2002). Different binding modes may also result in the activity discrepancies. However, the molecular level understanding of the binding mechanisms between HO-PBDEs and the ER protein still remains limited.

Molecular modeling methods such as molecular docking and molecular dynamics (MD) simulations are powerful tools to reveal detailed information of the protein–ligand interactions at the

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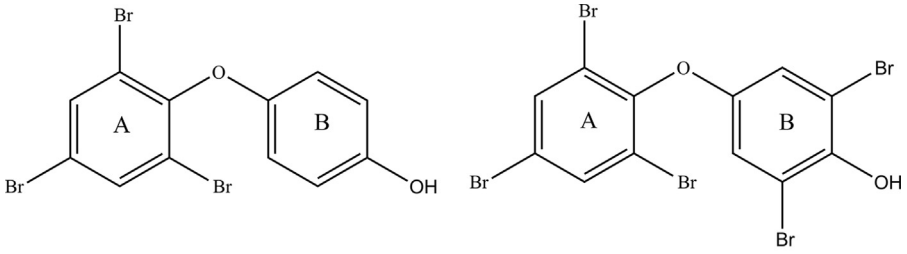

4'-HO-BDE30 4'- HO-BDE121

Fig. 1. Chemical structures of 4′-HO-BDE30 and 4′-HO-BDE121.

molecular and atomic levels (Li et al., 2013; Rajasekaran et al., 2011; Shahlaei et al., 2011; Thorsteinson et al., 2009; Xu et al., 2012). In this study, a molecular computational study was carried out through integrated molecular docking, MD simulations and molecular mechanics-generalized Born surface area (MM-GBSA) calculations. The goal was to: (1) investigate the binding modes between HO-PBDEs and estrogen receptor α (ERα), (2) determine the major contributions to the binding free energy, and (3) reveal how these binding modes affect the activities of HO-PBDEs. To facilitate disclosing the impact of meta-substituents in B benzene ring of HO-PBDEs, two ligands (4′-HO-BDE30 and 4′-HO-BDE121) with the same 2,4,6-bromine on the A benzene ring and 4′-hydroxyl on the B benzene ring were selected (Fig. 1). Their estrogenic activities were reported by Meerts et al (2001) using the T47D.Luc-based ER-CALUX (chemically activated luciferase gene expression) transactivation assay, where the gene for luciferase was under transcriptional control of the response elements for activated ER receptors. The 4′-HO-BDE30 showed high estrogenic potency (EC_{50} was 0.1 μM). In contrast, 4′-HO-BDE121 demonstrated no estrogenic effect within tested concentrations. The binding modes were analyzed in detail and the binding free energies were calculated based on the MD trajectories. Furthermore, the contributions of some important residues (such as Glu353 and Gly521) to the binding were obtained. It was intended that the information acquired from this study would improve our understanding of the binding mechanisms of structurally similar ligands to ERα, and facilitate identification of the structural and conformational characteristics of emerging estrogenic endocrine disrupting chemicals.

2. Methods

2.1. Molecular docking

2.1.1. Preparation of the ligands

The 3D structures of 4′-HO-BDE30 and 4′-HO-BDE121 were constructed using the Sketch Molecule module in SYBYL 7.3 molecular modeling software package (Tripos Inc, St. Louis, MO). Energy minimization and conformational search were performed using the Tripos force field (distance dependent-dielectric function) by the Powell method with a convergence criterion of 0.001 kcal/mol Å and a maximum iteration of 1000. Partial atomic charges were calculated by the Gasteiger–Hückel method (Gasteiger and Marsili, 1980). The minimized structures were used as initial conformations for the molecular docking studies.

2.1.2. Preparation of the protein

The crystal structure of the ERα (PDB code: 1ERE) with the natural ligand 17 β-estradiol (E2) was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/). The structure was prepared in the following procedures: (1) removing the co-crystallized ligand and structural water molecules from the crystal structure, (2) adding hydrogen atoms to the protein by the Biopolymer module implemented in the SYBYL software, and (3) assigning the Kollman-All atom charges to the protein atoms. Finally, the resultant structure was converted from the PDB format to MOL2 and used for the molecular docking experiments.

2.1.3. Molecular docking

After the structures of ligands and protein were prepared, molecular docking was performed using the Surflex-Dock program (Jain, 2003, 2007) implemented in the SYBYL software. In this program, ligands were automatically docked into the binding site of the protein using a protomol based approach with an empirical scoring function and a patented search engine. The detailed algorithm for Surflex-Dock has been described in the literature (Jain, 2007). In this work, the automated docking approach was employed. During this process, two important parameters, i.e., the protomol_bloat and protomol_threshold, which can significantly affect the volume and extent of the protomol, were specified with default values of 1.00 and 0.50, respectively. With other parameters setting default, Surflex-Dock produced the top 10 options of binding conformations for both ligands ranked by total scores.

2.2. MD simulations

In order to detect the conformational changes of the ligands in the binding pocket and validate the binding stability, two 400 ps MD simulations were performed on the docking complexes of 4′-HO-BDE30–ERα and 4′-HO-BDE121–ERα considering the effects of the flexibility of the receptor protein and the water solvation. The MD simulations were performed with the Sander module, implemented in the AMBER 10 software package (Case et al., 2005). The FF03 AMBER force field (Duan et al., 2003) was used to describe the parameters of the protein, whereas the force field parameters for the ligands were generated by the general AMBER force field (Klionsky et al., 2012) using the Antechamber program (Wang et al., 2004). The partial charges for the ligands were calculated by the AM1-BCC charge scheme (Jakalian et al., 2000, 2002). Sodium ions were added to neutralize the system. Each complex was solvated in a cubic box of the TIP3P (Jorgensen et al., 1983) water molecules with a margin of 29 Å in each direction from the ligand. The particle mesh ewald (PME) method (Essmann et al., 1995) was performed to treat long-range electrostatic interactions. A 12 Å cut-off distance was set for the non-bonded interactions (Sun et al., 2011).

Prior to MD simulations, the solvated systems were energy-minimized by a three-step minimization procedure to eliminate possible bad contacts. First, the hydrogen atoms, the solvent, and the ligand and solvent were successively minimized, while restraining the rest with a force constant of 10 kcal/mol $Å^2$. In each procedure, 1000 steps of the steepest descent following 9000 steps of conjugate gradient were performed. Second, the temperature of the entire solvent system was gradually increased from 0 to 200 K by 25,000 steps running to ensure that water molecules were fully optimized. Finally, the whole system was minimized with no restraint by 1000 steps of the steepest descent and 49,000 steps of conjugate gradient. Afterward, 400 ps MD simulations were performed as follows: First, the system was gradually heated from 0 to 300 K over a period of 50 ps and maintained at 300 K followed by 100 ps of constant pressure equilibration. Second, a production run for 250 ps was performed at 300 K with 1.0 atm pressure. The time step used for the MD simulations was set to 1.0 fs. During the MD simulations process, snapshots (coordinates) were collected every 1 ps. The dynamics equilibration was monitored by checking the stability of the system temperature and pressure.

2.3. Calculations of binding free energy and energy decomposition

The MM-GBSA approach implemented in the Amber software was applied to compute the binding free energy (ΔG_{bind}, kcal/mol) of the protein–ligand complexes. The last 250 snapshots of the MD trajectory were used in the calculations. The binding free energy was evaluated as follows:

$$\Delta G_{bind} = G_{cpx} - (G_{pro} + G_{lig}) = \Delta E_{MM} + \Delta G_{sol} - T\Delta S \quad (1)$$

$$\Delta E_{MM} = \Delta E_{val} + \Delta E_{ele} + \Delta E_{vdw} \quad (2)$$

$$\Delta G_{sol} = \Delta G_{p} + \Delta G_{np} \quad (3)$$

where ΔG_{cpx}, ΔG_{pro}, and ΔG_{lig} represent the free energy changes of the protein–ligand complexes, protein, and ligands, respectively. The molecular mechanical