Published in final edited form as:

Chem Biol Interact. 2008 September 25; 175(1-3): 187–191. doi:10.1016/j.cbi.2008.05.006.

Reactivation of model cholinesterases by oximes and intermediate phosphyloximes: A computational study

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Abstract

Phosphyloximes (POX) are generated upon the reactivation of organophosphorus (OP) inhibited cholinesterases (ChEs) by pyridinium oximes. These POXs are known to be potent inhibitors of the ChEs following reactivation. However, they can also decompose to give an OP derivative and a cyano derivative of the oxime when a base abstracts the benzylic proton. Using density functional theory, thermodynamic properties were calculated for the reactivation and decomposition pathways of three different oximes (2-PAM, 3-PAM and 4-PAM) with six different OPs (cyclosarin, paraoxon, sarin, tabun, VR and VX). For reactivation purposes, 2-PAM is predicted to be more efficient than 3- and 4-PAM. Based on atomic charges and relative energies, 2-POXs were found to be more inclined towards the decomposition process.

Keywords

Cholinesterase; phosphyloximes; inhibition; decomposition; density functional theory

Introduction

Reactivation of organophosphorus (OP) inhibited cholinesterases (ChEs) can be accomplished by using pyridinium based oximes.¹ Many oximes have been designed and tested against a variety of OPs in the past, but a general and efficacious remedy against all of the OPs has yet to be discovered. Phosphyloximes (POXs) are formed during the reactivation process by the reaction of the oxime with the OP, and these products are potent inhibitors of ChEs and can remain in the active site. In addition to inhibiting ChEs, POXs can also decompose to generate a cyano derivative as well as a phosphate analogue of the OP – a process initiated by base-induced abstraction of the benzylic hydrogen depicted in Figure 1.

To minimize the inhibition pathway for POXs, a thorough understanding of the thermodynamic and kinetic parameters governing the two competing processes is required. Doctor et al.² have reported extensive data on the inhibition of ChEs by POXs formed upon reactivation of various OP-inhibited enzyme using 2-, 3- and 4- [hydroxyimino methyl]-1-methylpyridinium chloride (PAM) as shown in Figure 1. They concluded that POXs generated from 2-PAM (2-POXs) have a significantly higher rate of decomposition than the corresponding compounds formed from 3- and 4-PAM (3- and 4-POXs, respectively). However, the inhibition pathway was also highly facile for 2-POXs compared to the others. In addition, they performed some semi-

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empirical calculations, the results of which suggested that acidity of the benzylic hydrogen might play an important role in POX reactivity. However, at present there are no *ab initio* studies examining the relative energetics of the two pathways of POX activity which could be used to modulate the reactivation and inhibitory activity of novel oximes. In this study, we present a systematic computational investigation of the thermodynamic parameters for both pathways with six OPs (cyclosarin, paraoxon, sarin, tabun, VR and VX; Figure 2) and three oximes (2-, 3- and 4- PAM).

Computational Details

All calculations were performed using the Gaussian 03 program suite³ at the Ohio Supercomputer Center. The geometries were optimized with density functional theory using Becke's three parameter exchange functional and the correlation functional of Lee, Yang, and Parr (B3-LYP),⁴ with a 6-31+G(d) basis set.⁵ For computing the thermochemistries, the ChE was represented by an ethoxide group, simulating the catalytic serine. All structures were confirmed to be minima via vibrational frequency analyses; zero-point vibrational energy corrections were not scaled. Thermodynamic parameters discussed herein are ΔH values and were calculated at the B3LYP/6-311+G(d,p)//B3LYP/6-31+G(d) level of theory at 298K, unless noted otherwise. Electrostatic potential charges were calculated using the CHELPG⁶ method, and the inclusion of implicit solvation effects was performed using the polarizable continuum model (PCM) model for water.⁷

Structures

2-PAM is a widely used therapeutic for the reactivation of OP-inhibited ChEs. However, there are no comparative studies of the three PAM derivatives, which attempt to correlate computed properties with their reactivation efficacies. To this end, we have optimized the geometries of six energetically favorable conformers for 2-PAM and 3-PAM and three conformers of 4-PAM (Figure 3). It has been proposed that the **ESA** conformer of 2-PAM is the biologically active form.⁸ Recently, Harel et al.⁹ solved an x-ray crystal structure (2.71 Å resolution) with 2-PAM bound to AChE, and this study also suggested that 2-PAM is in the ESA or ESS conformation. However, the geometry of the oxime group about the exocyclic carbon atom is quite unusual with a C-C=N angle of ~172°. According to our calculations, the **EAA** and **ESA** conformers for 2- and 3-PAM were found to differ by only 0.5 kcal/mol (Table 1). The **EAA** (**EA** for 4-PAM) conformers were determined to be the most stable structures for all three PAMs. These results are consistent with the available X-ray structure¹⁰ and previous calculations performed for 2-PAM.¹¹ Hence, the **EAA** conformers were used in calculating the overall reaction thermodynamics. All of the POXs prefer *syn* stereochemistry at the O-N=C(H)-C(Pyridine) bond, consistent with the conclusions from NMR experiments.¹²

Reactivation Process

In the gas phase, all of the reactivation processes were found to be endothermic except for tabun, which is just slightly exothermic, as shown in Table 2 (the reverse process, inhibition, will thus be exothermic for all OPs except tabun). Although the enthalpy of reactivation for tabun is close to being thermoneutral, it is interesting to note that based on *in vitro* experiments performed by Szinicz et al.,¹³ these authors reported that reactivation was more favored for tabun relative to sarin. However, we should note that the present calculations are only thermodynamic predictions, and kinetic information will be needed to provide more insight to differentiate between this set of OPs. (Such studies are currently under investigation.) Further, these reactions became more endothermic upon solvation, due to an increase in charge delocalization for the POX compared to the parent oxime, which results in differential stabilization of the parent oximes over the POXs. 2-PAM was the best oxime for the reactivation process as suggested by the gas phase enthalpies. In contrast, enthalpic data for

the reactivation in the aqueous phase suggested that 2-PAM was the least suitable oxime. This is likely the result of reduced solvent stabilization for 2-PAM and corresponding POXs, as they are less polar than their corresponding 3- and 4-substituted structural isomers. Magnitudes of the dipole moments of these species were found in the order 2-PAM < 3-PAM $\approx$ 4-PAM.

Charge analyses are summarized in Table 3 for the oximes studied. Because the trend in the charge analysis is similar for both the gas phase and aqueous solution, we have shown only the aqueous phase results. A correlation was observed between increased charge separation at the oxime O–H bond and reaction exothermicity for the reactivation pathway. 2-PAM was found to have the highest charge separation at the oxime O–H bond, indicating that it is the most acidic among the three oximes studied. These results suggest that 2-PAM is also the most nucleophilic oxime in the set and therefore is a better candidate for increased reactivation than the others.

Decomposition Process

The POXs can decompose in the presence of a base as shown in Figure 1. The reaction generates a negatively charged OP analogue and the conjugate acid of the base used, which is highly unfavorable from an energetic standpoint. Thermodynamic calculation were carried out using either water or ethoxide ion as a base, which yielded the reaction enthalpies for the decomposition pathway of about 160 kcal/mol and –55 kcal/mol respectively. These seemingly contradictory energetics for the decomposition process are consistent with the proton affinities of each nucleophile, water and ethoxide, respectively. Thus proton transfer to the OP analogue will take place simultaneously to the generation of the conjugate acid. Therefore, we hypothesize that the decomposition process occurs via a proton-shuttle mechanism as shown in Figure 4 and the corresponding reaction enthalpies are summarized in Table 2.

All of the decomposition processes were found to be exothermic overall, confirming that decomposition is a thermodynamically viable pathway for POX species. Based on the decomposition enthalpies, 3-POXs were found to be more favorable toward the decomposition pathway. This result is supported by the fact that 2- and 4-POXs are resonance stabilized but 3-POXs are not. The exothermicities of the decomposition process were nearly doubled in the aqueous phase, because two molecules were solvated on the product side which yielded higher solvation energy compared to the parent POX at the reactant side. This was confirmed by comparing the gas phase and the solution phase energies.

By comparing the energetics among the sets of POXs, we found that 2-POXs were 3–5 kcal/mol less stable than 3- and 4-POXs. This could be explained by the steric bulk provided by the pyridinium methyl group around the oxime-OP linkage, which is not present in the case of 3-POXs and 4-POXs. This observation was also supported by previous work² which showed that 3- and 4-POXs were 100–500 times more stable than the 2-POXs. Additionally, we performed CHELPG charge analysis for all of the POX species to gain insight into the parameters governing the decomposition pathway of the POXs (Table 4.) The trends in the charges were similar to the thermochemical results: charge separation at the O–N bond was about 3–5 fold higher in the 2-POXs than the others, indicating a higher propensity for decomposition, and thus lower stability. In addition, the benzylic proton had the highest positive charge concentration in the case of 2-PAM, which correlates with the increased propensity for decomposition in 2-POXs.

Conclusions

In summary, the reactivation process was found to be endothermic for all systems and 2-PAM was concluded to be a more efficient reactivator than 3- and 4-PAM for the reactivation process, due to higher charge separation at the oxime bond. From an enthalpic point of view, the

protein's active site environment will provide an electrostatic environment which will be somewhat in between the gas phase and aqueous phase; quantum mechanics/molecular mechanics calculations are in process to obtain more refined results as to the electrostatic effect of the active site. The 2-POXs were found to be less stable than the others, which we attributed to the steric effects of the pyridinium methyl group and also reflected by the higher acidity of its benzylic proton which encouraged the decomposition. The calculations performed herein indicate that 2-substitution improves oxime performance with regard to reactivation and decomposition. Further calculations to characterize the transition states for the processes to complete a kinetic picture are currently in progress.

Acknowledgement

Support of this work by a generous grant from US Army Medical Research Institute of Chemical Defense (MRICD) and computational facilities from the Ohio Supercomputer Center is gratefully acknowledged.

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Figure 1.

Reactivation of the OP-inhibited ChEs by oxime and POX formation (top); POX decomposition pathway leading to cyano compound and OP analogue (bottom). (2-PAM and 2-POX are shown as the oxime in the top figure, 3-PAM and 3-POX, and 4-PAM and 4-POX are meta- and para- positioned oxime groups with respect to the pyridinium methyl group, respectively.)

Cyclosarin **Paraoxon** **Sarin** **Tabun** **VR** **VX**

Figure 2.
Organophosphorus (OP) compounds under study

Figure 3. Different conformers of 2-PAM considered for this study. (**EAS** conformer shows the naming convention of the conformers; **EAA** conformer shows the atomic numbering).

Figure 4.
Hypothesized decomposition pathway for POXs via proton-shuttle mechanism.

Table 1

Relative energies (ΔE in kcal/mol) of different conformers of the PAMs at the B3LYP/6-311+G**//B3LYP/6-31+G* level. (Values in parentheses are ΔE including aqueous solvation at the PCM level.)

	2-PAM	3-PAM	4-PAM
EAA	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
ESA	0.5 (0.1)	-0.4 (0.2)	---
EAS	8.0 (3.7)	7.3 (3.5)	7.0 (3.5)
ESS	7.2 (3.7)	6.2 (3.6)	---
ZSA	4.1 (2.9)	1.6 (2.2)	---
ZAA	3.7 (3.2)	2.6 (2.4)	3.5 (3.2)

Table 2

Calculated ΔH (298K) for the reactivation and decomposition processes in kcal/mol at the B3LYP/6-311+G**//B3LYP/6-31+G* level.
(Values in parentheses are ΔH at 298K including aqueous solvation at the PCM level.)

	Reactivation Process			Decomposition pathway		
	2-PAM	3-PAM	4-PAM	2-PAM	3-PAM	4-PAM
Cyclosarin	0.0 (5.7)	0.4 (4.7)	1.2 (4.9)	-19.3 (-39.6)	-19.7 (-40.0)	-18.6 (-38.6)
Paraoxon	4.9 (10.0)	4.5 (8.4)	7.4 (10.3)	-20.1 (-40.4)	-19.8 (-40.1)	-20.7 (-40.4)
Sarin	2.9 (8.3)	4.8 (9.3)	5.7 (9.7)	-17.8 (-37.1)	-19.7 (-39.5)	-18.7 (-38.2)
Tabun	-1.5 (6.3)	-1.1 (5.3)	-0.1 (5.7)	-17.9 (-38.1)	-18.4 (-38.4)	-17.4 (-37.2)
VR	2.7 (8.0)	2.9 (7.0)	3.9 (7.3)	-19.0 (-39.3)	-19.2 (-39.6)	-18.3 (-38.2)
VX	1.1 (8.1)	1.9 (7.6)	2.9 (7.8)	-20.3 (-39.8)	-21.1 (-40.6)	-20.1 (-39.3)

CHELPG charges (in units of electrons) at the B3LYP/6-311+G**//B3LYP/6-31+G* level in the aqueous phase for the oximes. (Atom labels are shown in Figure 1.)

Table 3

Conformer	H1	O2	N3	C4	H5	C6	N7
2-PAM/EA	0.504	-0.451	-0.217	0.222	0.144	0.047	0.129
3-PAM/EA	0.495	-0.469	-0.265	0.264	0.122	-0.083	0.197
4-PAM/EA	0.494	-0.447	-0.230	0.205	0.132	0.097	0.120

Table 4
CHELPG charges (in units of electrons) at the B3LYP/6-311+G**//B3LYP/6-311+G* level in the aqueous phase for the POXs. (Atom labels are shown in Figure 1.)

POXs	O2	N3	C4	H5	C6	N7
2-Cyclosarin	-0.346	-0.104	0.106	0.148	0.145	0.134
3-Cyclosarin	-0.324	-0.235	0.237	0.115	-0.041	0.188
4-Cyclosarin	-0.303	-0.212	0.172	0.128	0.139	0.125
2-Paraoxon	-0.323	-0.089	0.106	0.147	0.178	0.107
3-Paraoxon	-0.311	-0.256	0.261	0.122	-0.050	0.175
4-Paraoxon	-0.313	-0.226	0.219	0.122	0.125	0.124
2-Sarin	-0.336	-0.104	0.096	0.148	0.166	0.119
3-Sarin	-0.323	-0.230	0.222	0.121	-0.036	0.177
4-Sarin	-0.303	-0.218	0.187	0.124	0.123	0.140
2-Tabun	-0.290	-0.095	0.072	0.143	0.210	0.089
3-Tabun	-0.272	-0.245	0.206	0.119	0.119	0.119
4-Tabun	-0.244	-0.238	0.187	0.115	0.115	0.136
2-VR	-0.348	-0.096	0.098	0.147	0.160	0.116
3-VR	-0.316	-0.245	0.235	0.116	-0.039	0.176
4-VR	-0.304	-0.235	0.205	0.120	0.121	0.134
2-VX	-0.350	-0.094	0.096	0.150	0.165	0.122
3-VX	-0.318	-0.248	0.270	0.104	-0.068	0.170
4-VX	-0.297	-0.226	0.192	0.117	0.151	0.135