

18. ORGANOPHOSPHORUS COMPOUNDS AND ESTERASES: CURRENT RESEARCH TOPICS CONCERNING TOXICITY OF, AND PROTECTION AGAINST ORGANOPHOSPHATES

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INTRODUCTION

Two groups of esterases react with organophosphorus (OP) compounds: serine esterases and phosphoric triester hydrolases (PTHs; EC 3.8.1). The acute toxicity of OP compounds is primarily due to inhibition of acetylcholinesterase (AChE; EC 3.1.1.7). The delayed polyneuropathy, induced by some OP compounds, is related to inhibition of the neuropathy target esterase (NTE). So far, NTE has not been classified in the Enzyme Nomenclature (1). AChE and NTE are both serine esterases. Unlike to serine esterases, OP compounds are not inhibitors but substrates of phosphoric triester hydrolases (PTHs): paraoxonase (PON; EC 3.1.8.1) and DFPase (EC 3.1.8.2). The hydrolysis products of the OP compounds are not toxic, and PTHs therefore play an important role in OP detoxification.

The structural formulae of several OP compounds are given in Figure 1. Sarin, soman, tabun, and VX are nerve agents. Paraoxon and DFP are characteristic substrates of the PTHs. Both serine esterases and PTHs react with OP compounds on the same ester or anhydride bond marked in the figure by an undulated line.

For detailed information, readers are invited to consult books and conference proceedings listed in references 2-13 at the end of this brief review. Reference 14 summarizes the early literature on the subject. Individual papers are not quoted in the text, because their number would grossly exceed the length of the review.

REACTIONS OF ORGANOPHOSPHORUS COMPOUNDS WITH SERINE ESTERASES

The reactions of serine esterases with OP compounds are shown in Scheme 1. Inhibition of serine esterases is due to phosphorylation of the active-site serine. This reaction proceeds via an intermediate Michaelis-type complex between the enzyme and OP compound. The phosphorylated enzyme (EP) is catalytically inactive. Dephosphorylation with water (spontaneous reactivation) is very slow, while it is faster with oximes (oxime reactivation). Depending on the substituents on the phosphorus, EP can undergo dealkylation, which is termed ageing. The aged enzyme cannot be reactivated.

AChE is a globular protein and its three-dimensional structure is known. Its physiological substrate is acetylcholine. A schematic drawing of the AChE molecule is shown in Figure 2. The active site of AChE is in the center of the molecule accessible through a narrow gorge lined with water molecules. The catalytic triad (serine, histidine and glutamic acid), a choline-binding pocket and an acyl-binding pocket form the active site. Furthermore, AChE has an allosteric (peripheral) site close to the rim of the gorge. The allosteric site is catalytically inactive. However, reversible binding of substrates or other ligands to that site affects catalysis in the active site. The effect is usually inhibitory. There is some evidence that the AChE molecule might have a "back door" through which products of substrate hydrolysis leave the enzyme.

Research on AChE is now directed towards identification of individual amino acids that participate in the individual steps of catalysis. The experimental approach consists in preparing site-directed mutants, correlating the catalytic properties with the structure of the mutant and modeling the enzyme/ligand complexes. The results are expected to further elucidate the mechanism of action of AChE. This in turn might facilitate a more rational design of compounds, which would prevent phosphorylation of the active site by OPs, facilitate reactivation of EP and slow down ageing. The toxicity of OPs is primarily determined by these three reactions.

NTE has only recently been cloned and was shown to be unrelated to any known serine hydrolases. Its physiological role is not known. OP compounds are inhibitors of NTE, but only some OPs cause delayed polyneuropathy. These OPs phosphorylate the active-site serine, which in turn is followed by ageing of the inhibited NTE. Polyneuropathy does not develop if ageing does not occur. This could indicate that the axonal maintenance is sensitive to the negative charge on the aged enzyme (Scheme 1) and not to the activity of NTE. Phosphorylation and ageing of NTE occur within minutes, but degeneration of the long axons takes weeks to develop.

Present studies aim at clarifying the link between NTE and the OP-induced delayed polyneuropathy. Studies are focused on the molecular structure of NTE and on compounds that promote OP-induced polyneuropathy without causing polyneuropathy themselves. Promoters known so far are NTE inhibitors, but it seems unlikely that NTE is

the target enzyme.

HYDROLYSIS OF ORGANOPHOSPHORUS COMPOUNDS BY PHOSPHORIC TRIESTER HYDROLASES

The mechanism of reaction of OP compounds with PTHs is different from the reaction of OPs with serine esterases. PTHs require divalent cations for catalysis as shown in Scheme 2. The cation (M^{++} in Scheme 2) is embedded in histidine residues and it binds the water molecule required for OP hydrolysis. So far nothing is known about the intermediate steps leading to hydrolysis, except for the kinetic evidence that a Michaelis complex is formed between the enzyme and OP. The physiological role of PTHs is also not known.

PTHs hydrolyse a broad range of OP compounds. The substrate specificities of paraoxonase and DFPase are different, and the same holds for the cation required for catalysis. Paraoxonase in mammalian sera requires calcium ions, while DFPase in microorganisms usually depends on magnesium, zinc or manganese cations. Recently DFPase was also found in plants and its activity was stimulated by manganese ions. PTHs are stereoselective like AChE. The OP enantiomer, which is more quickly hydrolysed by PTHs, is less inhibitory for AChE, and vice versa. PTHs have been shown to hydrolyse carboxylic acid esters such as phenylacetate, which is a characteristic substrate of the arylesterase (EC 3.1.1.2) (1).

The search for the natural substrate(s) and physiological role of PTHs continues. Mammalian serum paraoxonases seem to act against cellular damage from toxic agents and oxidized lipids, and there are some indications that human serum paraoxonases might be markers of lipid metabolism disorders. The polymorphism of these enzymes in human sera is well established. PTHs have recently been cloned and mutants prepared. This has greatly enhanced studies on substrate specificity and mechanism of substrate hydrolysis. The *in vivo* role of PTHs in detoxification of OPs and their use in decontamination from OPs constitutes a very lively field of research.

OXIMES AS ANTIDOTES AND PROTECTORS AGAINST ORGANOPHOSPHORUS COMPOUNDS

The primary mechanism of action of oximes as antidotes is to reactivate the phosphorylated AChE (Scheme 1). The pyridinium oximes PAM-2 and HI-6 (Figure 2), and the bis-pyridinium dioxime toxogononin, are therapeutic drugs in OP poisoning. Not a single oxime prepared so far is active against a very broad range of OPs. Moreover, none has been known to act against all four nerve agents. The search for better antidotes is still based on the trial and error approach. The same applies to oximes and other compounds used as prophylactic agents. Imidazolium oximes, and more recently quinuclidinium derivatives, are under evaluation; two compounds from those groups are shown in Figure 2.

The products of dephosphorylation by oximes are phosphorylated oximes (Scheme 1). Phosphorylated oximes are potent AChE inhibitors. Many, but not all, are very unstable compounds. *In vivo* phosphorylation of the reactivated AChE by phosphorylated oximes has seldom been reported so far. Oximes themselves are also toxic. They bind to AChE as reversible inhibitors.

Reversible inhibitors form complexes with AChE either in the active site or in the allosteric site or in both sites of the enzyme. Reversible inhibitors, including oximes, protect thereby AChE from phosphorylation. When the reversible inhibitor binds to the active site, the protection is due to direct competition between the OP compound and reversible inhibitor. Binding of a reversible inhibitor to the allosteric site induces indirect protection of the active site. This has been well documented by *in vitro* studies, but was so far less evaluated in experimental toxicology.

ESTERASES AS ANTIDOTES, PROTECTORS AND DECONTAMINATING AGENTS AGAINST ORGANOPHOSPHORUS COMPOUNDS

Phosphorylation of serine esterases by OP compounds occurs on a 1:1 molar basis. This reaction inhibits the enzyme, but detoxifies the OP compound. Phosphorylation of AChE causes toxicity, because phosphorylated AChE cannot hydrolyse its physiological substrate acetylcholine. On the other hand, butyrylcholinesterase (BChE; EC 3.1.1.8) and carboxylesterase (EC 3.1.1.1) in mammalian sera and tissues can be almost completely inhibited without an apparent toxic effect. This means that cholinesterases and carboxylesterases act as scavengers of OPs. However, once phosphorylated, the enzymes cannot detoxify another OP molecule unless oximes are present which reactivate the enzyme. Consequently, cholinesterases and carboxylesterases act as stoichiometric scavengers, while combined with oximes they become catalytic scavengers.

The feasibility of using serine esterases, such as AChE and BChE combined with oximes, as drugs against OPs has been demonstrated in rodents and in non-human primates. Protein engineering techniques have now enabled an intensive search for enzyme mutants, which ideally should meet the requirement to react rapidly with OPs, to age

slowly and to be easily reactivatable by oximes.

PTHs also play a major role in the detoxification of OPs. The high toxicity of OPs for avian species has been attributed to the low activity of endogenous PTHs in these species. Rabbits, which have a very high paraoxonase activity, are more resistant to paraoxon than other mammals. Human serum paraoxonases exhibit a substrate dependent polymorphism, with low and high activity modes for each substrate. Analysis of polymorphisms in population groups is suggested to identify individuals at risk. Studies on rodents have shown that administration of purified paraoxonase significantly reduces the toxicity of paraoxon and other OPs. These promising results stimulate present research on the use of PTHs and their mutants as drugs against OPs.

Finally, the use of esterases is suggested for the decontamination of skin, clothing and equipment. PTHs from a microorganism immobilized to cotton wipes detoxifies nerve agents and OP pesticides. Purified AChE or BChE immobilized to polyurethane sponge does the same. Rinsing the sponge with oxime solutions restores the enzyme activity. PTHs can be co-immobilized on the same sponge thus increasing the decontaminating capacity. Enzymes immobilized to matrices are more stable than in solution and can therefore be used repeatedly.

SUMMARY

Reactions of organophosphorus compounds with cholinesterases, neuropathy target esterase and phosphoric triester hydrolases are discussed with respect to the toxicity of organophosphates. Antidotes, protectors and decontaminating agents against organophosphates are described.

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KEYWORDS

Serine esterases; Cholinesterases; Acetylcholinesterase; Butyrylcholinesterase; Neuropathy target esterase; Phosphoric triester hydrolases; Paraoxonase; DFPase; Oximes

FIGURES AND TABLES

Figure 1. Structural formulae of several organophosphorus compounds

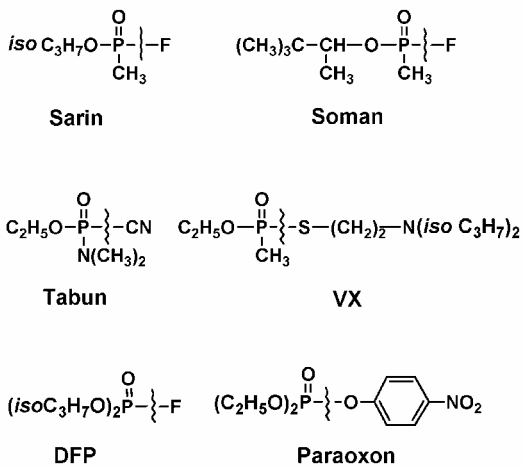


Figure 2. Schematic drawing of the AChE molecule (prepared by Zoran Radic, UCSD, USA)

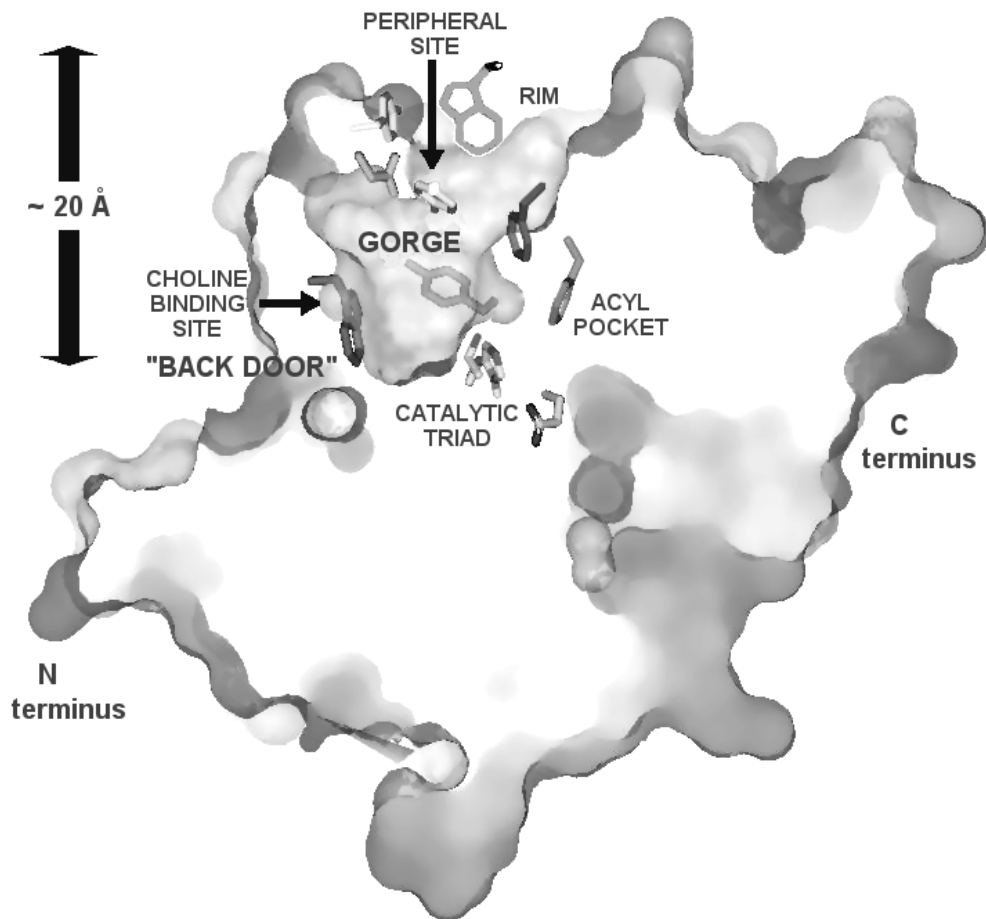
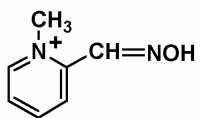
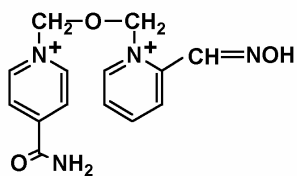


Figure 3. Structural formulae of several oximes

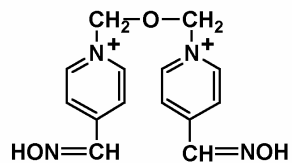
Pyridinium oximes



PAM-2

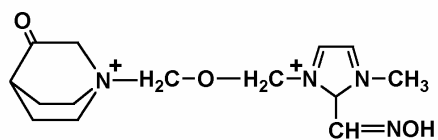
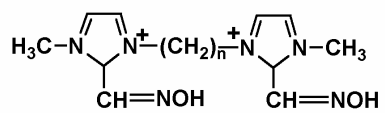


HI-6



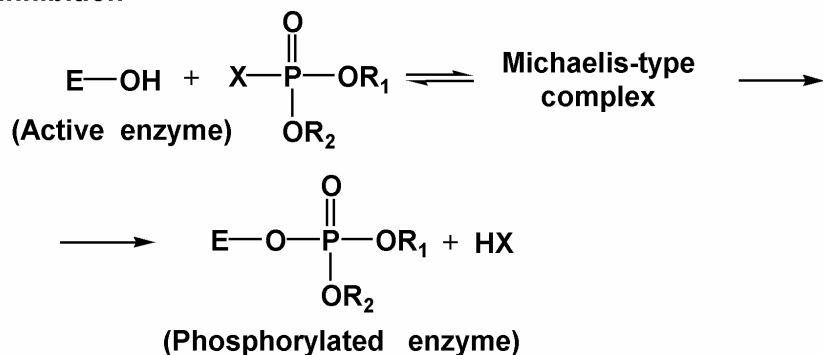
Toxogonin

Imidazolium oximes

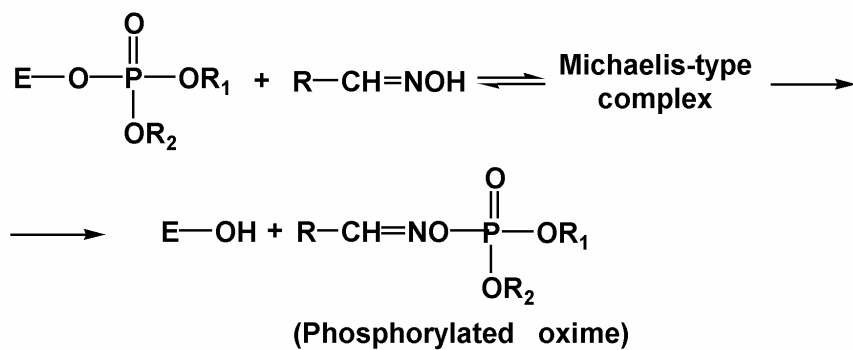


Scheme 1. Reactions of serine esterases with organophosphorus compounds

Inhibition



Oxime reactivation



Ageing (dealkylation)

