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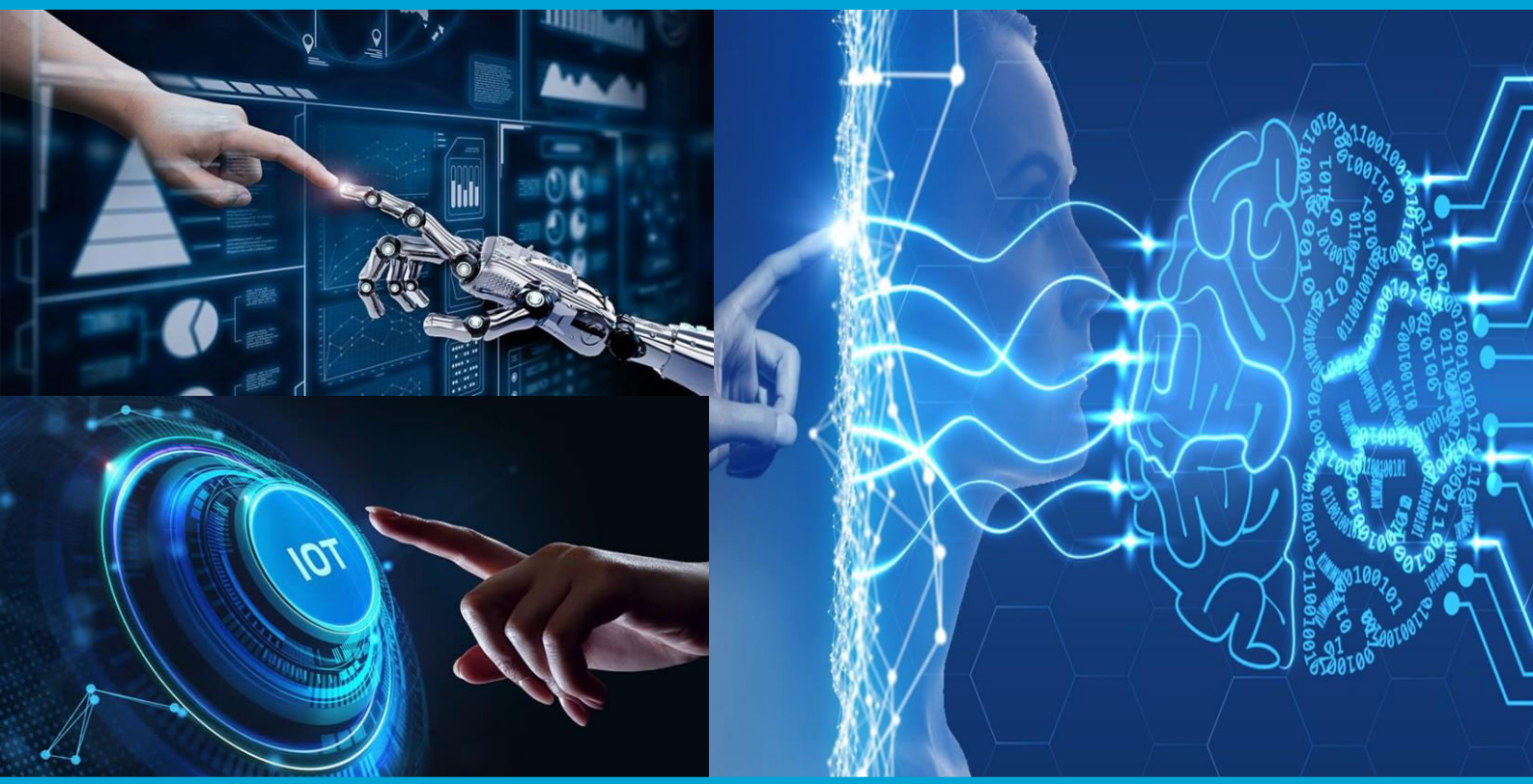

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Enzymatic Reactions of Organophosphorus Compounds

Dr. Dinesh Chandra Sharma

Department of Chemistry, SCRS Government College, Sawai Madhopur, Rajasthan, India

ABSTRACT: Organophosphorus compounds are derivatives of phosphoric, phosphonic or phosphinic acids whose oxygen atoms bound directly to the phosphorus atom can be substituted by sulphur or nitrogen atoms. These compounds represent a large group of organic compounds used primarily as pesticides. Some are used as drugs and the most toxic compounds as nerve agents. Acute toxicity of organophosphorus compounds is due to the inhibition of acetylcholinesterase, the critical enzyme in neurotransmission. Organophosphorus compounds whose sulphur atom creates a coordinative covalent bond with the phosphor atom are not acetylcholinesterase inhibitors. To become biologically active these compounds must transform into their oxo analogues, passing through spontaneous or biotransformation reactions. Biotransformation reactions of organophosphorus compounds involve a large number of enzymatic reactions that can make them more or less toxic, or even non-toxic for acetylcholinesterase. The classification of organophosphorus compounds in this paper considers the nature of groups bound directly to the central phosphorus atom. The paper describes the enzymes taking part in biotransformation of organophosphorus compounds and gives examples of their reactions.

KEYWORDS-enzymatic,organophosphorus,compounds,toxicity,biotransformation

I. INTRODUCTION

Organophosphorus compounds (OPCs) are able to interact with various biological targets in living organisms, including enzymes. The binding of OPCs to enzymes does not always lead to negative consequences for the body itself, since there are a lot of natural biocatalysts that can catalyze the chemical transformations of the OPCs via hydrolysis or oxidation/reduction and thereby provide their detoxification. Some of these enzymes, their structural differences and identity, mechanisms, and specificity of catalytic action are discussed . Organophosphorus compounds (OPCs) are extremely dangerous and have a wide variety of chemical structures mainly presented by phosphotriester, thiophosphotriester, and phosphorothioester moieties [1]. Pesticides currently approved for application among OPCs contain sulfur instead of phosphoryl oxygen. Such substitution is believed to reduce the level of acute toxicity of these compounds to humans. Unlike pesticides, all chemical warfare agents belonging to OPCs are chiral phosphonates with a C–P bond. The most famous nerve agents among them are sarin and soman, both containing a labile fluoride group, and V-gases, containing branched alkylthiols.

The main hazard of OPCs to living beings is their reactivity towards Ser residue which is present in active centers of numerous enzymes [2]. For example, acetylcholinesterase necessary for the normal transmission of nervous signals is completely and rapidly inhibited in an irreversible manner by the most OPCs. Other serine hydrolases are also affected [3]. Besides, OPCs can randomly bind to other proteins [4], proteinaceous receptors [5], etc. That totally leads to multiple dysfunctions of cellular regulation [6], oxidative stress, and apoptosis [7]. To avoid these negative outcomes, biocatalytic detoxification of OPCs seems to be the most reliable.[2,3,5]

Despite the structural diversity of OPCs, a large number of enzymes have been discovered to be catalytically active with these compounds. Most of these enzymes (organophosphorus hydrolase, OPH; methyl parathion hydrolase, MPH; organophosphorus acid anhydrolase, OPAA; etc.) belong to hydrolases, but there are oxidoreductases and lyases also, and some representative examples of these enzyme classes will be discussed later. Various pro- and eukaryotic organisms are sources of these enzymes, and new ones are constantly discovered. Over the past decades, the catalytic characteristics of these enzymes, as well as crystallographic structures of many of them, have been determined, and the mechanisms of their action have been explained.

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II. DISCUSSION

Organophosphate compounds are ubiquitously employed as agricultural pesticides and maintained as chemical warfare agents by several nations. These compounds are highly toxic, show environmental persistence and accumulation, and contribute to numerous cases of poisoning and death each year. While their use as weapons of mass destruction is rare, these never fully disappear into obscurity as they continue to be tools of fear and control by governments and terrorist organizations. Beyond weaponization, their wide-scale dissemination as agricultural products has led to environmental accumulation and intoxication of soil and water across the globe. Therefore, there is a dire need for rapid and safe agents for environmental bioremediation, personal decontamination, and as therapeutic detoxicants. Organophosphate hydrolyzing enzymes are emerging as appealing targets to satisfy decontamination needs owing to their ability to hydrolyze both pesticides and nerve agents using biologically-derived materials safe for both the environment and the individual. As the release of genetically modified organisms is not widely accepted practice, researchers are exploring alternative strategies of organophosphate bioremediation that focus on cell-free enzyme systems. In this review, we first discuss several of the more prevalent organophosphorus hydrolyzing enzymes along with research and engineering efforts that have led to an enhancement in their activity, substrate tolerance, and stability. In the later half we focus on advances achieved through research focusing on enhancing the catalytic activity and stability of phosphotriesterase, a model organophosphate hydrolase, using various approaches such as nanoparticle display, DNA scaffolding, and outer membrane vesicle encapsulation.[7,8,9]

III. RESULTS

Biotransformation of a series of organophosphorus compounds by the 9,000-g supernatant of rainbow trout (Oncorhynchus mykiss) liver was tested in an in vitro system fortified either with NADPH-generating cofactors or with reduced glutathione (GSH). Elimination rate constants for both systems were calculated from linear decay curves when substrate concentrations were used that were considerably lower than the K_m values of the concerned enzymatic reactions. The results reveal a large variation in both the oxidative and the glutathione-mediated biotransformation rate of the organophosphorus compounds. Half-lives ranged from 25 to 1,216 min in the NADPH system and from 18 to 381 min in the GSH system. Elimination rate constants in the GSH system were related to Hammett σ constants or reactivity toward 4-nitrobenzylpyridine, which substantiates the assumption that electrophilicity is the controlling variable for the reaction with GSH within this particular class of compounds. A remarkable analogy was observed between compounds that were metabolized relatively quickly by glutathione S-transferases and compounds that showed a reduced bioconcentration factor in guppies. A significant improvement of the relationship between the bio-concentration factor in guppies and the octanol/water partition coefficient was obtained when the rate constant with GSH was introduced in this relationship. Such an improvement was not obtained with the rate constants from the oxidative system. These observations are discussed in view of the differences in the activities of the involved enzyme systems in the test species and in view of the possible relevance of the different biotransformation pathways for the in vivo situation.[10,11]

Our present work is focused on the degradation mechanisms of organophosphorus pesticides. In contrast to P–O bond containing compounds, which are efficiently decomposed by phosphotriesterase, the rate of hydrolysis of P–S bond, present in many organothiophosphate pesticides, is significantly lower. (8) As evidenced by the experimental kinetic isotope effects for alkaline and enzymatic hydrolysis of the P–O bond in paraoxon, (14) both reactions fit to the characteristics of an S_N2-like concerted associative mechanism. The resemblance of enzymatic and nonenzymatic phosphotriester degradation suggests that the knowledge of the mechanism of alkaline hydrolysis could provide a valid starting point for computational approaches directed toward improvement of PTE properties.

In the case of hydrolysis of phosphotriester compounds, nucleophilic substitution at the phosphorus center appears to follow an associative pathway, for which two limiting scenarios exist (15, 16) (Scheme 1). Addition–elimination mechanism involves the presence of a pentacoordinate phosphorane intermediate resulting in the triple-well shape of the potential energy surface. It can then be described as a two-step process composed of intermediate formation and its further decomposition. The direct-displacement pathway leads through a single S_N2-like transition state directly toward products, which is described by a double-well energy profile. The approach of the nucleophilic hydroxide ion is accompanied by the leaving group expulsion. Independently of the number of steps along the hydrolysis pathway, the

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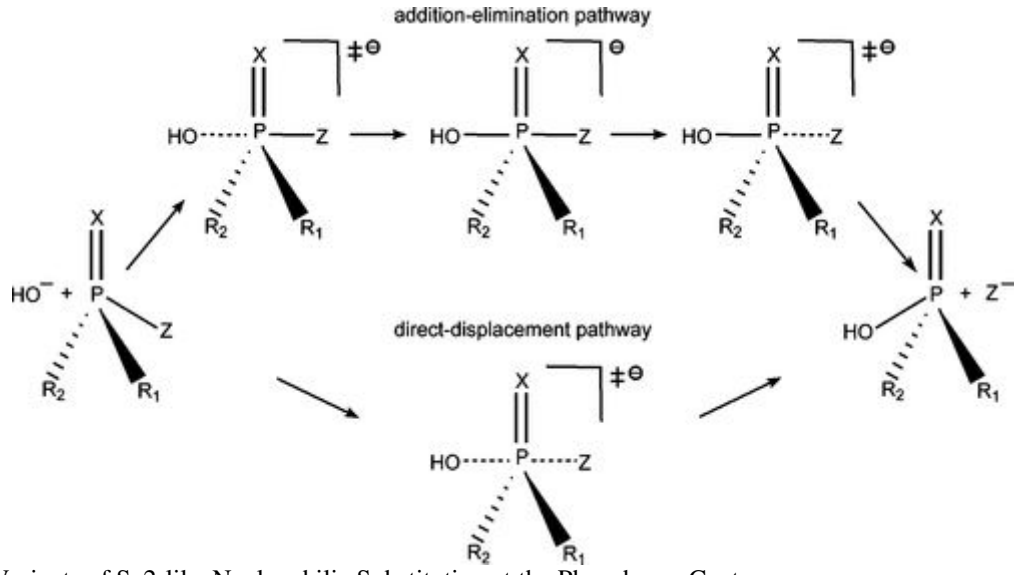
favorable mechanism involves the entering and leaving groups positioned on the opposite sides of the plane formed by the three remaining atoms bonded to the phosphorus center. (17)

Scheme 1



Scheme 1. Variants of S_N2-like Nucleophilic Substitution at the Phosphorus Center

The multistep pathway was shown to occur in the case of P–F bond hydrolysis of phosphofluoridate compounds (e.g., sarin, O,O-diisopropyl phosphorofluoridate,) as well as acephate (P–S bond), O,S-dimethyl methylphosphonothiolate (VX model compound; P–S bond) and tabun (P–CN bond). (12, 17-19) The two-step addition–elimination process described for these compounds involves a trigonal bipyramidal intermediate, the formation of which constitutes the rate-determining step. In contrast, P–O bond-containing compounds (e.g., paraoxon, parathion, fenitrothion) along with demeton-S (P–S bond) appear to be hydrolyzed via a single-step mechanism. (12, 18, 20)

According to our previous results, alkaline hydrolysis of organophosphorus compounds may proceed along two alternative pathways that differ in the conformation of the attacking hydroxide ion. (12) In particular, the lower energy barrier pathway corresponds to the organophosphate species with hydroxide proton pointing in the direction of a phosphoryl oxygen atom. The similar conclusion was reached in the study of model systems of nucleophilic substitution at phosphorus atom, where this effect was ascribed to an intramolecular hydrogen bond with the phosphoryl oxygen atom. (21)

In this work we extend our previous study of alkaline hydrolysis of paraoxon, acephate, and demeton-S hydrolysis (12) by employing a more accurate description of the reaction mechanism. Higher level of theory for the analysis of the potential energy surface enabled identification of new stationary points, changing the overall characteristics of the reaction pathway. For further study of the possible mechanisms of P–S bond hydrolysis, we selected the following organothiophosphate pesticides: malathion, phosalone, and azinophos-ethyl (Chart 1). Similarly to acephate and demeton-S, these compounds are decomposed by phosphotriesterase at a lower catalytic rate compared to paraoxon and the related P–O bond containing pesticides. (8) To the best of our knowledge, there is no experimental data concerning the PTE ability to hydrolyze fenitrothion, another commonly used pesticide. (21) Since fenitrothion differs from experimentally verified PTE substrate methyl parathion, (8) only by the presence of additional methyl group at the phenyl ring (Chart 1), it is likely that PTE is capable of cleaving the P–O bond in fenitrothion as well. Thus, both fenitrothion and methyl parathion were also included in our study.[12]

Overall, organophosphorus pesticides considered in this work include organophosphate (paraoxon), phosphoramidothioate (acephate), and organothiophosphate compounds. Considering the type of the leaving group, organothiophosphate pesticides can further be classified into phenyl (methyl parathion, fenitrothion), heterocyclic (phosalone, azinophos-ethyl), and aliphatic (demeton-S, malathion) compounds. These species also differ by the number of phosphorus-bonded sulfur atoms. In particular, phosalone, azinophos-ethyl, and malathion containing two sulfur atoms belong to phosphorodithioates, while methyl parathion, fenitrothion, and demeton-S possess a single sulfur atom (phosphorothioates). Considering the sulfur substitution site, P–S bond hydrolysis occurs in the case of demeton-

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S, whereas methyl parathion and fenitrothion feature phosphoryl sulfur atom (Chart 1). Such a choice of organophosphorus pesticides enables analysis of the influence of both sulfur substitution and the type of leaving group moiety on the mechanism of phosphotriester hydrolysis.[13,15,17]

Chart 1

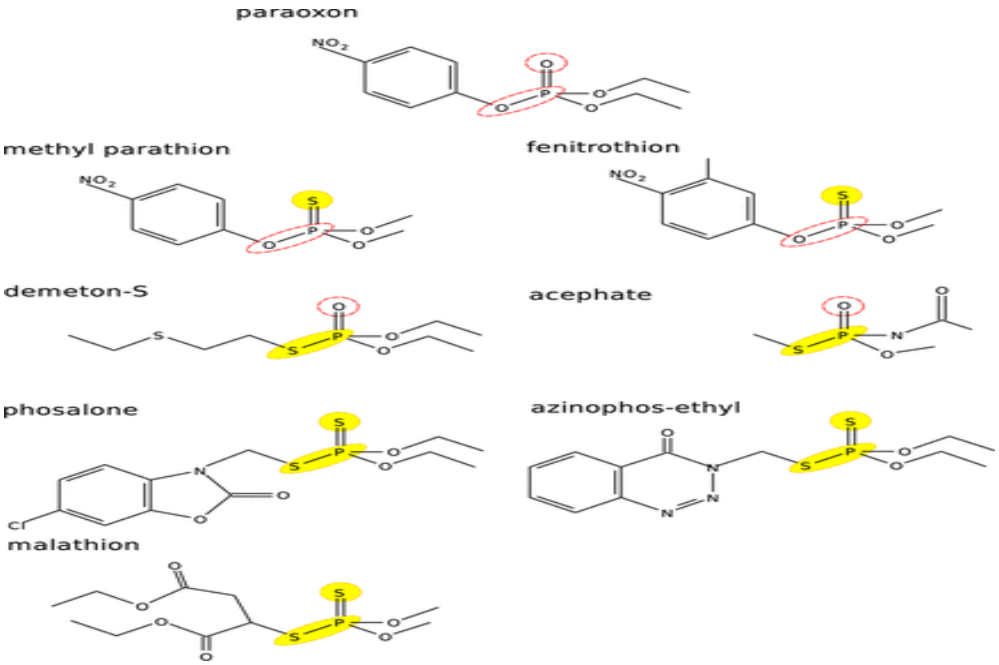

Chart 1. Structures of the Organophosphorus Pesticides Considered in This Study

IV. CONCLUSIONS

Organophosphorus compounds are among the most widely used pesticides worldwide. The accumulation of these highly toxic agents in water supplies and food products, increasing the risk of human exposure, raises environmental and health concerns regarding the availability of safe and economically feasible methods of detoxification. (1) The nonharmful removal of organophosphate is therefore a matter of urgent need, as it would be applicable for not only environment decontamination, but also postexposure treatment or prophylactics.[18,19,20]

A promising detoxification approach involves enzymatic biodegradation. (2-4) Among the several organophosphate degrading enzymes, the best characterized is a bacterial enzyme phosphotriesterase (PTE), (5, 6) which makes it a prospective candidate due to its ability to cleave various phosphorus-ester bonds (7, 8) and to hydrolyze one of its substrates, paraoxon, at the rate approaching the diffusion-controlled limit. (9) Noticeably, the PTE catalytic activity can be modified with the aim of tailoring enzyme properties toward specific targets. (10) The introduction of the rational biocatalyst design into the process of mutant development would save a significant amount of time-consuming and expensive laboratory work. In the case of de novo enzyme design or redesign allowing for the novel catalytic activity to be introduced, computational approaches are required to provide a reasonable starting scaffold structure, that could further be refined with experimental techniques. (11) However, prior to the computational enzyme design, a molecular level understanding of the reaction is needed. Our earlier computational results (12) concerning mechanisms of decomposition of organophosphate neurotoxins have already been utilized in the design process of a mononuclear zinc metalloenzyme capable of performing the hydrolysis of a coumarinyl analogue of the nerve agent cyclosarin. [21]

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REFERENCES

1Raushel, F. M. Chemical Biology: Catalytic Detoxification Nature 2011, 469, 310– 311[Crossref], Google Scholar
2Singh, B. K.; Walker, A. Microbial Degradation of Organophosphorus Compounds FEMS Microbiol. Rev. 2006, 30, 428– 571[Crossref], [PubMed], [CAS], Google Scholar
3Zhang, J. L.; Qiao, C. L. Novel Approaches for Remediation of Pesticide Pollutants Int. J. Environ. Pollut. 2002, 18, 423– 433[Crossref], Google Scholar
4Raushel, F. M. Bacterial Detoxification of Organophosphate Nerve Agents Curr. Opin. Microbiol. 2002, 5, 288– 295 [Crossref], [PubMed], [CAS], Google Scholar
5Dumas, D. P.; Caldwell, S. R.; Wild, J. R.; Raushel, F. M. Purification and Properties of the Phosphotriesterase from Pseudomonas diminuta J. Biol. Chem. 1989, 264, 19659– 19665 [Crossref], Google Scholar
6Ghanem, E.; Raushel, F. M. Detoxification of Organophosphate Nerve Agents by Bacterial Phosphotriesterase Toxicol. Appl. Pharmacol. 2005, 207, S459– S470 [Crossref], Google Scholar
7Dumas, D. P.; Durst, H. D.; Landis, W. G.; Raushel, F. M.; Wild, J. R. Inactivation of Organophosphorus Nerve Agents by the Phosphotriesterase from Pseudomonas Diminuta Arch. Biochem. Biophys. 1990, 277, 155– 159 [Crossref], [PubMed], [CAS], Google Scholar
8Lai, K.; Stolowich, N. J.; Wild, J. R. Characterization of P–S Bond Hydrolysis in Organophosphorothioate Pesticides by Organophosphorus Hydrolase Arch. Biochem. Biophys. 1995, 318, 59– 64 [Crossref], Google Scholar
9Caldwell, S. R.; Newcomb, J. R.; Schlecht, K. A.; Raushel, F. M. Limits of Diffusion in the Hydrolysis of Substrates by the Phosphotriesterase from Pseudomonas Diminuta Biochemistry 1991, 30, 7438– 7444 [ACS Full Text], [CAS], Google Scholar
10Hill, C. M.; Li, W.-S.; Thoden, J. B.; Holden, H. M.; Raushel, F. M. Enhanced Degradation of Chemical Warfare Agents through Molecular Engineering of the Phosphotriesterase Active Site J. Am. Chem. Soc. 2003, 125, 8990– 8991 [ACS Full Text], [CAS], Google Scholar
11Röthlisberger, D.; Khersonsky, O.; Wollacott, A. M.; Jiang, L.; DeChancie, J.; Betker, J.; Gallaher, J. L.; Althoff, E. A.; Zanghellini, A.; Dym, O.; Albeck, S.; Houk, K. N.; Tawfik, D. S.; Baker, D. Kemp Elimination Catalysts by Computational Enzyme Design Nature 2008, 453, 190– 195 [Crossref], Google Scholar
12 Dyguda-Kazimierowicz, E.; Sokalski, W. A.; Leszczyski, J. Gas-Phase Mechanisms of Degradation of Hazardous Organophosphorus Compounds: Do They Follow a Common Pattern of Alkaline Hydrolysis Reaction as in Phosphotriesterase? J. Phys. Chem. B 2008, 112, 9982– 9991 [ACS Full Text], Google Scholar
13 Khare, S. D.; Kipnis, Y.; Greisen, P. J.; Takeuchi, R.; Ashani, Y.; Goldsmith, M.; Song, Y. F.; Gallaher, J. L.; Silman, I.; Leader, H.; Sussman, J. L.; Stoddard, B. L.; Tawfik, D. S.; Baker, D. Computational Redesign of a Mononuclear Zinc Metalloenzyme for Organophosphate Hydrolysis Nat. Chem. Biol. 2012, 8, 294– 300 [Crossref], [PubMed], [CAS], Google Scholar
14 Caldwell, S. R.; Raushel, F. M.; Weiss, P. M.; Cleland, W. W. Transition-State Structures for Enzymic and Alkaline Phosphotriester Hydrolysis Biochemistry 1991, 30, 7444– 7450 [ACS Full Text], Google Scholar
15 Knowles, J. R. Enzyme-Catalyzed Phosphoryl Transfer Reactions Annu. Rev. Biochem. 1980, 49, 877– 919 [Crossref], [PubMed], [CAS], Google Scholar
16 Cox, J. R., Jr.; Ramsay, O. B. Mechanisms of Nucleophilic Substitution in Phosphate Esters Chem. Rev. 1964, 64, 317– 352 [ACS Full Text], [CAS], Google Scholar
17 Xiong, Y.; Zhan, C.-G. Reaction Pathways and Free Energy Barriers for Alkaline Hydrolysis of Insecticide 2-Trimethylammonioethyl Methylphosphonofluoridate and Related Organophosphorus Compounds: Electrostatic and Steric Effects J. Org. Chem. 2004, 69, 8451– 8458 [ACS Full Text], Google Scholar
18 Zheng, F.; Zhan, C.-G.; Ornstein, R. L. Theoretical Studies of Reaction Pathways and Energy Barriers for Alkaline Hydrolysis of Phosphotriesterase Substrates Paraoxon and Related Toxic Phosphofluoridate Nerve Agents J. Chem. Soc., Perkin Trans. 2001, 2, 2355– 2363 Google Scholar
19 Šeckute, J.; Menke, J. L.; Emnett, R. J.; Patterson, E. V.; Cramer, C. J. Ab Initio Molecular Orbital and Density Functional Studies on the Solvolysis of Sarin and O,S-Dimethyl Methylphosphonothiolate, a VX-like Compound J. Org. Chem. 2005, 70, 8649– 8660 [ACS Full Text], Google Scholar

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|| Volume 8, Issue 1, January 2021 ||

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20Mandal, D.; Mondal, B.; Das, A. K. Nucleophilic Degradation of Fenitrothion Insecticide and Performance of Nucleophiles: A Computational Study J. Phys. Chem. A 2012, 116, 2536– 2546

[ACS Full Text], Google Scholar

21 van Bochove, M. A.; Swart, M.; Bickelhaupt, F. M. Nucleophilic Substitution at Phosphorus Centers (S_N2@P) ChemPhysChem 2007, 8, 2452– 2463

[Crossref], Google Scholar

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