Ecotoxicology and Environmental Safety 138 (2017) 215–222

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

An enantiomer-based virtual screening approach: Discovery of chiral organophosphates as acetyl cholinesterase inhibitors

Aiqian Zhang[a], Yunsong Mu[b,*], Fengchang Wu[b]

[a] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

[b] State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China

ARTICLE INFO

Keywords:
Chiral organophosphates
Enantioselectivity
In vitro testing
Molecular docking
Virtual screening

ABSTRACT

Chiral organophosphates (OPs) have been used widely around the world, very little is known about binding mechanisms with biological macromolecules. An in-depth understanding of the stereo selectivity of human AChE and discovering bioactive enantiomers of OPs can decrease health risks of these chiral chemicals. In the present study, a flexible molecular docking approach was conducted to investigate different binding modes of twelve phosphorus enantiomers. A pharmacophore model was then developed on basis of the bioactive conformations of these compounds. After virtual screening, twenty-four potential bioactive compounds were found, of which three compounds (Ethyl *p*-nitrophenyl phenylphosphonate (EPN), 1-naphthaleneacetic anhydride and *N*,4-dimethyl-*N*-phenyl-benzenesulfonamide) were tested by use of different *in vitro* assays. *S*-isomer of EPN was also found to exhibit greater inhibitory activity towards human AChE than the corresponding *R*-isomer. These findings affirm that stereochemistry plays a crucial role in virtual screening, and provide a new insight into designing safer organ phosphorus pesticides on human health.

1. Introduction

Organophosphates (OPs) have been used to treat various neurological diseases such as Alzheimer's disease, as well as being used to prepare insecticides and fire retardant materials (Brown et al., 1989). Given that OPs are used in such a wide range of applications, questions continue to be asked regarding their biological effects, duration of action and toxic mechanisms (Liu et al., 2005). Modern OPs-based pesticides that contained one or more chiral atoms were reported to show different toxic potency to insects or human (Tenberken et al., 2010; Tsai et al., 2010; Wang et al., 2010; Zhang et al., 2014). Currently, the OPs stockpile worldwide is estimated at 200,000 t (Singh, 2009). These concerns about health and the environment have encouraged the search for efficient tools for the environmentally benign detection of OPs (Li et al., 2014; de Castro et al., 2016). The mechanism of action of organophosphates (OPs), as well as their metabolites, has been studied using experimental and computational methods. They indicated that hydrogen bonds between P=O and oxyanion hole was critical for AChE inhibition through covalent bond (Zhang et al., 2002; Lee and Barron, 2016). The oxyanion hole (*i.e.* Gly120, Gly121 and Gly122) contributed to the functional architecture and to the hydrolytic efficiency of human AChE (Ordentlich et al., 1998). Compared to P=S forms, P=O compounds are more active. The reason is that oxidation of P=S to the corresponding P=O results in a more electronegative phosphorus atom.

X-ray crystal structure of acetyl cholinesterase (AChE) has been solved together with several structurally diverse co-crystal structures, including tacrine (Harel et al., 1993), 1-benzyl-4-[(5,6-dimethoxy-1-indanon-2-yl)methyl]piperidine (Kryger et al., 1998) and several OPs (Millard et al., 1999a, 1999b). It was a major way to acquire crucial information on the binding modes in the active site of the enzyme. The active site of AChE comprised a catalytic subsite and an anionic subsite, which binds the quaternary group of acetylcholine (Quinn, 1987). Like other serine hydrolases, the catalytic triad is located at the bottom of a deep and narrow cavity, named the "aromatic gorge". The primary site of interaction of the quaternary group of acetylcholine is with the aromatic ring of the Trp84. The x-ray crystallographic evidence showed the presence of Trp84 and Phe330 in the "anionic" subsite of the active site and a distal residue, Trp279, near the top of the gorge, was also a part of the "peripheral" anionic site (Sussman et al., 1991). Molecular simulation approach was another way to investigate the orientation of these inhibiters to AChE (Rosenberry et al., 1999; Pilger et al., 2001; Dvir et al., 2002; Cavalli et al., 2004). This process occurred in an enantioselective manner, as exemplified by the *S*-enantiomer of an

* Correspondence to: State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, 8 Dayangfang, Beiyuan Road, Chaoyang District, Beijing 100012, China.
E-mail address: muyunsong323@163.com (Y. Mu).

http://dx.doi.org/10.1016/j.ecoenv.2016.12.035
Received 29 September 2016; Received in revised form 23 December 2016; Accepted 27 December 2016

extremely poisonous gas called sarin, which phosphorylated the tyrosineosine within the active site of AChE at least 4200-fold faster than the corresponding *R*-enantiomer (Boter and Dijk, 1969). A more complicated array of interactions is involved in the phosphorylation of this enzyme to diastereoisomers of soman (Ordentlich et al., 1999). It demonstrates that AChE can discriminate between different enantiomers of OPs in terms of their toxicity towards the active site of AChE.

Enantiospecific toxicity is considered as a part of the risk assessment and regulatory decision-making processes underlying the development and application of chiral pesticides. Several quantitative structure–activity relationship (QSAR) studies have been reported pertaining to the specificity and orientation of tetrahedral alkyl phosphate in cholinesterase (Millard et al., 1999a; Ordentlich et al., 2005). Authors of these studies tried to explain the relationship between the shape of the active site of the AChE enzyme and the stereo selectivity of its binding interactions. These events were dependent on the dimensions of the acyl pocket, which governed the leaving group orientation and the productive association of the phosphonyl oxygen with the oxyanion hole (Wong et al., 2000). Comparative molecular field analysis has also used to model the binding affinities of 30 OPs, but the researchers neglected to consider the differences in the stereo chemistries of the OPs when overlaying the different conformations (Guo et al., 2006). With this in mind, the aim of the current study was to develop a new molecular modeling method for the discovery of new AChE inhibitors. Subsequent determination of the inhibitory activities of these compounds against AChE by use of an *in vitro* bioassay would be used to confirm the validity of this approach. Different enantiomers of chiral OPs can be used to probe the enantioselectivity of the binding pocket of AChE towards these compounds. The molecular simulation approach developed in this study could therefore be used to predict the enantiospecificity of AChE towards various chiral compounds, which can be otherwise difficult to determine experimentally.

2. Materials and methods

2.1. Reagents

Ethyl *p*-nitrophenyl phenylphosphonate (EPN, 96.5%) was purchased from the Dr Ehrenstorfer Co. (Augsburg, Germany). Human AChE (EC 3.1.1.7) expressed in human embryonic kidney 293, acetylthiocholine iodide (≥98%), 1-naphthaleneacetic anhydride (96%), *N*,4-dimethyl-*N*-phenyl-benzenesulfonamide (≥96%), tacrine hydrochloride (≥99%), dimethyl sulfoxide (DMSO, ≥99%), 2,2′-dinitro-5,5′-dithiobenzoic acid (DTNB, ≥97.5%) and sodium dodecyl sulfate (≥98.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffer solution (pH 8.0) was prepared by use of solutions of NaCl, KCl, KH_2PO_4 and Na_2HPO_4. All of the reagents used in this study were prepared as the analytical grade.

2.2. Theoretical calculations

Six pairs of chiral organophosphates evaluated in this study (Hosea et al., 1995; Yen et al., 2003; Liu et al., 2006; Nillos et al., 2007) were shown in Table 1, where the *S*/*R*-activities had been described as the quotient of the *S*- and *R*-activities. Three dimensional 3D structures of these OPs were constructed by use of the SYBYL X1.1 molecular modeling software package (Tripos, Princeton, NJ 08540, USA). Chemical structures were first geometrically optimized by use of the standard Tripos force field with a conjugate-gradient energy convergence criterion of 0.05 kcal/mol. Atomic charges were calculated by use of the Gasteiger + Hückel charge field. The X-ray crystal structure of human AChE was obtained from the Protein Data Bank (http://www.rcsb.org/pdb), in which the protein data file entitled accession number 2 × 8B (Carletti et al., 2010). Biopolymer package was used in the current study to identify the key amino acid residues involved in the binding of the co-crystallized ligand to the protein. Amino acid residues within 6.5 Å around the ligand were considered during this process. Different binding modes of the chiral OPs to AChE were investigated by use of the Surflex-Dock program (Jain, 2003). Surflex-Dock's scoring function, which was based on hydrophobic, polar, repulsive, entropic and salvation terms, was trained as the total score (TS). Total score was then used to estimate the dissociation constant K_d, which was expressed in units of $-\log K_d$. In addition to the automated docking procedure, this function was recently enhanced by the incorporation of a base fragment matching algorithm. In this case, the fragments are allowed to shift from their original position during the pose optimization process.

A DISCOtech program was used to align bioactive conformations, including those of *R*-trichloronate, *S*-leptophos, *R*-fonofos, *S*-cycloheptyl thiocholine, *S*-isopropyl thiocholine, and *S*-cycloheptyl methyl S-ethyl phosphonyl thioate. Bioactive conformations of these compounds generated from the Surflex-Dock program were treated as the initial configurations for establishing 50 low energy conformations. The DISCOtech program initially assigned as a series of pharmacophore features, such as hydrogen bond donor atoms, hydrogen bond acceptor atoms, charged centers, mass centers of the hydrophobic rings, and the most likely locations of the binding sites. In some cases, the chirality check option was switched on prior to performing the search process, although this was dependent on the multi-search method. In this study, the most common substructure for elements within a series of aligned conformations was superimposed by use of the categories algorithm. The 'features by class' option was defined and the number of pharmacophore features required in each class was set to more than two donor sites, as well as one acceptor atom, and one hydrophobic center. Distance tolerance between two pharmacophore features in the overlaid structures was set to 0.25 Å, which was increased up to 0.5 Å if no model was found by use of the lower tolerance settings (step size 0.25 Å). The National Cancer Institute database was searched by use of the UNITY program, which contained two hundred thousand chemical structures at the time of this search.

2.3. AChE competitive inhibition assay

A colorimetric assay based on the DTNB-mediated reduction of acetylthiocholine iodide was used to measure the inhibitory activity of

Table 1
Observed *vs.* predicted *S*/*R*-enantiomers of organophosphates docked with acetyl cholinesterase (AChE).

OPs	*S-activity*	*R-activity*	*Observed S/R*	*S-docking*	*R-docking*	*Predicted S/R*
trichloronate	8. 40 ± 0.12[a]	9.31 ± 0.13[a]	0.90	4.82	5.37	0.90
leptophos	10.05[a]	8.74[a]	1.15	5.46	4.88	1.12
fonofos	7.85 ± 0.01[a]	9. 04 ± 0.10[a]	0.87	4.13	4.62	0.89
cycloheptyl thiocholine	8.28 ± 0.07[b]	5.91 ± 0.05[b]	1.40	7.65	5.78	1.32
isopropyl thiocholine	7.20 ± 0.03[b]	5.15 ± 0.10[b]	1.40	3.99	2.92	1.37
cycloheptyl methyl S-ethyl phosphonyl thioate	4.88 ± 0.03[b]	2.25 ± 0.05[b]	2.17	5.40	3.23	1.67

Note:
[a] The logarithm of the concentration that caused 50% mortality of the test population (μg/L) (Yen et al., 2003; Liu et al., 2006; Nillos et al., 2007);
[b] The logarithm of bimolecular rate constants ($min^{-1}\ M^{-1}$) determined for the inhibition of cholinesterase by *S*- and *R*-enantiomers (Hosea et al., 1995).