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Hydrolysis of Nerve Agents by Model Nucleophiles: A Computational Study

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Abstract

Density functional theory calculations were employed to study the reaction of five nerve agents with model nucleophiles, including EtX^- and EtXH ($\text{X} = \text{O}, \text{S}, \text{Se}$) for serine, cysteine and selenocysteine, respectively. Calculations at the B3LYP/6-311++G(2d,p) level of theory predict an exothermic reaction between ethoxide and all of the nerve agents studied. As compared to EtO^- as a nucleophile, these reactions become ~ 30 kcal/mol more endothermic for EtS^- , and by ~ 40 kcal/mol for EtSe^- . The equivalent reactions with the neutral nucleophiles (EtXH) were more endothermic. The effect of solvation on the reaction thermochemistry was determined using a polarizable continuum model simulating the dielectric constant of chloroform. While there was a large exothermic shift for reactions involving charged nucleophiles with solvation modeling, the corresponding shift was minimal for the reaction with neutral nucleophiles.

Keywords

Nerve Agent; Computational Chemistry; Acetylcholinesterase

Introduction

Nerve agents such as soman (GD), sarin (GB) and VX belong to a family of compounds known as organophosphorus compounds (OPs). The OPs are known to inhibit the activity of acetylcholinesterase (AChE), a protein responsible for the hydrolysis of the neurotransmitter acetylcholine. The catalytic center of AChE is a triad comprised of serine-200, histidine-440, and glutamate-327.¹ Although acetylcholine is hydrolyzed by AChE at nearly diffusion-controlled rates,² nerve agents bind to the catalytic serine, thereby inhibiting cholinesterase activity. The inhibited enzyme could be reactivated by a sufficiently strong nucleophile, such as oximes, which are currently employed as therapeutics for OP exposure.³ However, the efficacy of AChE reactivation by various oximes is dependent on the nerve agent with which the cholinesterase was inhibited as well as on the ChE source.³ OPs bound to AChE can also undergo dealkylation,⁴ a process referred to as aging, which leads to irreversible enzyme inhibition. Due to the rapid aging rates of some nerve agents (with half lives ranging from minutes to days)³ as well as the varying selectivity of oximes towards reactivation of inhibited AChE, it is desirable to develop a protein capable of hydrolyzing a broad spectrum of nerve agents to act as a prophylactic for those in high-risk positions of nerve agent exposure.

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Previous studies have shown that the substitution of selenocysteine (U) for cysteine (C) in enzymatic systems has yielded increases in activity varying from 2-fold to several thousand-fold.⁵ We have therefore determined the variable binding of OPs to serine, cysteine, and selenocysteine analogues (replacing the nucleophile oxygen with sulfur or selenium, respectively) to glean the effects of varying nucleophilic strength on hydrolysis.

Unfortunately, computational modeling of the reaction between OPs and AChE is extremely complex due to the requirement for high accuracy and the size of the protein. Numerous theoretical studies have been conducted on phosphate ester hydrolysis with model nucleophiles; however, most have not addressed the actual mechanism of hydrolysis, but focused instead on the existence of dianionic phosphorane intermediates.⁶ Similarly, while many studies have examined the general reaction pathway for phosphate ester hydrolysis,^{6,7} the number of studies that provide data for OP binding to AChE are far more sparse.^{7,8}

It is important to benchmark the predictive abilities of model systems for evaluating the thermochemistry for the reaction between a family of nerve agents and AChE due to the computational expense of treating the full protein structure. In addition, the reaction energetics of OPs with model nucleophiles will serve as a foundation for later calculations, revealing the perturbations created by the protein environment near the active site of cholinesterases. To that end, this study will evaluate the reaction thermochemistry for the binding of 5 common nerve agents with model nucleophiles, including novel cysteine (C) and selenocysteine (U) mutations of the catalytic serine (S200).

Computational Methodology

The reaction of selected OPs, soman (GD), VX, VR, paraoxon, and tabun (Figure 1) with ethoxide was explored as a model for the catalytic serine of AChE. In addition, ethyl thiolate and ethyl selenate were utilized as representations for proposed S200C and S200U mutations. Each nucleophile was considered in its neutral (EtXH) and anionic (EtX⁻) form as depicted in Figure 2.

Several prior studies have illustrated the aptitude of Density Functional Theory (DFT) simulations using Becke's 3-parameter exchange functional⁹ combined with Lee, Yang and Parr's correlation functional (B3LYP)¹⁰ for evaluating the thermochemistry of reactions similar to those presented herein. We have selected the B3LYP functional for our simulations due to its relatively low computational cost and accuracy in thermochemical evaluation of S_N2-type reactions.

Geometry optimization of each compound was carried out using the B3LYP functional as implemented in the Gaussian 03 software package¹¹ with a 6-31+G* basis set.¹² Second derivatives of the potential energy surface were evaluated at each stationary point to verify that each structure was a minimum on the potential energy surface. Energies were then further evaluated at the B3LYP/6-311++G(2d,p) level using the B3LYP/6-31+G* optimized geometries. Conformations were sampled manually, and the lowest energy conformers were used for evaluation of the thermochemistry. Results were calibrated using accurate CBS-QB3¹³ calculations for tabun and GD. Energies presented herein are $\Delta H(0K)$ with scaled (0.964)¹⁴ zero-point vibrational energy corrections.

Results and Discussion

DFT calculations predict that the hydrolysis of all OPs by ethoxide is a favorable process, ranging in exothermicity from -2.4 (GD) to -57.7 kcal/mol (paraoxon), as shown in Table 1. Competitive cleavage of the P-S and P-O bond for VX and VR is predicted to heavily favor P-S bond cleavage by 35 kcal/mol in the gas phase. While no gas-phase results are available

to compare this value to experimentally, the P–S bond cleavage is experimentally favored over P–O bond cleavage in aqueous solution.⁷ The hydrolysis of the C^+P^-/C^-P^+ isomers of GD is observed to be less favorable due to increased steric interactions between the larger alkyl group and the incoming nucleophiles, interactions which are less pronounced in the C^+P^+/C^-P^- isomers.

Interestingly, the substitution of a stronger nucleophile in the form of ethyl thiolate or ethyl selenate for ethoxide does not yield a more favorable reaction, as both nucleophiles render the reaction to be more endothermic. However, hydrolysis by ethyl selenide is slightly more favorable than ethyl sulfide. One explanation for this discrepancy can be found in the acidities of the nucleophiles; the side-chain pK_a values for serine, cysteine, and selenocysteine are 15, 8.3 and 5.2, respectively.⁵ Similarly, the experimental gas-phase acidities of EtOH and EtSH are 378.3¹⁵ and 355.2¹⁶ kcal/mol at 298K, respectively. Calculations by our methods determined the acidity of EtSH relative to EtOH to be –24.4 kcal/mol (experimental: –23.1) and EtSeH at –33.7 kcal/mol below EtOH. The relative instability of ethoxide in the gas phase destabilizes the reactants, resulting in a more exothermic reaction than for the thiolate and selenate derivatives. This effect is attenuated for OP hydrolysis by the protonated form of each nucleophile (Reaction Model 2), also presented in Table 1.

In contrast to the thermochemical data involving an anionic nucleophile, hydrolysis of OPs using EtSeH is slightly more favorable than with EtSH in most cases. This trend is consistent with the experimental observation that selenium has only a slightly larger atomic radius than sulfur, but Se is a moderately stronger nucleophile.⁵

The effect of implicit solvation on the thermochemistry was evaluated using the Polarizable Continuum model using chloroform as a solvent as implemented in Gaussian '03.¹⁷ Chloroform was selected due to its dielectric constant of 4.9, a value frequently chosen as a model for the interior of a protein.¹⁸ With this dielectric, hydrolysis of GD by ethoxide was very exothermic, –22 kcal/mol, compared to only –2.4 kcal/mol in the gas phase as indicated in Table 2. Unfortunately, experimental values for heats of hydrolysis are unavailable, although our value is very similar to previously reported theoretical studies of the hydrolysis of sarin by hydroxide, which predicted a $\Delta H(0K)$ of –30.8 kcal/mol using PCM (water).⁷ Interestingly, the reaction thermochemistries for the remaining OPs are predicted to be more endothermic than in the gas phase. The shifts in energetics are the result of differential stabilization of reactants and products by the solvent, and in particular, the dramatic stabilization of the anionic species. This is further illustrated by the reactions using neutral nucleophiles, in which the solvent shift is attenuated and the reaction energetics are very similar to the gas phase results. In addition, our value for P–S bond cleavage of VX in PCM (chloroform, –30.8 kcal/mol) is in close agreement with the previously calculated value for the base-catalyzed hydrolysis using an implicit (PCM) consideration of water(–35.5).⁷

Conclusions

The data presented herein demonstrate that the hydrolysis of all OPs with ethoxide is a favorable process. When the protonated form of each nucleophile is considered, it is predicted that cysteine and selenocysteine would be only slightly less favorable nucleophiles than serine for the reaction with nerve agents. Solvent effects, as obtained by implicit solvation (PCM) in chloroform, predict that the energetics for hydrolysis are comparable to the gas-phase $\Delta H(0K)$ values, with only minor thermochemical changes for the anionic nucleophiles in the presence of the dielectric constant. While the model reactions with anionic nucleophiles represent more reasonable energetics, simulations using neutral nucleophiles help mitigate the large solvation effects with the three different nucleophiles, and further are more reasonable mimics of the protein environment and the entire catalytic cycle for OP processing. The results for the

protonated nucleophiles indicate that overall, cysteine and selenocysteine mutations would be less favorable nucleophiles for OP reactions in AChE, although secondary perturbations to the active site have not yet been considered. These effects are currently under investigation.

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Figure 1.
Structures of OPs with Leaving Groups in Brackets: VX and VR can undergo hydrolysis of either the P-S or P-O Bonds

Reaction Model 1 **Reaction Model 2**

Figure 2.
Reaction Models

$\Delta H(0K)$ for Gas Phase Hydrolysis of OPs by Model Nucleophiles evaluated at the B3LYP/6-311++G(2d,p)//B3LYP/6-31+G* and CBS-QB3 (parentheses) Level of Theory (kcal/mol)

	Anionic Nucleophiles		Neutral Nucleophiles		
	EtO ⁻	EtS ⁻	EtOH	EtSH	EtSeH
GDC ⁺ P ⁺ /C ⁻ P ⁻	-2.4 (-5.9)	28.5	5.0	14.4	13.9
GDC ⁺ P ⁺ /C ⁻ P ⁺	-2.4	28.8	5.0	14.7	14.4
VX (P-S)	-35.5	-4.2	-12.1	-2.3	-6.8
VX (P-O)	0.0	28.7	0.0	7.3	7.5
VR (P-S)	-31.1	0.9	-9.8	0.7	1.4
VR (P-O)	-1.2	27.6	1.7	9.1	8.5
Tabun	-40.6 (-47.6)	-8.4 (-9.9)	-13.7	-2.9	-3.1
Paraoxon	-57.7	-25.5	-4.6	6.1	6.4

$\Delta H(0K)$ for Solution-phase (Chloroform) Hydrolysis of OPs by Model Nucleophiles evaluated at the B3LYP/6-311++G(2d,p)//B3LYP/6-31+G* Level of Theory (kcal/mol) using the PCM solvation method

	Anionic Nucleophiles		Neutral Nucleophiles		EtSeH
	EtO ⁻	EtS ⁻	EtOH	EtSH	
GDC ⁺ P ⁺ /C ⁻ P ⁻	-22.2	5.0	4.4	9.9	10.0
GDC ⁺ P ⁺ /C ⁻ P ⁺	-22.6	4.9	3.9	9.8	9.8
VX (P-S)	-30.8	-2.5	-10.9	-4.2	-7.3
VX (P-O)	0.0	25.1	0.0	3.5	3.3
VR (P-S)	-30.1	-1.9	-8.6	-2.0	-1.6
VR (P-O)	2.7	27.8	1.7	5.2	4.6
Tabun	-41.8	-13.5	-12.3	-5.7	-5.5
Paraoxon	-42.4	-13.8	-7.2	-0.3	-0.2