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Improved 3D-QSAR analyzes for the predictive toxicology of polybrominated diphenyl ethers with CoMFA/CoMSIA and DFT ☆, ☆☆

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ABSTRACT

With the popular methods of CoMFA and CoMSIA, three-dimensional quantitative structure–activity relationships (QSARs) were newly developed for the toxicity of polybrominated diphenyl ethers (PBDEs). The choice of optimized geometries by density functional theory (DFT) as molecular template and the RMSD-based molecular alignment strategy might mostly contribute to the QSAR improvement, which was highlighted specifically by the increased q^2 of 0.870 for CoMFA, 0.887 for CoMSIA, respectively. QSARs analyzes indicated that the steric effects from *ortho*- and *meta*-substitution and the correlated hydrophobicities have the greatest impact on the binding affinities of aryl hydrocarbon receptor (AhR) to PBDEs. Though the effects of electrostatics were comparatively inferior in the AhR binding, the aromatic interaction and possible charge transfer proved to be indispensable for toxicity mediation. Consistent with that proposed previously for other structurally similar compounds, such as dioxins and polychlorinated biphenyls, the predictive toxicology was helpful to understand the congener-specificity of toxicity of PBDEs.

1. Introduction

Due to the superior performance in reducing or inhibiting flammability, polybrominated diphenyl ethers (PBDEs) are widely used as additives into a variety of plastics, textile products and electrical appliances at a general concentration of 5–30% (WHO, 1994; Renner, 2000; De Wit, 2002; Watanabe and Sakai, 2003; Peters et al., 2006). With continuous usage in commercial products since the late 1960s (Luross et al., 2000; Ikonomou et al., 2000, 2002), PBDEs including a variety of congeners have been ubiquitously detected in biotic and abiotic matrices, such as sediment (Sellström et al., 1998; Allchin et al., 1999; Covaci et al., 2005), fish (Hale et al., 2001; Borghesi et al., 2008), birds (Polder et al., 2008; Vetter et al., 2008), adipose tissue (Haglund et al., 1997; Covaci et al., 2008), human plasma (Klasson-Wehler et al., 1997; Sjödin et al., 1999) and even mother milk (Meironyté et al., 1998; Norén and Meironyté, 2000). Through long-term exposure to PBDEs, a series of adverse effects such as the thyroid hormone disruption, immunosuppression and the possible carcinogenicity (Zhou et al., 2002; Branchi et al., 2003; Darnerud, 2003, 2008) are gradually imposed on humans and wildlife. Like polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs), some PBDEs are persistent, lipophilic and bioaccumulative in nature (Meerts et al., 2000), and they are suggested to be more dangerous than previously considered (Peters et al., 2006; Wahl et al., 2008). Thus, PBDEs have been an important group of environmental pollutants, and raised more concerns of scientific community.

In general, the mixtures of PBDEs used commercially are mainly comprised of highly brominated congeners, e.g. *octa*- and *penta*-BDEs, whereas they only account for a small part of the whole PBDE group. In the true environment, the highly brominated congeners can be readily degraded into the less ones because of chemical instability under photocatalysis or enzyme catalysis (Bezares-Cruz et al., 2004; Keum and Li, 2005). As such, the presence of PBDE compounds in the environment is much more complex. Centered on a small number of congeners, the study conducted previously may not satisfy the need of whole risk

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☆☆ *Ethical statement*: The experimental bioactivities used in this study are derived from the *in vitro* rat hepatocytes assay by Chen et al. (2001); as such, no human and experimental animals are involved in this study. We make assurance that the research is in accordance with the national and institutional guidelines for the *protection of human subjects and animal welfare*.

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assessment. More relevant data are urgently demanded to characterize the toxicological features of the theoretically defined 209 congeners. Restricted to the analytical difficulty or the unavailability of pure chemicals, however, the toxicological data obtained only from experiments are still rather limited (Chen et al., 2001). Due to the datum limitation, the origin of significant toxicity variance of PBDE congeners is not yet well understood. For the structural similarity with PCBs or other halogenated aromatic compounds (HACs), PBDEs are basically believed to share the same pathway of toxicity expression with them: they might activate the signal transduction pathway of aryl hydrocarbon receptor (AhR) to exhibit agonist and antagonist activities (Safe, 1990; Okey et al., 1994; Meerts et al., 1998; Behnisch et al., 2003; Wahl et al., 2008). The affinity of ligand binding to AhR plays a pivotal role in the exhibition of toxicity, that is, the greater affinity generally yields the higher toxicity. In contrast to 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TCDD), the binding affinities of PBDEs to AhR are all relatively low (Chen et al., 2001). In the experiment by Chen et al. (2001), 2,2′,3,4,4′-*penta*-BDE (PBDE-85) was determined to be more potent in the AhR binding, whereas the induction potency of it for 7-ethoxyresorufin *O*-deethylase (EROD) or cytochrome P-450 isozyme CYP1A1 (Whitlock, 1993; Okey et al., 1994; Hu and Bunce, 1999) was not shown as other PBDE congeners.

In order to well interpret the toxicological mechanism of PBDEs, the theoretical approach relative to experiment can be considered as a useful alternative. Among the various theoretical methods, quantitative structure–activity relationships (QSARs) have been widely used to the studies on many HACs. In earlier work, Harju et al. (2002) firstly developed QSAR models for luciferase *in vitro* activity, which were measured for 17 *di*- through *hepta*-BDE congeners. For the toxicity, the corresponding QSAR was not yet successful with the statistical analysis of multivariate linear regression (MLR) (Xu et al., 2007); the 3D-QSARs from comparative molecular field analysis (CoMFA) and comparative molecular similarity index analysis (CoMSIA) were also moderate in performance (Wang et al., 2005). By comparison, the analyzes of support vector machine (SVM) and radial basis function neutral network (RBFN) gave the relatively satisfactory QSARs, and the seven quantum chemical descriptors introduced significantly implied that the electrostatic interaction played a key role in the AhR binding (Zheng et al., 2007). With the influential factors considered in heuristic method (HM), the QSARs of Wang et al. (2006) confirmed the prominent role of electrostatics. However, the common interpretation for toxicity variance of HACs (e.g. dioxins or PCBs) suggested that the steric or dispersion interaction rather than the electrostatics effectively dominate the binding affinity (Mhin et al., 2002; Hirokawa et al., 2005; Ashek et al., 2006; Gu et al., 2007a, b). Thus, it is clearly known that the interpretation drawn from the limited research for PBDEs is remarkably different from that for HACs, and more careful studies on PBDEs are necessary to be conducted for further clarification.

The purpose of present work herein is to improve the performance of QSARs for PBDE toxicology with CoMFA and CoMSIA. For precisely predicting the toxicity of PBDEs, the template structure used in molecular alignment was determined with the full geometry optimization by density functional theory (DFT) (Parr and Yang, 1989; Seminario and Politzer, 1995). With the improved QSARs, the toxicological mechanism of PBDEs was further reasonably clarified, and it proved to be consistent with that for the structurally similar HACs. In combination with the contour map analyzes of different fields, the obtained QSARs can not only provide clearer insight into the nature of congener-specific toxicity and ligand–AhR binding interactions, but also serve as a useful tool for assessing the potential risks of PBDEs through toxicity prediction.

2. Materials and methods

2.1. Data set for analysis

To perform the structure-based QSAR study, 18 PBDE congeners, which have been synthesized and tested for the relative binding affinities (RBAs) to AhR in rat hepatocytes were taken from the literature (Chen et al., 2001). As a measure of PBDE toxicity, each binding affinity was calculated as the ratio of EC_{50} of individual congener to that of the reference compound, *viz.* [^{3}H]-TCDD in 1.0 nM, where EC_{50} was the molar concentration of chemical necessary to inhibit 50% of the specific binding of radio-labeled TCDD. In Table 1, they were expressed as the negative of logarithm range $-\log(\mathrm{RBA})$, i.e. pRBA for QSAR development. Obviously the selected experimental values span a range of at least 3-log units and comprise of a broad and homogenous dataset (McKinney et al., 2000).

2.2. Molecular modeling

The molecular modeling and 3D-QSAR studies described herein were implemented in SYBYL 7.3 (2007) software package on Red Hat Enterprise Linux workstation. As CoMFA and CoMSIA require the necessary molecular alignment of the target compounds before analyzes, a core conformational template needs to be given firstly; and then the geometrical structure of it should be determined in virtue of the X-ray crystallography of active ligand or well optimized considering the effect of superimposition by other congeneric molecules. The optimized structure corresponding to the global energy minimum is often accepted as a starting geometry of the template in CoMFA studies (Cramer III et al., 1988b) when the X-ray crystallographic data about the complex of ligand–AhR are not available. However, for PBDEs the flexible rotation of bridged C–O bonds with much low energy barriers makes it difficult to obtain the lowest energetic minimum. The recent study on the chemical properties of 14 PBDEs indicated that the optimized structures by DFT were in conformity with the published X-ray crystallographic data (Zhao et al., 2008). Thus, it is possible to apply DFT as optimization method to make the obtained structure similar to the bio-actively interacting ligand. The template was optimized at the higher level of B3LYP/6-311G(d, p) (Lee et al., 1988; Becke, 1993) within DFT approach. Vibrational analyzes were performed to ensure the optimized structure was definitely stable and corresponded to the relative minimum point on the potential energy surface. The initial optimization was fulfilled with Gaussian 03 suite of programs (Frisch et al., 2003).

As described by Pasha et al. (2008), the optimized template by DFT was used to design other molecules of concern by fixing common moiety, *viz.* the oxygen-bridged diaryl rings in SYBYL 7.3 (2007). With the distance-dependent dielectric constant set as 1.0, the designed molecules were further minimized with Powell method by assigning with the standard Tripos force field (Clark et al., 1989), Merk molecular force field 94 (MMFF94) charges. The repeated minimization would not be ceased until the gradient value of 0.05 kcal $(\mathrm{mol\ \AA})^{-1}$ was achieved. As such, the optimized structures by final minimization instead of time-consuming systematic search were used for QSAR development. As for the template choice, PBDE-85 was the optimal candidate in view of its bioactivity and high toxicity. As the molecular orientation and QSAR performance is sensitive to the molecular alignment (Cho and Tropsha, 1995), an appropriate choice of alignment scheme is also crucial. In the study, the data-based fitting procedure was finally adopted through careful comparison and each analog was superimposed to the template based on the common substructure of oxygen-bridged diaryl ring moiety. The aligned molecules were illustrated in Fig. 1.

2.3. CoMFA and CoMSIA analyzes

In CoMFA analyzes, the aligned structures of PBDEs were placed in a three-dimensional cubic lattice of 2 Å spacing, with an extension of 4 Å units beyond the molecules in each direction. The sp3 carbon atom with van der Waals radius of 1.52 Å and a formal charge of +1 served as the probe atom. The steric and electrostatic field energies, which were represented by the potentials of Lennard-Jones 6–12 and Coulomb, respectively, were generated using standard Tripos force field (Clark et al., 1989). With the selected distance-dependent dielectric constant, the calculated steric and electrostatic energies were truncated to the threshold of 30 kcal mol^{-1}. The CoMFA fields created automatically were scaled by the CoMFA-STD method, and the electrostatic contribution was ignored at the lattice intersections with maximum steric interactions.

As a complementary 3D-QSAR technique, CoMSIA is less sensitive to the grid spacing and relative orientation of aligned molecules. The application of distance-dependent Gaussian-type function in CoMSIA can avoid the inherent singularities at atomic positions and the dramatic changes of potential energies for those grids in the vicinity of surface. Thus, no arbitrary cutoffs are required generally. The similarity index fields of CoMSIA were computed with the same alignment and lattice box as used for CoMFA (Klebe et al., 1994). The attenuation factor (α) that determined the steepness of Gaussian-function was assigned with a default value of 0.3 (Bohm et al., 1999). The similarity indices were presented by different physicochemical properties, e.g. the steric, electrostatic and hydrophobic fields,