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ORIGINAL ARTICLE

CHEMICAL WARFARE: PERSPECTIVES ON REACTIVATING THE ENZYME ACETYLCHOLINESTERASE INHIBITED BY ORGANOPHOSPHATES*

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Summary

It is known that nerve agents are potent inhibitors of acetylcholinesterase (AChE), the enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine and, thus, transmission of nerve impulses. The process of AChE inhibition by nerve agents can be reversed by a nucleophile able to dephosphorylate the enzyme. In this sense, oximes exhibit this characteristic and are able to remove the neurotoxic and reactivate AChE. Here, we review experimental and theoretical results involving docking and quantum mechanical-molecular mechanics hybrid methods (QM/MM), using Molegro® and Spartan® softwares to analyze the interaction of different nerve agents and oximes with AChE and to evaluate kinetic constants of reactivation.

Key words: Chemical warfare; organophosphates; oximes; docking; QM/MM

INTRODUCTION

Chemical Warfare

Weapons of Mass Destruction (WMD), namely the nuclear, chemical and biological weapons, are capable of provoking a great number of deaths in just one attack [1] and represent, today, an unimaginable threat to society [2]. The chemical and biological agents constitute low cost classes of WMD but are hard to detect and control [3,4,5]. Differently from the nuclear agents, there are no significant technical obstacles in the production of such agents. Biological agents, for example, can be cultivated using basic techniques of microbiology and materials of easy acquisition. Samples of these agents are routinely traded for research purposes and can, also, in some cases, be obtained directly from infected animals in nature [6]. Chemical agents, in turn, can be easily produced in large amounts in any industrial facility.

The term "chemical warfare" was first used in 1917 because of the large scale use of chemicals du-

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ring World War I [7, 8]. According to the Organization for the Prevention of Chemical Weapons (OPCW) [9], chemical warfare agents are defined as chemical substances that can be used due to their toxic effects on human beings, animals or plants.[10] In a military context there are two basic goals of using chemical weapons: 1) to inflict causalities (deaths and injuries) and 2) to reduce the operational performance of the enemy troops due to the prolonged use of protective equipment [10].

The ease of production and the low cost have raised the interest of terrorist groups on chemical weapons, turning the defense against chemical warfare into a global concern and not anymore an essential military issue [9, 11-13].

The neurotoxic agents

Neurotoxic agents are organophosphates (OP), a class of chemicals with great military importance and extremely toxic inhibitors of the enzyme acetylcholinesterase (AChE) [14]. These compounds block the AChE activity in the synapses by binding a phosphoryl group to its active site, resulting in an accumulation of the neurotransmitter acetylcholine (ACh) and an uncontrolled activation of the cholinergic synapses [15].

The neurotoxic agents (or nerve agents) are the most lethal group among the OP. Tabun (Ethyl *N,N*-dimethylphosphoramidocyanidate), sarin [(*RS*)-Propan-2-yl methylphosphonofluoridate], soman (3,3-Dimethylbutan-2-yl methylphosphonofluoridate), cyclosarin (cyclohexyl methylphosphonofluoridate) and VX (Ethyl ({2-[bis(propan-2-yl)amino]ethyl} sulfanyl)(methyl)phosphinate (Figure 1) are the most known members of this family [16-21].

The recent attack with sarin in Syria (august of 2013) drew again the attention of the world public opinion and the scientific community, on the danger represented by chemical weapons. It is estimated that more than 1,400 people lost their lives, including 426 children. According to Syrian doctors the limited amounts of antidotes {pralidoxyme [(2-hidroxiimino) methyl]-1-methylpyridin-1-ium] and diazepam [7-cloro-1,3-dihydro-1-metil-5-fenil-1,4-benzodiazepin-2(2H)-one)]} available contributed to this high mortality rate [16].

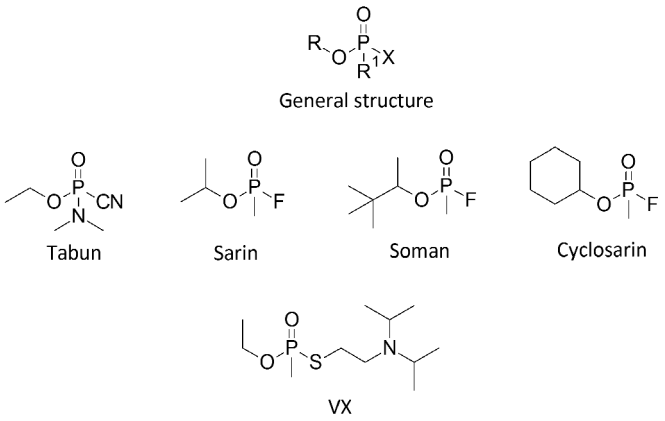

Figure 1. Structures of some nerve agents. In the general structure: X = F, CN or SR, R^1 = alkyl or alkylamine and R = alkyl, cycloalkyl, H or $(CH_2)nN+R_3$.

The nerve agents react directly with the OH group of the serine residue of the catalytic triad in the active site of AChE, forming an OP-AChE complex, relatively stable, and leading to fatal toxic effects [22]. These compounds collapse the Peripheral and Central Nervous Systems (PNS and CNS). Symptoms are anguish, loss of coordination, spasms and seizures followed by death [23].

The treatment against poisoning by nerve agents today includes the administration of an anticholinergic, usually intravenous atropine, to reduce the effects of the accumulation of ACh, combined with a CNS depressor, like diazepam, to reduce the spasms and seizures, and a cationic oxime to reactivate AChE. These three components are combined in self injection syringes that can be taken to the battlefield [5, 21, 24].

AChE, or acetylcholine acetyl-hydrolase, is a regulatory enzyme responsible for the transmission of nerve impulses in synapses through the hydrolysis

of ACh. This is a key role in the regulation of the nerve impulse transmission that, if inhibited, usually leads quickly to death [14, 25]. AChE is present in the PNS and CNS and in the neuromuscular synapses. It is one of the most efficient enzymes, being able to quickly hydrolyze ACh in the neuromuscular and cholinergic junctions of the brain [26, 27].

Each AChE monomer has one catalytic center containing 04 domains. The first domain is the catalytic triad [Ser203, Glu334 and His447 in human AChE (*Hss*AChE)] located at the bottom of the active site gorge [26, 28]. The second is the anionic sub site, located around 4.7 Å from Ser203, where the ammonium group of ACh interacts, electrostatically, with Glu334 [28, 29]. The role of the anionic sub site is to guide the charged part of the substrate entering the active site.[14] The third domain is constituted of a hydrophobic region, important to the binding of cyclic substrates [28, 29], and the fourth domain is responsible for interactions with cationic and some neutral ligands. This domain, called peripheral anionic site, is located at more than 20 Å from the active site and have residues Asp74 and Trp286 (in *Hss*AChE) as common nucleus.

It is important to notice that the binding of ligands with the peripheral site frequently causes conformational changes in the active site. Interactions of the ligands with these residues can be the key to the allosteric modulation of the catalytic activity of AChE [14, 28, 30]. Due to its key biological function, AChE is a molecular target vulnerable to neurotoxic agents, pesticides, snakes venoms and, also, drugs against illnesses like Parkinson and Alzheimer diseases [28, 29].

Nerve agents bond covalently to Ser203, interfering with the catalytic mechanism of AChE. These compounds block the hydrolysis of ACh, disturbing the cholinergic transmission [26, 28, 29]. After inhibition, AChE can be aged or spontaneously reactivated, a process significantly accelerated by a strong nucleophile like an oxime [31]. In the absence of an oxime the reactivation of most OP-AChE complexes goes at an insignificant rate and the aging prevails [26]. In this process an elimination reaction or spontaneous dealkylation of the P atom complexed with AChE, through the break of the O-alkyl bond, results in a very stable anionic complex, resistant to the reactivation by known oximes and irreversibly bonded to the enzyme [26, 31].

The AChE reactivation (Figure 2) is based on the dephosphorylation of Ser203. The positively charged N of the oxime is attracted by the anionic site of AChE, allowing the active part of the oxime positioning on the phosphorylated site of AChE to perform the nucleophilic attack [22, 32].

It is important to point out that the efficiency of this reactivation reaction depends on factors like the chemical structures of the nerve agent and the oxime. The complex soman-AChE, for example, is highly resistant to reactivation, while the complexes XV-AChE and sarin-AChE can be reactivated by a series of different compounds [20].

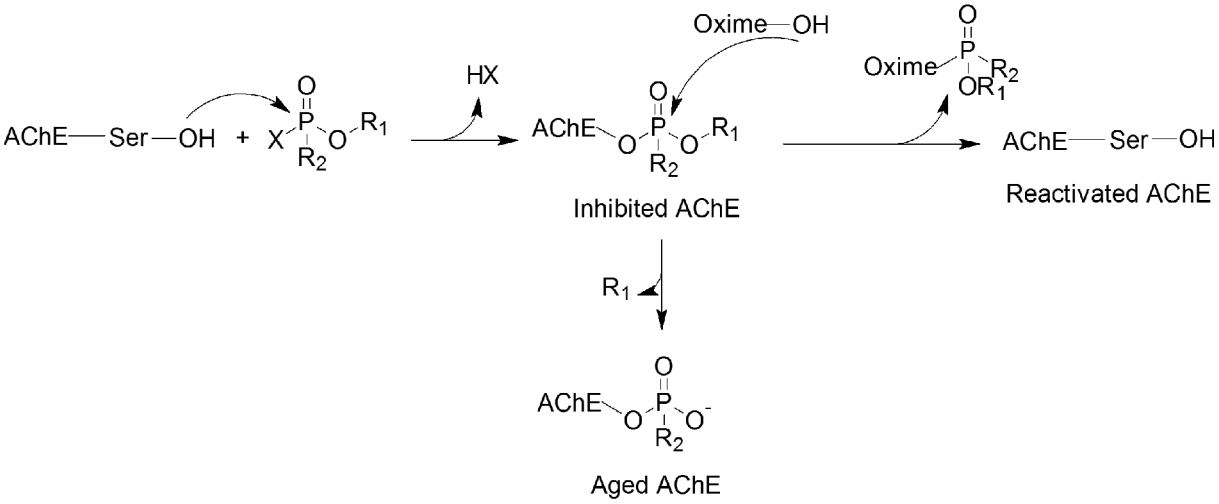

Figure 2. Inhibition and reactivation of AChE. R_1 = Alkyl, O-alkyl or amide; R_2 = O-alkyl, or amide and X = leaving group.

In the last century several oximes were synthesized and tested as antidotes against neurotoxic agents but, unfortunately, none of them showed to be efficient with all of them [35- 38]. This confirms the importance of the search for new oximes.

Here we revisited some former results of our research group through experimental and theoretical studies [docking and hybrid quantum mechanics/molecular mechanic methods (QM/MM)] in 2 research lines: 1) The study of the reactivation of AChE, inhibited by different nerve agents, by the oxime BI-6 [39] (1-2-hydroxyimino methyl pyridinium-4-carbamoyl pyridinium-2-butene) and 2) Study of the reactivation of the complex cyclosarin-AChE by different oximes [23].

METHODOLOGY

Docking studies

Docking is one of the most important techniques for the investigation of molecular interactions between a protein and a ligand when the 3D structure of the protein has been elucidated by crystallography, NMR or homology modeling [6, 40-42].

The value of the performance function E_{score} in docking is defined by equations 1, 2 and 3. Where E_{inter} corresponds to the interaction energy ligand-protein and E_{PLP} represents the potential energy of the inhibitor with two different sets of parameters: one for the approximation of the steric effect

$$E_{score} = E_{\text{int}\,er} + E_{\text{int}\,ra} \qquad \text{Eq. 1}$$

$$E_{\text{int}\,er} = \sum_{i=ligand} \sum_{j=protein} \left[E_{PLP}\left(r_{ij}\right) + 332.0 \frac{q_i q_j}{4 r_{ij}^2} \right] \qquad \text{Eq.2}$$

(van der Waals), between atoms, and the other for H-bond. The second term describes the electrostatic interactions between charged atoms. It is the Coulomb potential with a distance dependent dielectric constant [D(r) = 4r]. The numeric value of 332.0 fixes the electrostatic energy units in kcal.mol^{-1} [43-45]. and Eintra is the internal energy of the ligands (Equation 3).

$$E_{\text{int}\,ra} = \sum_{i=ligant} \sum_{j=ligand} E_{PLP}\left(r_{ij}\right) + \sum_{flexiblebonds} A\left[1 - \cos\left(m\theta - \theta_0\right)\right] + \mathrm{E}_{\text{clash}} \qquad \text{Eq. 3}$$

The two first terms in Equation 3 are related to all pairs of atoms of the ligand, excluding the pair of atoms connected by two bonds. The second term refers to the torsion energy, where θ is the torsion angle of the bond. The last term, E_{clash} (called correction term), computes a penalty of 1,000 if the distance between two heavy atoms (more than two bonds distant) is less than 2.0 Å, punishing nonexistent conformations of the ligand. In short these functions are used to automatically dock a flexible molecule in a template (protein) [43].

It is important to keep in mind that usually the molecules drawn in the 3D form are not necessarily in the most stable conformation. Distortions can occur during the creation of the 3D structure, leading to unfavorable bond angles and dihedrals. Also, non-bonded atoms can interact in a same region in space causing steric and electrostatic repulsion. In order to correct such distortions, the molecules are optimized by two mathematical models: (i) molecular mechanics or (ii) quantum mechanics. Non predictable interactions, related to the superposition of molecular orbitals, distribution of electronic density or steric interferences can be solved by the computational methods. The energy minimization and conformational analysis are used interactively to optimize the geometry of a molecule [20]. However, to understand a drug mechanism of interaction, it is essential to know the tridimensional positioning appropriate to its molecular interaction with the target, considering that the prediction of the geometry and energy of bonding is of great interest in drug design. In this sense the docking technique can be extremely useful. In fact this technique finds an average of possible stable structures of the ligand in the target and calculates the relative stability. In order to find

the lower energy structure, without any previous insight, it is necessary to analyze all modes of interaction, considering the conformational flexibility of the ligand. As these two issues are interconnected, they can be solved at the same time. However, the number of combinations involved is too large [46].

Despite the great importance and countless applications, the docking technique is limited when the phenomena involves breaking and formation of bonds. In this case the QM/MM hybrid techniques are employed.

Study of the interactions between BI-6 and AChE inhibited by different OP

The *in vitro* studies with BI-6 involved a set of standard experimental procedures [47]. Rat brains were used as source of AChE. They were obtained from anesthetized rats (narcosis does not influences the AChE activity [16]) after decapitation and homogenization in water. The efficiency of reactivation was tested with homogenate of rat brain (p/v) incubated with the appropriate nerve agent for 30 minutes in order to achieve 96% of AChE inhibition. After, BI-6 was added for 10 minutes. Measures were done at 25 oC, pH 8 and concentrations between 10^{-3} and 10^{-5} M. The activity of AChE was measured by a potenciostatic method using a titrator RTS 822 (Radiometer, Denmark). Data on the initial rate (i.e. initial part of the curve) of the reaction enzyme-substrate (acetylcholine iodate 0.02 M) were used to calculate the percentage of reactivation from Equation 4. Where a_o is the activity of the free enzyme, ai is the activity of the enzyme inhibited by the nerve agent and a_r is the activity of the inhibited enzyme after incubation with BI-6.

$$\%reactivation = 100 - 100(a_o - a_r)/(a_o - a_i) \qquad \text{Eq. 4}$$

The coordinates of AChE from *Mus Musculus* (*Mm*AChE) phosphorylated by sarin and complexed with HI-6 [({4-[(Iminocarbonyl)pyridiniomethoxy) methyl]}-2-[(hydroxyimino)methyl]pyridinium dichloride were downloaded from the Protein Data Bank (PDB), under the code 2WHP [48]. The structure of HI-6 inside this crystal was modified to BI-6 using the software Spartan08® [49], in order to obtain the 3D structure of AChE complexed with BI-6.

The 3D structures of tabun (GA) sarin (GB), soman (GD) and cyclosarin (GF), were constructed based on the crystallographic structure of sarin from the crystal, using Spartan08® [49]. Subsequently, the geometry optimizations and calculations of partial atomic charges were performed with the same program, using the semi empirical method AM1.

The docking studies were performed using the software Molegro Virtual Docker 2006 (MVD)®, [43] according to the instructions. The binding sites were restricted to a sphere with 7 Å of radius centered in the BI-6 structure and including all residues of the active site. Due to the stochastic nature of the search algorithm, around 15 solutions were recorded for each run. The poses with the best superposition related to HI-6 from the crystal were selected to the analysis of the present work.

Study of the interactions of different oximes and AChE inhibited by cyclosarin

The *in vitro* data of the oxime/$AChE_{inhibited}$ binding constant (KR) and rate constant of the reactivation process (kR) for the oximes studied here (Figure 3) with MmAChE inhibited by GF were reported by Kassa *et al.* (2007) [50].

The crystallographic coordinates of *Mm*AChE phosphorylated by GA and complexed with HI-6 (PDB code: 2WHP) were downloaded from PDB [48] and had the crystallographic water molecules removed. The structure of GA inside *Mm*AChE was converted to GF by the introduction of the group $–CH_2CH_2CH_2–$, using spartan08® [49], in order to obtain the 3D structure of *Mm*AChE phosphorylated by GF. The 3D structures of each oxime in Figure 3 were constructed based on the HI-6 structure from the crystal.

The QM/MM hybrid methods

Due to the large number of atoms in proteins and the fact that chemical reactions always involve breaking and formation of chemical bonds, enzymatic catalysis is a big challenge in computational chemistry [52].

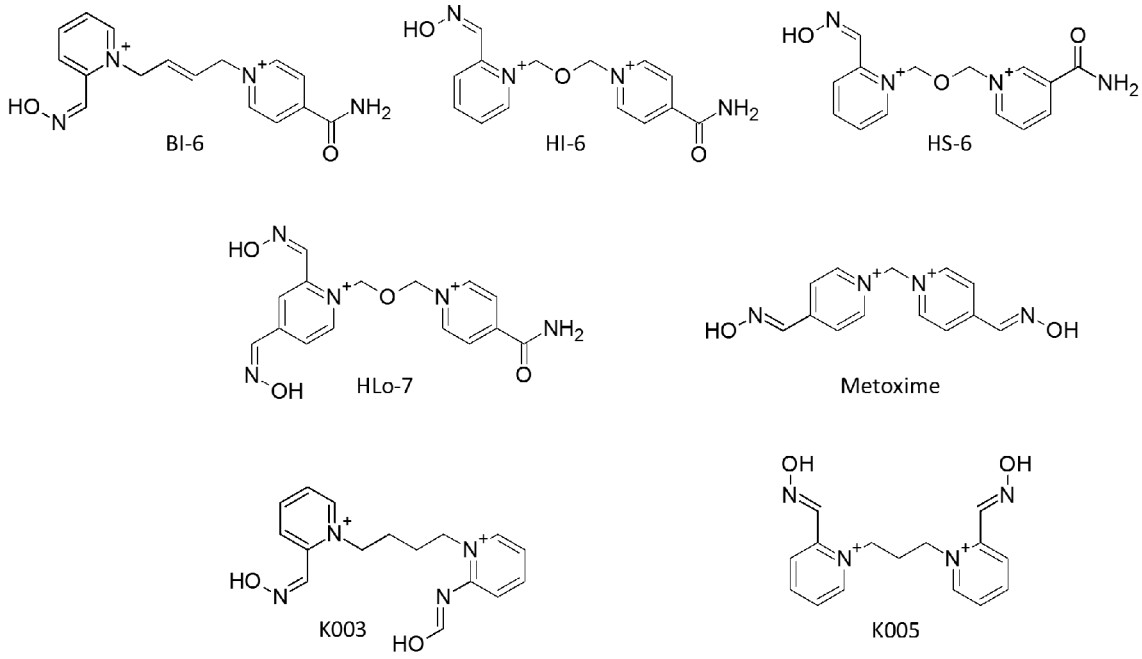

Figure 3. Oximes studied.

QM methods of high theoretical level are limited to systems with a relatively small number of atoms. Combination of QM and MM methods extends the dominium of the QM calculations to macromolecules. The basic strategy of this methodology, introduced by Warshel and Levitt [53], can be described assuming that the system can be divided into two subsystems: the QM and the MM regions. The MM region contains all atoms explicitly treated in the calculation while in QM region the atoms are represented by nucleus and electrons and the potential surface is constructed under the Born-Oppenheimer approximation [54].

Study of the interactions between BI-6 and AChE inhibited by different nerve agents

Calculations of the mechanism of reaction and DFT were performed with software Spartan® [49]. and Gaussian09® [55], respectively. QM regions were cut off from the docking results using SPDBViewer [51]. This region consists on the catalytic triad (Ser203, Glu334 and His447), residues involved in H-bond with BI-6 and the cofactor. All transition states (TS), intermediates and precursors involved were calculated and characterized by calculating the imaginary frequency. Each conformer was totally optimized in both levels, PM3 and DFT with conjugate gradient and quasi-Newton-Raphson algorithms. The final geometry was obtained by the DFT method using B3LYP/6-31G+(d,p) [56].

QM/MM study of the interactions between different oximes and AChE inhibited by cyclosarin

The methodology used here is similar to the former one with small differences. The QM calculations were performed with the packages Spartan® [49] and Gaussian98 [57], the QM regions were delimited inside a sphere with a radius between 9 and 15 Å and each conformer was totally optimized in DFT level with B3LYP/6-31G [56].

RESULTS AND DISCUSSION

Study of the interactions between BI-6 and AChE inhibited by different nerve agents

Table 1 presents the experimental results, the values of intermolecular interaction energy and the residues interacting with BI-6 for the complexes *Mm*AChE-OP in the docking studies.

It is clear that there is not a significant difference among the interaction energy values. This was already expected considering the similarity among

Table 1. Docking results of BI-6 inside *Mm*AChE.

Complex	Residue	ΔE* (kcal.mol^{-1})	H-bond energy (kcal.mol^{-1})	Experimental values of reactivation (%)
AChE-GF	Tyr124 Tyr124 Tyr124	-159.44	-5.52	1
AChE-GD	Tyr124 Tyr124 Tyr124 Glu285	-152.14	-9.37	9
AChE-GA	Tyr124 Tyr124	-155.54	-4.34	22
AChE-GB	Tyr124 Tyr124 Tyr124	-152.21	-3.61	32

*ΔE = Energy of intermolecular interaction.

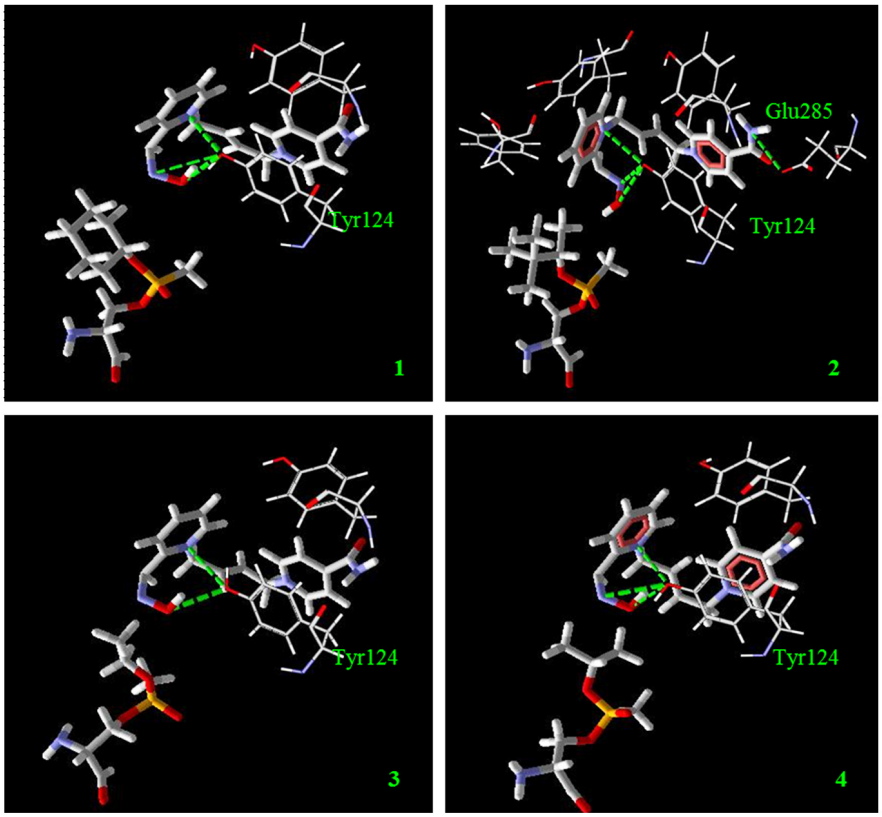

Figure 4. Interactions observed for each system. 1 – AChE-GF, 2 – AChE-GD, 3 – AChE-GA and 4 – AChE-GB.

the systems. Except for the complex AChE-GD (that interacted also with Glu285), the other systems interacted only with Tyr124 (Figure 4) and a possible π-stacking interaction between the pyridine ring of BI-6 and Tyr124 was observed. Also, long range interactions could be contributing with extra stability to the TS. This interaction was also observed in a former study of our research group [23].

It is important to point out that theoretical results of interaction energy do not explain experimental results well. Based on this we also performed

theoretical calculations to determine the relative $\Delta\Delta E^{\#}$ for each system studied where we compared the values of $\Delta E^{\#}$ at the TS and at the initial configuration of each system. So we could draw the tendency of reactivation of each oxime, thus avoiding the direct calculation of the absolute values of energy.

Several studies have investigated the mechanism of action of oximes in the last decades and there are convincing evidences of the oximes' action, mainly as AChE reactivators [3, 5]. The reactivation process happens in two steps (Equation 5). First there is the for-mation of a reversible Michaelis like complex AChE-phosphyl with a penta-coordinated TS (in the case of inhibition by nerve agents) followed by the displacement of the phosphyl group from the TS. The K_R is, therefore, similarly to the dissociation constant, inversely proportional to the oxime affinity for the phosphorylated AChE and the oxime's reactivity can be expressed in relation to the rate constant k_R.

$$EI + Ox \underset{}{\overset{K_R}{\rightleftharpoons}} EIOx \xrightarrow{k_R} E + I\text{-}Ox \qquad \text{Eq. 5}$$

Table 2 reports the kinetic parameters $\Delta\Delta E^{\#}$ predicted by theoretical calculations, the experimental values and the respective imaginary frequencies characteristic of each TS.

Table 2. Experimental results, intermolecular energy and activation energies of BI-6 with 4 different nerve agents.

System	% of reactivation	ΔE (kcal mol^{-1})	$\Delta\Delta E^{\#a}$(kcal mol-1)	Frequency (cm^{-1})
AChE-GF	1	-159.44	0.00	i97.88
AChE-GD	9	-152.14	-18.16	i95.83
AChE-GA	22	-155.54	-21.95	i100.25
AChE-GB	32	-152.21	-28.22	i97.95

[a]$\Delta\Delta E^{\#}$ (Reference: complex AChE-cyclosarin) = Δ_{LIG2} - Δ_{LIG1}

As observed before, the interaction energies obtained from docking studies are very similar for all systems. So the experimental data can't be rationalized based only on the ΔE values. The $\Delta\Delta E^{\#}$ values, on the other hand, can be used to rationalize, in part, the experimental results (R2 = 0.75 in Figure 5). We expect that a better correlation between theoretical and experimental values could be achieved when other effects related to the interaction process are included.

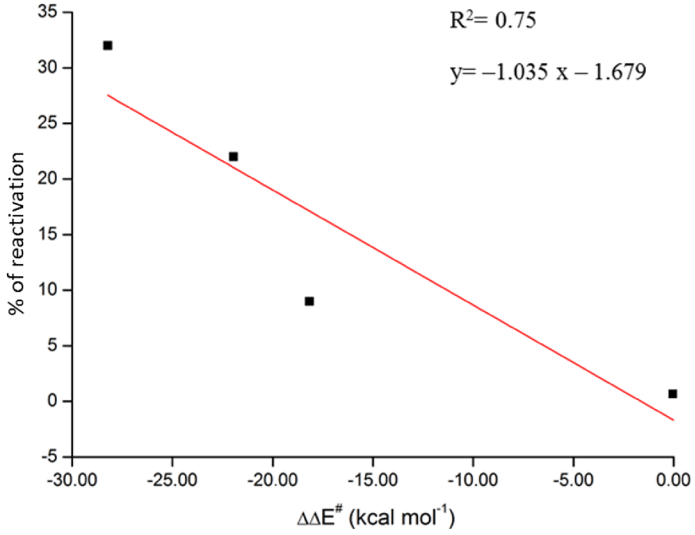

Figure 5. % of reactivation *versus* $\Delta\Delta E^{\#}$.

In fact the reactivation process depends on two issues: 1) The interaction of the oxime with the inhibited enzyme and 2) the chemical reaction between oxime and nerve agent. From data in Table 2, a mathematical model was constructed using the method of multiple linear regression (MLR), resulting in equation 6 that can be useful to predict the reactivation percentage of the systems studied.

$$\%reactivation = -2.39(\Delta E) - 1.62(\Delta\Delta E^{\#}) - 381.15 \qquad \text{Eq. 6}$$

The theoretical values for the reactivation percentage obtained from equation 6 showed a good correlation with the experimental results ($R^2 = 0.90$). The same correlation was not observed for the kinetics parameters versus the interaction energies separately ($R^2 = 0.07$ and $R^2 = 0.75$, respectively). Thus, our theoretical results evidence that the interaction and the mechanism of reaction have an important role in the reacti-vation process.

Equation 6 also shows that the lowest interaction energy values lead to a larger percentage of reactivation. It is noticeable that the values of ΔE and $\Delta\Delta E^{\#}$ in equation 6 are similar but the kinetic parameters are more sensible to small variations.

Study of the interactions between different oximes and AChE inhibited by cyclosarin

To evaluate the reactivation power of oximes, it is necessary to compare the interaction energies among the residues present in the active site of *Mm*AChE and GF. This will provide a better understanding of the interaction modes and factors responsible for the activities of each oxime. Table 3 and Figure 6 present the experimental values of pK_R and the interaction energy for the oximes studied.

Based on Figure 6 it is possible to observe that the largest value of pK_R correspond to a lower value of interaction energy. In this sense oxime K005 presents the best affinity for the active site of *Mm*AChE while BI-6 presents the worse affinity.

It was also observed that BI-6 makes the lowest number of H-bonds while HS-6 presents 03 interactions, K005 and HLö-7 present 5, K033 and HI-6 present 6 and metoxime present 8 interactions with the aminoacids of the active site.

It is important to stress that a minimal change in the molecular structure of the reactivators is enough to significantly change its reactivity and, consequently, the capacity to reactivate AChE.

After the QM/MM study we obtained the values of $\Delta\Delta E^{\#}$ and imaginary frequency for the oximes studied, described in Table 4.

Table 4 shows that the reactivation of GF inhibited *Mm*AChE by oximes Hlö-7 and HI-6, presented the lowest and largest energy barriers, respectively. A possible explanation is that the stabilization of the TS is favored by H-bonds with the residues close to the active site. These aminoacids (Tyr124, Trp286, Phe295, Arg296 and Phe338) guide the oxime towards the appropriate geometry of the TS. In these conformations the oximes interact more strongly with GF, increasing the probability of formation of the products.

Table 3. Experimental values of pk_R and interaction energy (IE) for the oximes studied.

Oxime	pK_R* (mmol L^{-1})	ΔE** (kcal mol^{-1})	Interacting residues
K005	5.30	–143.48	Phe338/Phe295/Tyr124/Arg296/Trp286
HI-6	4.92	–142.98	Tyr124/Tyr124/Tyr124/Tyr124/Ser298/Ser298
Metoxime	4.82	–142.32	Thr83/Tyr337/Tyr337/Tyr341/Tyr124/Tyr124/Ser298/Ser298
K033	4.70	–142.86	Trp286/Arg296/Arg296/Phe295/Tyr124/Tyr124
HS-6	4.35	–142.67	Tyr124/Tyr124/Phe295
HLö-7	3.00	–141.91	Arg296/Tyr124/Tyr124/Tyr124/Tyr124
BI-6	1.00	–140.21	Glu285/Tyr124

*Experimental data; **Theoretical data.

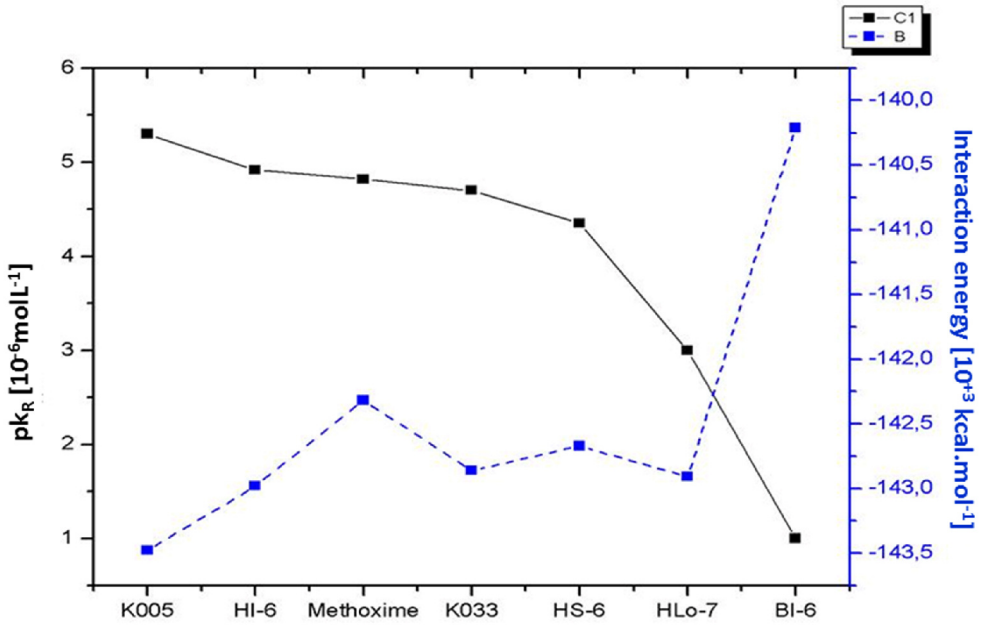

Figure 6. Values of the experimental constant of dissociation of the complex oxime-inhibited AChE ($pK_R = -\log K_R$, $\mu mol.L^{-1}$) [50] and intermolecular interaction ($kcal.mol^{-1}$) obtained from docking.

Long range interactions between oximes and other aminoacids can also occur, like cation-π interactions with Tyr34, Phe295, Tyr337 and Phe338, electrostatic interactions with Glu285 and, surprisingly, π-staking interactions between the pyridine ring of the oxime and residues Tyr124 and Trp286 that happen in the TS geometry.

The oxime K005 is considered to be one of the best GF inhibited AChE reactivators, presenting good K_R results (Figure 6) and, therefore, a high affinity for the free enzyme (Table 3). However, it is important to say that an excess of this oxime can also inhibit the reactivated AChE [50, 58].

CONCLUSIONS

In the first study reported here it was observed that the interaction process of BI-6 is favored by interactions with Tyr124 and Glu285. Analyzing the interaction energies obtained, we realized that they do not explain the experimental values. Thus further theoretical calculations of the activation energies were performed and showed a good correlation with the values of percentage of reactivation. However, the correlation between the interaction energies and the relative energies of reactivation showed a $R^2 = 0.90$, suggesting that our theoretical achievements evidenced that both the interaction step

Table 4. Activation energies related to the TS and kinetic parameters of the oximes studied.

Oxime	K_R[a] (min^{-1})	$\Delta\Delta E^{\#}$[b] (kcal mol-1)	Frequency (cm^{-1})
HI-6	0.350	220.85	i5.15
BI-6	0.150	170.43	i64.21
metoxima	0.240	132.00	i16.59
HS-6	0.156	131.12	i13.68
K033	0.095	82.34	i5.21
K005	0.010	31.96	i74.20
HLö-7	0.008	0.00	i2.31

[a] Constant of reactivation; [b] $\Delta\Delta E^{\#} = E_{LIGi} - E_{HLö-7}$.

and chemical reaction step have an important role in the reactivation process.

In the second study we observed that the interaction process of the oximes is favored by residues Tyr124, Trp286, Phe295 and Arg296, while the TS is stabilized by Tyr124, Trp286 and Arg296. Thus, we believe that the calculated kinetics parameters can be useful for the project and selection of new and more efficient oximes. It is important to notice that we observed, in both studies, that the number of H-bonds with residue Tyr124 is a key feature to determinate the interaction mode of each oxime.

The molecular modeling studies of oximes as AChE reactivators, available in literature, point to several important features to be considered for a better understanding of the mechanism of action and further design of new AChE reactivators. Our results showed a good correlation between theoretical and experimental data, suggesting that our methodology is useful to the prediction of ki-netics and thermodynamics parameters for AChE reactivators.

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