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REVIEW

ORGANOPHOSPHORUS COMPOUNDS AND ESTERASES: CURRENT RESEARCH TOPICS CONCERNING THE TOXICITY OF AND PROTECTION AGAINST ORGANOPHOSPHATES

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This brief review describes the reactions of organophosphorus compounds with cholinesterases, neuropathy target esterase, and phosphoric triester hydrolases with respect to their toxicity. It also describes antidotes, protectors, and decontaminating agents against organophosphates.

Key words:
acetylcholinesterase, butyrylcholinesterase, cholinesterases, DFPase, neuropathy target esterase, oximes, paraoxonase, phosphoric triester hydrolases, serine esterases

Two groups of esterases react with organophosphorus (OP) compounds: serine esterases and phosphoric triester hydrolases (PTHs; EC 3.1.8). 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 the 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 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 products of hydrolysis of OP compounds are not toxic, and PTHs play an important role in OP detoxification.

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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.

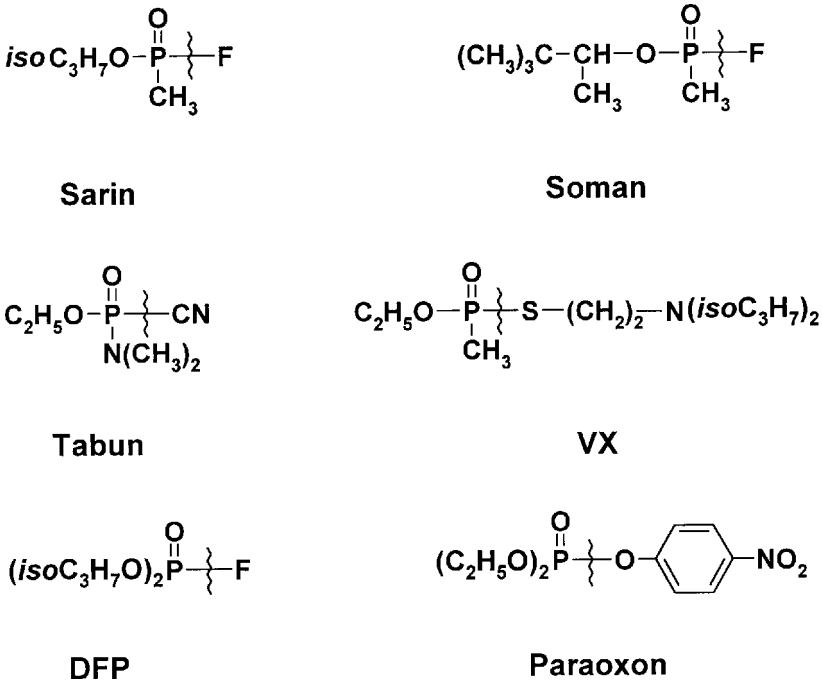


Figure 1 *Structural formulae of several organophosphorus compounds*

For detailed information, readers are invited to consult books and conference proceedings listed in references 2-12 at the end of this brief review. Reference 13 summarises 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 OP COMPOUNDS WITH SERINE ESTERASES

Figure 2 shows the reactions of serine esterases with OP compounds. The 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. 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*. An aged enzyme cannot be reactivated.

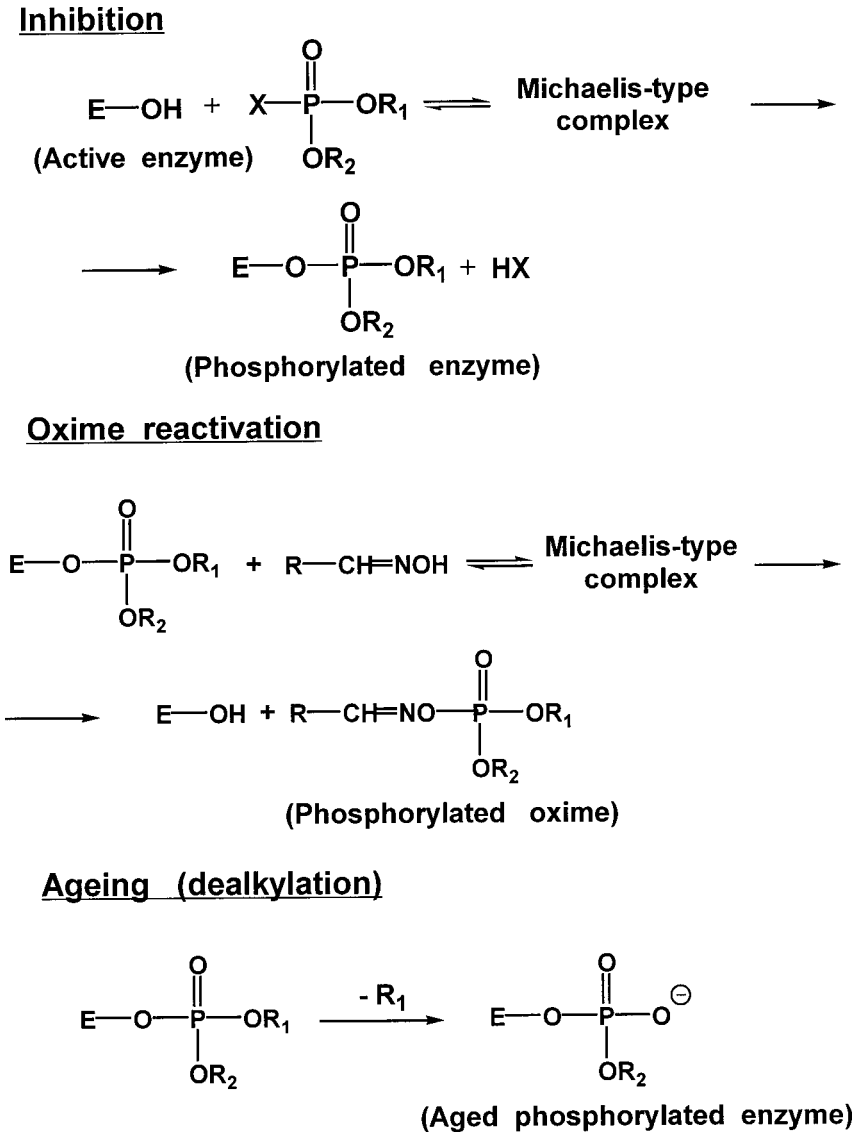


Figure 2 *Reactions of serine esterases with organophosphorus compounds*

Acetylcholinesterase is a globular protein and its three-dimensional structure is known. Its physiological substrate is acetylcholine. Figure 3 shows a schematic drawing of AChE molecule. The active site of AChE is in the centre 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 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.

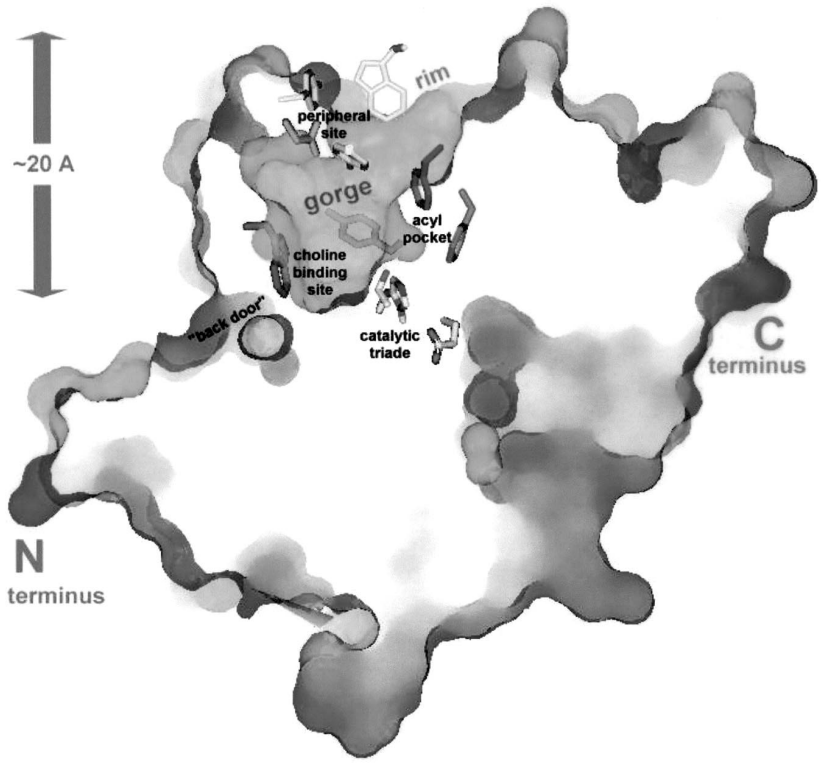


Figure 3 *Schematic drawing of the acetylcholinesterase molecule*
(prepared by Zoran Radić, UCSD, USA)

Research on AChE is now directed towards identification of individual amino acids which participate in the individual steps of catalysis. The experimental approach consists of preparing site-directed mutants, correlating the catalytic properties with the structure of the mutant and modelling 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 shown to be unrelated to any known serine hydrolases. Its physiological role is not known. The 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 NTE (Figure 2) 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 which promote OP-induced polyneuropathy without causing polyneuropathy themselves. The promoters known so far are NTE inhibitors, but it seems unlikely that NTE is the target enzyme.

HYDROLYSIS OF OP COMPOUNDS BY PHOSPHORIC TRIESTER HYDROLASES

The mechanism of reaction of OP compounds with PTHs is different from the reaction of OPs with serine esterases. The molecular structure of PTHs is also different. Phosphoric triester hydrolases require divalent cations for catalysis as shown in Figure 4. The cation (M^{++} in Figure 4) is embedded in histidine residues and 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.

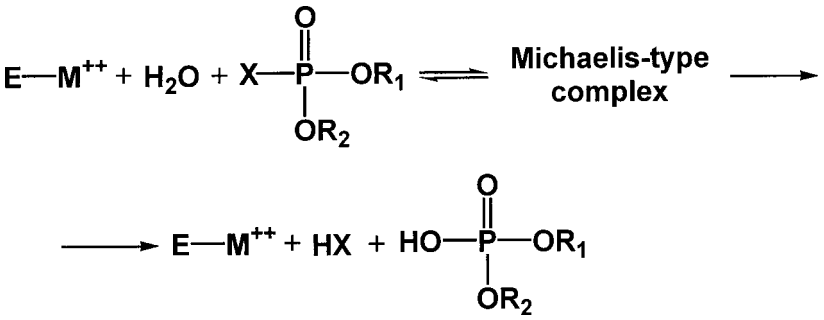


Figure 4 *Reactions of phosphoric triester hydrolases with* organophosphorus compounds

Phosphoric triester hydrolases 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. Phosphoric triester hydrolases are stereoselective like AChE. The OP enantiomer which is more quickly hydrolysed by PTHs is less inhibitory for AChE, and vice versa. Phosphoric triester hydrolases have recently been shown to hydrolyse carboxylic acid esters such as phenylacetate, which is a characteristic substrate of arylesterases (EC 3.1.1.2) (1).

The search for natural substrate(s) and the physiological role of PTHs continues. Mammalian serum paraoxonases seem to act against cellular damage from toxic agents and oxidised 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. Phosphoric triester hydrolases 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.

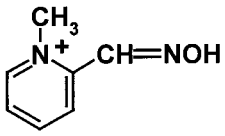
OXIMES AS ANTIDOTES AND PROTECTORS AGAINST OP COMPOUNDS

The primary mechanism of action of oximes as antidotes is to reactivate the phosphorylated AChE (Figure 2). The pyridinium oximes PAM-2 and HI-6 (Figure 5) 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 5.

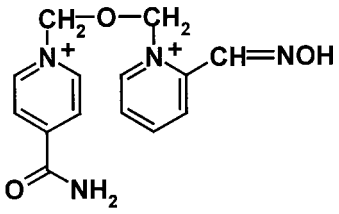
The product of dephosphorylation by oximes is the phosphorylated oxime (Figure 2). Phosphorylated oximes are potent AChE inhibitors, but as they are very unstable, phosphorylation of the reactivated AChE by phosphorylated oximes has seldom been reported.

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 AChE from phosphorylation. When the reversible inhibitor binds to the active site, the protection is due to direct competition between the OP 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 less evaluated in experimental toxicology so far.

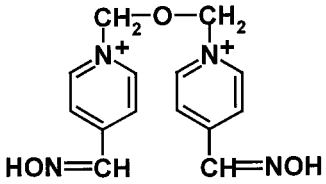
Pyridinium oximes



PAM-2

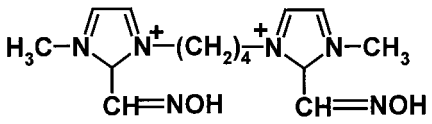


HI-6



Toxogonin

Imidazolium oximes



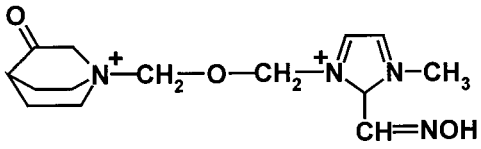


Figure 5 *Structural formulae of several oximes*

ESTERASES AS ANTIDOTES, PROTECTORS AND DECONTAMINATING AGENTS AGAINST OP COMPOUNDS

Phosphorylation of serine esterases by OP compounds occurs on a 1:1 molar basis. This reaction inhibits the enzyme, but detoxifies OP. 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 there are oximes which would 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 reactivated 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. Rabbits, which have a very high paraoxonase activity, are more resistant to paraoxon than other species. 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. PTH from a microorganism immobilised on cotton wipes detoxifies nerve agents and OP pesticides. Purified AChE or BChE immobilised on polyurethane sponge do the same. Rinsing the sponge with oxime solutions restores the enzyme activity. Phosphoric triester hydrolases can be co-immobilised on the same sponge, increasing the capacity of decontamination. Enzymes immobilised on matrices are more stable than in solution, and can be used repeatedly.

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Sažetak

ORGANOFOSFORNI SPOJEVI I ESTERAZE: STANJE SADAŠNJIH ISTRAŽIVANJA TOKSIČNOSTI I ZAŠTITE OD ORGANOFOSFATA

U ovom prikazu opisane su reakcije organofosfornih (OP) spojeva s kolinesterazama, hidrolazama fosfornih triestera (PTH) i esterazom koja je povezana s neuropatskim djelovanjem OP spojeva (NTE). Kolinesteraze i NTE su serinske esteraze. Organofosforni spojevi inhibiraju te enzime zato što fosforiliraju serin u aktivnom mjestu enzima. Inhibicija acetilkolinesteraze (AChE) je glavni uzrok akutne toksičnosti OP spojeva. Suprotno serinskim esterazama, PTH enzimi hidroliziraju OP spojeve i time ih detoksiciraju. Oksimi su antidoti kod akutnog otrovanja OP spojevima zato što defosforiliraju inhibiranu AChE. Oksimi dodatno štite slobodnu AChE od fosforiliranja. Hidrolaze fosfornih triestera, kao i butirilkolinesteraza (BChE), služe i za dekontaminaciju od OP spojeva, a oba enzima istražuju se i kao mogući protektori protiv otrovanja OP spojevima. U prikazu su opisane i molekulske strukture AChE i PTH.

Ključne riječi:
acetilkolinesteraza, butirilkolinesteraza, DFP-aza, esteraze fosfornih triestera, kolinesteraze, oksimi, paraoksonaza, serinske esteraze

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