Effects of substituent position on the interactions between PBDEs/PCBs and DOM

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Dissolved organic matters (DOM) have important influence on the environmental behavior and fate of organic pollutants, therefore rationalization of interaction mechanisms between pollutants and DOM has become a hot topic in the field of environmental studies. In this paper, polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs), widely detected pollutants, were chosen as target compounds. The effects of substituent position on the interaction between PBDEs/PCBs and DOM were explored. Equilibrium dialysis technique combined with quantum chemistry and molecular docking calculations were employed to reveal the interaction mechanism from the view of charge distribution and molecular conformation. It is shown that non-*ortho*-substituted isomers have larger volumes and stronger hydrophobicity than those of *ortho*-substituted, therefore non-*ortho*-substituted isomers bind more favorably with DOM by hydrophobic partition. By calculating the atomic charge distribution of target chemicals and Leonardite humic acid (LHA) molecular model, π - π interactions between the aromatic rings of target chemicals with LHA are proposed and further proved in molecular docking calculations. There were 10, 8, 6 docking conformations demonstrating π - π interaction with LHA for CB-77, BDE-77 and BDE-47, respectively, but none was found for CB-47. By comparing the change of dihedral angle of the aromatic rings and energy barrier, non-*ortho*-substituted PBDEs/PCBs have larger dihedral angle adjustment space and flexibility, which results in stronger interaction and binding capability with DOM than *ortho*-substituted isomers. This paper shed some lights on the effect of substituent position on the environmental behaviors of PBDEs and PCBs.

dissolved organic matter, polybrominated diphenyl ethers, polychlorinated biphenyls, substituent position, dihedral angle, π - π interaction

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The environmental behavior, ecological effects and pollution control of the persistent organic pollutants (POPs) are currently one of important worldwide environmental issues [1–4]. Polybrominated diphenyl ethers (PBDEs), widely used as additive flame retardants, and polychlorinated biphenyls (PCBs), mainly used as dielectric and coolant fluids, are typical POPs. In recent years, PBDEs and PCBs have been released into the environment through various means and detected in diverse environmental media (such as air, water,

soil and sediment) and biota samples [1,2]. There has been a growing scientific and societal concern about their environmental behavior and toxicological effects. With high octanol-water partitioning coefficient (K_{OW}) values [5,6], PBDEs and PCBs favorably partition to well-defined hydrophobic phase in environment, such as organic matters in soils and sediments. The partition behavior of pollutants in environment were not only influenced by their structures and intrinsic properties, but also were significantly affected by some environmental factors. As one of the most widely distributed ubiquitous natural materials in environment,

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many studies have reported that dissolved organic matter (DOM) can interact with hydrophobic organic pollutants (PAHs, PCBs, DDT, PBDEs, etc.) through hydrophobic partition or electrostatic effects (hydrogen bond or π - π bond), which would affect the adsorption, transport, bioavailability and other environmental behaviors of these compounds [7–9].

The binding interactions between organic contaminants and DOM depend on the source and nature of DOM [10,11], as well as the hydrophobicity and steric configuration of compounds [12,13]. Cornelissen et al. [14] found that the nonplanar 2,2'-CB absorbed much less to black carbon than planar compounds like anthracene, phenanthrene and 4-CB. Using equilibrium dialysis method, Qiao et al. [15] determined the binding constants of different isomers of trenbolone with two commercial humic acids, and found that the binding ability of β -isomer was significantly higher than that of α -isomer. For aromatic compounds, substituent groups make great impact on the distribution characteristics of atomic charges on the benzene ring, and further influence the interaction between molecules, such as π - π interaction [13,16]. Uhle et al. [13] reported that compared to non-*ortho*-substituted PCBs with the same number of chlorine substituent, the binding affinity of *ortho*-substituted PCBs was significantly weaker. They also speculated that owing to chlorine substituent in *ortho*-position, free rotation around carbon-carbon was inhibited, which could result in the less effectively interaction with fulvic acid substrate. However, there is no experimental support for this presumption in their study. In fact, it is challenging to reveal and characterize the interaction mechanisms between DOM and pollutants using experimental approaches currently. Instead, molecular simulation techniques are very helpful to study the molecular interactions [17].

In this paper, equilibrium dialysis approach was employed to determine the binding constants between DOM and two PBDEs comprised of different substituent positions, and the binding constants between two PCBs with different substituent positions and LHA were derived from references. Quantum chemistry calculations were applied to characterize the parameters of molecular structure and charge distribution of the target compounds, and molecular docking calculations were employed to reveal the effects of substituent position on the interaction mechanisms between PBDEs/PCBs and DOM. This study will be valuable for the assessment of the behavior and fate of organic pollutants with different halogenated substituents.

1 Experimental

1.1 Chemicals and reagents

Hexane, dichloromethane and acetonitrile (GC grade) were purchased from TEDIA Firm (USA). Potassium dihydrogen phosphate (KH_2PO_4) and potassium hydroxide (KOH) were

analytical reagent grade from Tianjin Bodi Chemical Company (China). Leonardite humic acid (LHA) was purchased from the International Humic Substances Society, and dialysis bags (Dialysis Membrane 2000 Daltons) were purchased from Spectra Laboratories firm. BDE-47, BDE-77 and F-BDE-47 were purchased from AccuStandard firm (USA).

1.2 Equilibrium dialysis experiment and sample analysis

Equilibrium dialysis technique was employed to determine the binding constants of two PBDEs comprised of different substituent positions with representative humic acid (LHA). This method is widely used to measure the binding ability of compounds with DOM, because of the reliability of test results [8]. Dialysis bags filled with 3 mL 200 mg/L humic acid were placed in the glass tubes containing solution with the target compound (2 $\mu\text{g/L}$) buffered with KH_2PO_4 . After an equilibration period of 48 h, samples of the solutions inside and outside within the dialysis bag were extracted with dichloromethane, and dichloromethane was volatilized and transformed into hexane. Final samples were analyzed with GC-MS.

DB-XLB was used as capillary column (30.0 m $\times$ 250 μm $\times$ 0.1 μm). Samples were injected to GC in splitless mode. The injector temperature is 290°C. The temperature program of the GC oven was as follows: initial temperature 90°C for 1 min, then at 30 °C/min to 200°C (held for 0 min), then at 2.5° C/min to 305°C (held for 2 min), and finally at 5 °C/min to 315°C (held for 5 min). Helium was used as carrier gas at 30 mL/min. BDE-47, BDE-77 were determined using BDE-28 and F-BDE-47 as recovery substitute and the internal standard respectively. The recoveries for the test procedure were higher than 90%, and the detection limit was 0.1 $\mu\text{g/L}$ for the compound tested.

The binding constant (K_{DOC}) is used to characterize the association affinity of PBDEs with DOM, K_{DOC} was calculated as

$$K_{\text{DOC}} = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{out}} \cdot [\text{DOC}]}, \quad (1)$$

where [DOC] is the concentration of DOM determined by TOC-VCSH analyzer and expressed in kg/L, C_{in} and C_{out} is the PBDEs concentrations in inside and outside of the dialysis bags respectively, and expressed in $\mu\text{g/L}$, K_{DOC} is expressed in L/kg.

1.3 Computation of the molecular structure parameter and charge distribution

The initial geometries of PBDEs, PCBs and LHA model were firstly optimized by PM3 method using the Chem3D software and then by density functional theory at the B3LYP/6-311+G(d,p) level. The molecular model of LHA was

suggested by Niederer et al. [18] according to the data derived from ^{13}C NMR and elemental analysis. Frequency analysis was performed on the optimized geometries of LHA and compounds to ensure that the system has no imaginary vibration frequencies using the Gaussian 09 program [19]. The IEFPCM model was used to simulate the solvent (water) effects. Based on the optimized structures, the structural descriptors including the balance parameter of surface potential and van der Waals molecular volume were calculated. Finally the optimized structures were also used for natural bond orbital (NBO) analysis at the B3LYP/6-311+G(d,p) level to calculate the electron distribution of target compounds.

1.4 Molecular docking

The CDocker protocol incorporated into Discovery Studio 2.5.5 (Accelrys Software Inc., San Diego, CA) was used to carry out the molecular docking. The optimized geometries of PCBs, PBDEs and LHA were used in molecular docking. The CHARMM force field and the default values of all other parameters were used. In the docking process, PBDEs and PCBs molecules are allowed to flex, and the LHA molecule is held rigid. In CDocker, random ligand conformations were generated from the initial ligand structure through high-temperature molecular dynamics followed by random rotations. After that, the random conformations were optimized by grid-based simulated annealing, and then the interaction energies between the ligands and receptors were calculated, finally the conformations of complexes were arranged according to the interaction energies. Then binding modes with the lowest interaction energies were obtained as stable modes.

2 Results and discussion

2.1 $\log K_{\text{OW}}$ and $\log K_{\text{DOC}}$

In this study, we determined the $\log K_{\text{DOC}}$ of BDE-47 and BDE-77 with LHA using equilibrium dialysis. The $\log K_{\text{DOC}}$ of PCBs with HA were derived from ref. [20] and listed in Table 1.

It was shown that the binding constants ($\log K_{\text{DOC}}$) of the non-*ortho*-substituted PBDEs and PCBs with the same humic

acid were significantly higher than those of *ortho*-substituents, and this trend was in correspond with $\log K_{\text{OW}}$, indicating that the binding affinity of the compounds with DOM has good relationship with hydrophobicity. Previous studies have reported that the interactions between molecules not only related to the hydrophobicity of compounds, but also related to the van der Waals forces, hydrogen bonding and π electron [21,22]. To reveal the difference between the binding affinities of *ortho*- and non-*ortho*-substituted compounds with HA, some molecular structure descriptors of target compounds such as the balance parameter of surface potential (τ) and van der Waals molecular volume (V_{cm}) were calculated. V_{cm} was used to characterize the size of molecule, and τ was used to represent the balance degree of the atomic charges, the closer the value of 0.25, indicating that the better balanced distribution of molecular charge [23]. It was reported that these parameters can reflect the integrity of target molecules and be related to the adsorption and distribution of compounds [15,24]. From the data in Table 1, it can be observed that compared with *ortho*-substituted CB-47, non-*ortho*-substituted CB-77 has better balanced distribution of molecular charge, however, non-*ortho*-substituted BDE-77 shows the opposite trend. The difference of charge distribution between PCBs and PBDEs maybe relate to the O atom on the ether bond of PBDEs. In addition, the non-*ortho*-substituted PBDEs/PCBs have larger molecule volumes than *ortho*-substituted isomers, which indicated larger cavity and stronger repulsion from aqueous phase. Therefore non-*ortho*-substituted isomers interact more effectively with DOM.

2.2 Atomic charge distribution

Previous studies have found that the π - π interactions are tending to occur between the aromatic compounds comprised of stronger electron donor and acceptor substitutes respectively [16,25]. The atomic charge distribution of PBDEs, PCBs, and LHA were obtained and demonstrated in Figure 1 and Table 2 to test the possibility of π - π interactions between PBDEs/PCBs and DOM. Compared with Br atoms, Cl atoms possess greater electron withdrawing ability, which result in larger reduction on the net charge of linked C atoms. However, the O atom on the ether bond of PBDEs

Table 1 $\log K_{\text{OW}}$, molecular structure parameters and $\log K_{\text{DOC}}$ of the target compounds

Compounds	Substituent position	$\log K_{\text{OW}}$	V_{cm} (cm ³ /mol)	τ	$\log K_{\text{DOC}}$	DOM ^{e)}
BDE-47	2,2',4,4'-	7.16 ^{a)}	198.5	0.188	5.80 ^{c)}	LHA
BDE-77	3,3',4,4'-	7.30 ^{a)}	207.6	0.174	6.21 ^{c)}	LHA
CB-47	2,2',4,4'-	5.85 ^{b)}	172.0	0.130	4.15 ^{d)}	SW
CB-77	3,3',4,4'-	6.36 ^{b)}	177.4	0.166	5.00 ^{d)}	SW

a) Calculated from Sparc on-line calculator <http://ibmlc2.chem.uga.edu/sparc/>. b) Data from Hawker et al. [6]. c) Data determined in this study. d) Data from Kukkonen et al. [20]. e) LHA is the Leonardite humic acid derived from the brown coal, and SW is the natural organic matter derived from surface water.

Figure 1 Atomic charge distribution of LHA, CB-47, CB-77, BDE-47 and BDE-77. The balls in red, green, blue, and gray represent oxygen, chlorine, bromine, and carbon atoms, respectively.

has stronger electron withdrawing ability than Cl atom, which leads to significant decrease in the charge of C atom connected (Table 2). Generally, benzene rings of both PBDEs and PCBs homologs have negative charge distribution, which might work as donors for π - π interactions. The LHA model comprised of four benzene rings, named as A, B, C and D ring respectively. Because of the existence of strong electron-withdrawing function groups (including $-\text{OH}$, $-\text{COOH}$, $-\text{C}=\text{O}$, etc.) on the aromatic rings of LHA model, significant net charge changes are found for C atoms on the

rings, and rather positive net charge were shown on some C atoms. Therefore, from the view of charge difference, π - π interactions were highly expected between aromatic rings of PBDEs/PCBs and LHA, which act as electron donor and electron receptor respectively.

2.3 Molecular docking

To further investigate how the substituent position affects the binding interactions between PBDEs/PCBs and DOM

Table 2 The atomic charges of benzene ring of PBDEs, PCBs and LHA

C atom in molecule	BDE-47	BDE-77	CB-47	CB-77	LHA model			
					A ring	B ring	C ring	D ring
C1	0.275	0.311	-0.082	-0.040	0.394	0.519	0.310	-0.188
C2	-0.125	-0.242	-0.004	-0.196	-0.244	-0.058	0.282	-0.240
C3	-0.239	-0.127	-0.248	-0.064	-0.116	-0.612	-0.186	0.347
C4	-0.106	-0.154	-0.024	-0.071	-0.388	0.822	-0.200	-0.235
C5	-0.211	-0.193	-0.223	-0.201	-0.117	-0.388	0.010	-0.206
C6	-0.219	-0.233	-0.151	-0.176	-0.197	-0.116	-0.261	0.290

from the view of steric conformations, molecular docking studies were conducted. The stable binding sites of each target compound with LHA were acquired through molecular docking, and there are 10, 8, 6 conformations showing π - π interactions for CB-77, BDE-77 and BDE-47 respectively (Figure 2). Under the same docking parameters, the stable binding mode of CB-47 with LHA could not be found.

Previous studies proposed that dihedral angle was one of the dominant parameters affecting the interactions between PCBs and DOM [13]. The dihedral angles of PBDEs and PCBs before and after docking were calculated. Before

docking, the dihedral angle of the two benzene rings in CB-47 and CB-77 are 80.83° and 141.41° , respectively, and the dihedral angle of BDE-47 and BDE-77 are 107.34° and 108.75° , respectively. In the docking modes, CB-77 has the maximum change range of dihedral angle (34.48° – 141.42°), revealing the molecule can easily twist and fold, thereby making the molecule interact effectively with LHA. The adjustment ranges of dihedral angle for BDE-77 and BDE-47 are 64.56° – 108.75° and 91.05° – 107.34° , respectively. To some extent, the adjustment ranges of dihedral angle between the two benzene rings seem to be one of the main factors to affect the binding of PCBs or PBDEs with DOM.

Figure 2 Main binding modes of CB-77 (left) and BDE-77 (right) with LHA in molecular docking.

In addition, it was also found that if orientation vdw energy threshold of the docking parameters adjusted from 300 to 400, the stable binding modes of CB-47 with LHA can also be obtained, which indicated that the rotation or twist of *ortho*-substituted PCB needs more energy to find the stable binding site and conformation with DOM. Though, the LHA model used in this study is not the best represent for diverse DOM in environment, it can provide us with some insights on the binding interactions between organic pollutants and DOM.

3 Conclusions

The effects of substituent positions on the interaction between organic pollutants and DOM have been mentioned in previous studies. However, there are short of experimental data to explain the mechanism of substituents induced difference, since it is a challenge to reveal and characterize the interaction mechanisms between DOM and pollutants using experimental approaches. Using equilibrium dialysis experiment, quantum chemical and molecular docking calculations, it was found that substituent positions had important influence on the binding affinities of organic pollutants with DOM. Compared with the *ortho*-substitutes isomers, non-*ortho*-substituted PBDEs/PCBs have larger molecular volume and stronger hydrophobicity. Moreover, the dihedral angle of the two aromatic rings for non-*ortho*-substituted PCBs or PBDEs demonstrate larger rotation space and flexibility, thereby interact more effectively with Leonardite humic acid (such as π - π interactions). With the aid of quantum chemical and molecular docking calculations, our studies give some insight into the influence of halogen atomic substituent position on the interaction between aromatic compounds with DOM. These findings will be helpful in the assessment of environmental behavior and ecological risk of PBDEs/PCBs.

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