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Perspectives for the structure-based design of acetylcholinesterase reactivators

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ABSTRACT

Rational design of active molecules through structure-based methods has been gaining adepts during the last decades due to the wider availability of protein structures, most of them conjugated with relevant ligands. Acetylcholinesterase (AChE) is a molecular target with a considerable amount of data related to its sequence and 3-dimensional structure. In addition, there are structural insights about the mechanism of action of the natural substrate and drugs used in Alzheimer's disease, organophosphorus compounds, among others. We looked for AChE structural data useful for *in silico* design of potential interacting molecules. In particular, we focused on information regarding the design of ligands aimed to reactivate AChE catalytic activity. The structures of 178 AChE were annotated and categorized on different subsets according to the nature of the ligand, source organisms and experimental details. We compared sequence homology among the active site from *Torpedo californica*, *Mus musculus* and *Homo* sapiens with the latter two species having the closest relationship (88.9% identity). In addition, the mechanism of organophosphorus binding and the design of effective reactivators are reviewed. A curated data collection obtained with information from several sources was included for researchers working on the field. Finally, a molecular dynamics simulation with human AChE indicated that the catalytic pocket volume stabilizes around 600 Å^3, providing additional clues for drug design.

1. Introduction

Acetylcholinesterase (AChE; EC 3.1.1.7) is a serine hydrolase with a primary role in the body: the regulation of acetylcholine and other choline molecules as neurotransmitters [1]. The enzyme has been studied as an important therapeutic target for Alzheimer's disease based on its role in the cholinergic nervous system [2]. Moreover, the enzyme is the target of different poisonous chemicals such as organophosphorus compounds (OPs), carbamates, among others [3]. The irreversible inhibition of AChE by OPs is due to the covalent bond formed between the compound's phosphorous and the oxygen belonging to the catalytic serine hydroxyl group [4]. This high affinity interaction leads to the accumulation of acetylcholine at neuronal synapses promoting the overstimulation of cholinergic receptors and severe toxic effects such as paralysis and respiratory failure [5]. There are two clinically important isoforms of the human enzyme (hAChE), the erythrocytic (hAChE-E) and the synaptic (hAChE-S). The hAChE-S is the canonical form and the most studied isoform [6].

From a computational perspective, structural information is crucial to understand the catalytic engine of AChE and to support the rational design of competitors and reactivators [7,8]. The first AChE structure was described in 1993 from the electric organ of *T. californica* (TcAChE) [9]. More than 100 structures have been resolved so far for this organism and others such as *Mus musculus* (mAChE), *Drosophila melanogaster* (dAChE) and *Homo sapiens* (www.pdb.org, accessed in November 2015). These structures are categorized in native conformations of the whole protein and particular domains (fragments), configured both in *apo* or *holo* states with ligands of medical importance [10].

The OPs are developed as esters of phosphoric or phosphonic acid and their thio-analogues (*e.g.* paraoxon, dimpylate) [3,11], and they act through AChE inhibition [12]. These compounds are widely used as pesticides, mainly in developing countries without strict regulatory policies, and poisoning is a major health problem related

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to careless manipulation, suicidal attempt and chemical warfare [13]. In Asia, there are two million OPs intoxications per year with 10% to 20% mortality. Of note, respiratory failure secondary to this poisoning demands the use of mechanical ventilators as supportive measure, requiring around 1.7 million days of ventilation every year [14].

The inhibition of the enzyme is initially reversible and this state can be regenerated by oxime drugs [15], but then there is a spontaneous process of deamidation or dealkylation of the phosphorous in the OPs-AChE conjugate, named "aging" [16]. Currently, there are no antidotes to reactivate "aged" AChE [17]. Oxime reactivators are the most frequently used molecules for OPs intoxication since the 1950s, and acceptable results have been obtained with quaternary heteroaromatic compounds with an oxime moiety [18], such as pralidoxime [18,19]. For finding novel reactivators, it is important to study the reaction mechanism between reactivator and the OP compounds. There are two steps involved in this mechanism, the first is the combination of the oxime (Ox) with the inhibited enzyme (EI) to produce a complex (EIO_x); the second one focuses on the reactivation that takes place by the (IO_x) complex leaving the system [20].

$$EI + O_x \overset{k_R}{\underset{}{\leftrightarrow}} EIO_x \overset{k_R}{\underset{}{\rightarrow}} E + IO_x \quad (1)$$

where k_R is the dissociation coefficient that represents the interaction between the oxime and the inhibited AChE. k_R stands for the rate constant of the chemical reaction between the oxime and the OP inside the AChE active site [21].

Additional attempts have looked for novel scaffolds to reactivate (*e.g.*, non-oximes as AW00554 and AW00565) [22] and/or inhibit reversibly AChEs, mainly supported by computational techniques such as molecular docking, molecular dynamics and 3D-QSAR [23–25].

Here, we describe an analysis of AChE structures (resolved by x-crystallography studies) and their interactions with different classes of ligands, in order to model the dynamics within the active site to provide clues for further design of more effective antidotes (competitors or reactivators).

2. Methods

2.1. Systematic search

AChE structures of different organisms were obtained from Protein Data Bank (PDB) and categorized by source (electric rays, mice and humans), crystal resolution, publication date, and co-crystallized ligands. Additional information as residue count, structure molecular weight, primary citation authors, bioactivity values and ligand structures, was also included. The data were curated and inspected manually in order to prioritize protein conjugates involving reactivators co-crystallized with or without toxins.

2.2. Structure and sequence comparison among organisms

For each source organism, the AChE with the best resolution by x-crystallography was chosen and the sequence and structure (complete protein and the active site) were compared. The PDB in Europe (PDBe) server [26] and the JOY program [27] were used for the multiple structure and sequence alignment, respectively. JOY provided additional information such as the presence of secondary structures (alpha helices, beta strands and coils), solvent accessibility, hydrogen bond to main-chain amide and carbonyl, disulfide bonds and positive *phi* torsion angles.

Table 1
Number of AChE structures per organism distributed by the crystal resolution and release date obtained from the Protein Data Bank.

Organism	Crystal resolution		Release date	
	<2.5 Å	≥2.5 Å	1990–2005	≥2005
Torpedo californica	54	30	42	42
Mus musculus	26	47	9	64
Homo sapiens	7	7	4	10
Drosophila melanogaster	–	3	3	–
Electrophorus electricus	–	3	3	–

2.3. Interaction patterns of OPs-AChE conjugates

Each structure was analyzed based on the active site conformation with the bound ligand (co-crystallized conjugates). The *apo* states of the enzymes were used as references to compare key movements within the pocket and the formation of different interactions (hydrogen bonds, hydrophobic interactions, electrostatic interactions and π-π stacking). Physicochemical properties of the active site and the interaction patterns of the conjugates were reviewed. PDB files were inspected using the UCSF Chimera version 1.10 [28], and the interactions with PoseView [29] and Discovery Studio 4.0.

2.4. Molecular dynamics (MD) simulation of hAChE binding site

The hAChE (PDB:4EY4) was selected because it is the most recent *apo* conformation of the enzyme. The purpose of this analysis was to describe how volume changes of the active site could impact the ligand binding in a molecular time scale. The structure was refined on the WHAT IF package [30] to correct errors from the experimental output such as missing atoms and undesirable location of side chains. The refined hAChE was subjected to a production MD simulation of 20 nanoseconds in GROMACS package [31]. Initially, the protein was minimized using the steepest descent algorithm, and then, the equilibration stage was run in two phases for temperature and pressure.

The simulation included the GROMOS96 united atom force field [32], periodic boundary conditions using a solvent-filled dodecahedral box, Langevin dynamics [33], Particle Mesh Ewald (PME) periodic electrostatic conditions [34], a time-step of 2 femtoseconds (fs) and the addition of ions to model the pH of the system. The resultant production was put into the Epock package [35] to describe the catalytic pocket volume, and the potential impact of small changes at and near the active site on ligand binding.

3. Results and discussion

3.1. Systematic search of available crystal structures of AChE

Since 1990, a total of 178 structures have been reported on the PDB website (www.rcsb.org; accessed November 2015). Most of the structures belong to *T. californica,* followed by the murine and human homologue. The number of the AChE structures deposited in PDB per organism, structure resolution obtained by X-ray crystallography, and the corresponding release date are shown in Table 1. Between 1990 and 2005, the most used organism to crystallize AChE was *T. californica* because it was the first standardized model used to design anti-Alzheimer's disease drugs [36], antidotes against OPs intoxications [37], and the development of insecticides [38]. During the following years, the availability of better techniques to solve more accurate crystals allowed the study of AChE in other organisms as mice (mAChE) and humans (hAChE). The latter was solved by Kryger et al. in 2000, with the purpose of elucidating the interactions between the enzyme and fasciculin-II toxin puri-