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Studies on the interaction of BDE-47 and BDE-209 with acetylcholinesterase (AChE) based on the neurotoxicity through fluorescence, UV–vis spectra, and molecular docking

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ABSTRACT

The neurotoxicity of polybrominated diphenyl ethers (PBDEs) has been of concern. Acetylcholinesterase (AChE) is a critical enzyme in the central and peripheral nervous system related to neurotoxicity. The interaction between BDE-47, BDE-209, and AChE was investigated through fluorescence and UV–vis spectra combined with molecular docking. Both BDE-47 and BDE-209 bound with AChE and changed the microenvironment of some amino acid residues, resulting in a change of AChE conformation. Hydrophobic interaction is the main binding force between BDE-47, BDE-209, and AChE, and electrostatic interaction exists according to the thermodynamic parameters of the interaction between them. A hydrophobic interaction of BDE-47-AChE and BDE-209-AChE has been confirmed through molecular docking to dominate the binding force. The binding constants of BDE-47-AChE and BDE-209-AChE were 4.2×10^4 and 4.1×10^4 L/mol, respectively, and the lowest binding energies of BDE-47-AChE and BDE-209-AChE were −7.8 and −5.9 kJ/mol, respectively. BDE-47 is more likely to bind with AChE than BED-209.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are used extensively as the flame retardants in various polymers, including construction materials, electronics, furnishings, plastics, polyurethane foams, and textiles (Alaee et al., 2003). PBDEs have been detected in many test samples, human serum, adipose tissue, and breast milk (Shaw et al., 2012; Wang et al., 2012). The influence of PBDEs on animals and human beings has been a topic of widespread concern. PBDEs have been revealed to affect estrogen/androgen interference (Ceccatelli et al., 2006), thyroid hormone interference (Zhou et al., 2002), and the interference effect of aryl hydrocarbon receptor (Wahl et al., 2010; Peters et al., 2006) and can cause reproductive toxicity (Huang et al., 2015) and neurotoxicity (Costa et al., 2015, 2016). The neurotoxicity of PBDEs has been paid more attention.

Both BDE-47 and BDE-209 receive attention for their widespread application and serious environmental toxicity. The neurotoxicity of these PBDEs has been extensively studied (Hendriks et al., 2012; Henrik et al., 2007; Angela et al., 2017). BDE-47 and BDE-209 can cause neurotoxicity in different ways (Garcia-Reyero et al., 2014). Lucio et al. demonstrated that BDE-47 induced oxidative stress and ensuing apoptotic cell death in mouse cerebellar granule neurons *in vitro* (Costa et al., 2015). Zhang et al. studied the neurotoxicity of BDE-209 through cultured rat neural stem cells (NSCs) *in vitro* and found that BDE-209 inhibited NSCs proliferation *in vitro* and was able to induce apoptosis, which might be associated with the activation of nuclear factor-κB (nF-κB) pathways (Zhang et al., 2016). However, the neurotoxicity caused by their inhibition of acetylcholinesterase (AChE) activity has rarely been reported.

AChE is closely related to the development and maturation of cells, and promotes the development of neurons and the regeneration of nerves (Zhang et al., 2016). In the human body, AChE, mainly distributed in the nervous system tissue, can quickly catalyze the hydrolysis of neurotransmitter acetylcholine and lead to the termination of nerve impulse transmission, so that the normal physiological function of the human body is guaranteed. AChE is one of the most important

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enzymes in the nervous system. Any substance affecting AChE activity may lead to neurotoxicity (Mrdaković et al., 2016). In the study of formaldehyde on the neurotoxicity of the process, Zendehdel et al. found that AChE activity and neurotoxicity are inseparable (Zendehdel et al., 2016). Neurotoxicity induced by PBDEs have been paid more attention.

Animal experiments are costly and time-consuming, and may also not be apt at explaining the toxic mechanism from the molecular perspective. Molecular docking and spectroscopy (ultraviolet-visible [UV-vis] and fluorescence spectrum) can be used as useful and rapid methods for studying the interaction between the small chemical molecules and the macromolecular proteins, which can reveal the toxic mechanisms at a deeper level.

Molecular docking, using the three-dimensional structure of two molecules, investigates whether they can be combined, and predicts the binding mode of the complex. Thermodynamics usually considers that the stable conformation of biomolecules is the lowest conformation of free energy; hence, the purpose of molecular docking is to find the lowest energy conformation of ligands and receptors in their active region (Chen et al., 2006). Additionally, docking procedure aims to identify the correct binding poses within the binding site of the proteins (Mascarenhas and Ghoshal, 2008). The interaction between hydroxy polybrominated diphenyl ethers (OH-PBDEs) and estrogen receptors was studied through molecular docking. For example, Lu et al. (Lu et al., 2014) found that OH-PBDEs could bind to the estrogen receptor and explain their binding patterns.

The UV–vis and fluorescence spectra have been used to study the interaction between small chemical molecules and biomolecules (Punith and Seetharamappa, 2012). The UV absorption peak of the protein changes with the microenvironment of the chromophore. That is, the peaks may show a certain degree of red shift or blue shift, and the absorbance and band may also change. According to the UV absorption spectrum of the characteristic absorption peak intensity, displacement and peak width, the changes in the proteins and small molecules could be determined before and after the interaction of small molecules and proteins. The spectra can also be used for studying the quenching and binding mechanism of the interaction between small molecules and proteins. The interaction between the proteins and small molecules generally includes the fluorescence quenching phenomenon and the fluorescence sensitization phenomenon, of which the fluorescence quenching phenomenon is more common. The interaction types (e.g. static quenching, dynamic quenching) can be confirmed by determining some relevant parameters (such as binding constant, number of binding sites, and thermodynamic constants) (Nan et al., 2016). Chaves et al. studied the binding of lophirone B with bovine serum albumin (BSA) based on the spectroscopic and molecular docking techniques (Chaves et al., 2017). Bayraktutan et al. reported the biophysical influence of coumarin 35 on BSA and confirmed the binding mechanism between them (Bayraktutan and Onganer, 2017).

Few studies have been conducted on the molecular interaction between PBDEs and AChE based on the neurotoxicity. There are 209 homologues of PBDEs, among which BDE-47 and BDE-209 are most widely used, while the molecular mechanism of neurotoxicity based on the activity change of AChE is not yet clear. In this study, the UV–vis and fluorescence spectra were used to investigate the interaction between BDE-47, BDE-209, and AChE. Additionally, molecular docking was performed to identify the probable bioactive conformations of ligands, and the ligand-receptor interactions were investigated at the molecular level based on the homology modeled 3D structure of AChE. The results of these two methods were mutually verified, and the final interaction types were confirmed. The findings enhance our understanding of the binding between PBDEs and AChE and aid in assessing the neurotoxicity risk of PBDEs.

2. Materials and methods

2.1. Materials

BDE-47 and BDE-209 (purity > 98%) were purchased from Wuhan Kaymke Chemical Technology Co., Ltd, China. A total amount of 48.85 mg of BDE-47 was dissolved in 100 mL of anhydrous ethanol, and the concentration was 1.0×10^{-3} mol/L (485.8 mg/L). 96 mg of BDE-209 was dissolved in 100 mL of dimethyl sulfoxide (DMSO, purity > 99.5%), and the concentration was 1.0×10^{-3} mol/L (960 mg/L). BDE-47 (or BDE-209) was diluted into the set concentrations of the solutions. The AChE (purity > 98%) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd, China. AChE solution (100 U/L) was prepared by Tris-HCl buffer solution. Tris-HCl buffer solution (pH 7.4) was formulated with 0.2 mol/L Tris (purity > 99.9%) and 0.1 mol/L HCl. The concentration of NaCl (purity > 99.5%) was 1 mol/L.

2.2. Methods

2.2.1. UV–vis and fluorescence spectra measurements

One milliliter of NaCl solution, a certain amount of AChE solution (25–800 μmol/L), and BDE-47 or BDE-209 solution were added to a 10-ml colorimetric tube. The solution volume was adjusted to 10 mL with Tris-HCl buffer solution. After the reaction at 300 K for 1 h, UV measurement was performed by a UV-2550 spectrometer (Shimadu, Japan), equipped with a 1-cm quartz pool. The emission spectra were recorded from 200 nm to 500 nm.

Fluorescence intensities were measured by F-7000 spectrofluorimeter (Hitachi, Japan), equipped with 1.0 cm quartz cells. The test samples were prepared as described above. The reaction was also conducted at 300 K and 310 K, respectively, for 1 h. The obtained data were used to calculate the binding constants. The protein intrinsic fluorescence of AChE, AChE-BDE-47, and BDE-209 systems were all measured at an excitation wavelength of 280 nm. The emission spectra were recorded over the wavelength from 300 nm to 500 nm, and the widths of excitation and emission slits were set at 5 nm. All fluorescence intensities were corrected to decrease the inner filter effect.

2.2.2. Molecular docking

ChemDraw was used to draw the structures of BDE-47 and BDE-209 (Fig. 1) and then OpenBabel2.3.2a_Windows_Installer was used to change the profile format to *pdbqt*, which was suitable for Auto Dock. Ligand molecules (BDE-47 and BDE-209) were properly pretreated using Graphical Interface (mgltool_win32_1.5.6rc3).

AChE includes a variety of enzymes. We chose some of them containing natural ligands, which are similar with the structure of PBDEs.

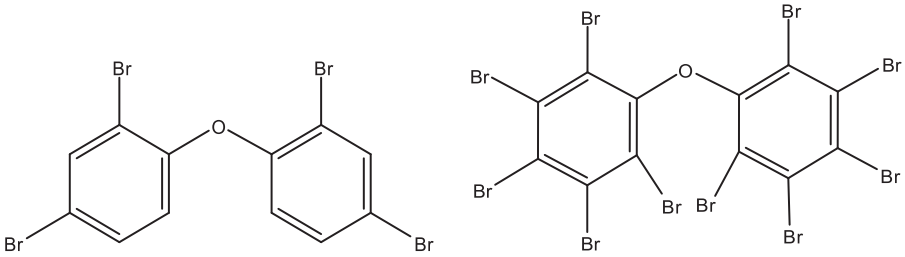

Fig. 1. Molecular structures of BDE-47 (Left) and BDE-209 (Right).