Journal of **A**pplied **B**iotechnology **R**eports

Review Article

A Review on Biodegradation of Toxic Organophosphate Compounds

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Abstract

Daily, organophosphorus compounds (OPs) in human life, has found wide applications. Although OPs have biodegradability potential, they induce clinical problems in humans and other organism. Different methods are used to detoxify these compounds. In the meantime, biodegradation is preferred as a compatible way to the environment since it produces less toxic compounds. Enzymes capable to degrade the OPs are of the most important items in the biodegradation. Genetic manipulation involved in the production of these enzymes has been employed in bacteria, and finally, is used for the mass production of recombinant microorganisms. In this paper, the role of organophosphates on human life and the ways to destroy toxic organophosphates are studied.

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Keywords: Organophosphates Toxicity, Biodegradation, Hydrolysis Enzyme,

Submission Date: 4/10/2015
Accepted Date:6/15/2015

Introduction

Organophosphate pesticides were recognized for the first time in 1850, when Moshchenine synthesized tetraethyl pyrophosphate (TEPP). Some years later, the initial synthesis of organophosphorus compounds including P-F bond was introduced by Long and Roger Vaughan. Gross firstly stated the performance of organophosphate in 1952 as an acetylcholine esterase (AChE) inactivator [1]. In the end of World War II, organophosphate compounds were widely used as insecticides and additives in plastic and oil industry [2]. Excessive use of organophosphates causes the pollution of soil and water in ecosystems around the world [3]. Although these compounds are degraded, they have high toxicity to mammals, and other animals and also for invertebrates and vertebrates [4]. The poisoning effect of these compounds takes place in terrorist attacks, the leakage to the environment and handling by workers and farmers. These compounds have acute toxicity for a wide range of disorders to nervous system and muscles [5]. Approximately three million cases of poisoning and three hundred thousand deaths in the world are caused by organophosphorus compounds [6]. This article briefly studies the influences of OPs on human life and provides some guidelines to detoxify these compounds in ecosystem. Organophosphate compounds are classified in two main categories:

I. Axons: Axons have a P=O double bond and include phosphotriestrases and phosphotulates.

- Phosphotuesters: The triester compounds derived from phosphoric acid which are considered as standard type of organophosphate pesticides, and are very active, in which all four phosphorus are bond to oxygen.
- Phosphotiolats: In addition to a P=O double bond, it contains a single P-S bond.

This category is more toxic, and commonly used as plant or soil systemic insecticides.

II. Thions: This group contains a P=S double bond and includes phosphorothionate and phosphorothionothiate.

- Phosphorothionates: This subgroup contains a P=S double bond and three O-P bonds, such as parathion, diazinon and chlorpyrifos.
- Phosphorothionothiate: This subgroup has a P=S double bond with a P-S single bound attached to the central phosphorus.
- Organophosphorus amides: This subgroup is derived from Phosphoric acid, such as phosphor amides and phosphoramidothionate.

There are also some other structures derived from phosphonats (P-CN), phosphofolridats (P-F) and phosphorocyanidats (P-CN) [7].

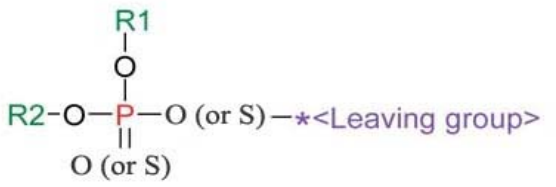

Figure 1. The overall structure of organophosphorus compounds [7].

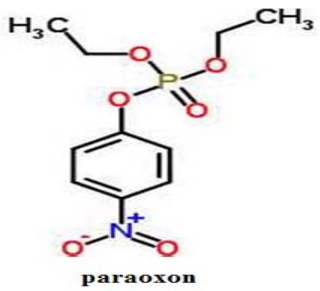

Figure 2. Chemical structure of organophosphorus compounds axon groups [7].

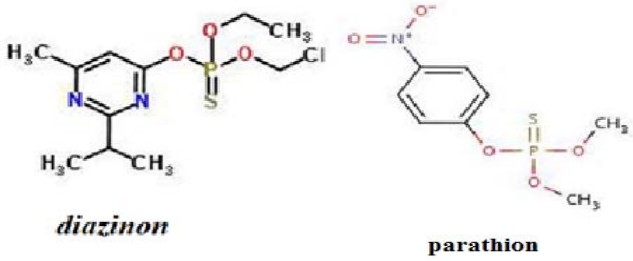

Figure 3. Chemical structure of organophosphorus compounds [6, 7].

The effect of organophosphate compounds on humans

Organophosphates can affect through oral, inhalation and also reacting with tyrosine residue present in the keratin of skin epithelium, and act as one of the most common poisoning factors on multiple biological systems [8].

Nervous system disorders

Nervous system is one of the major biological systems in animals that are influenced by, with various methods, mainly through the inhibition of cholinesterase [9]. Cholinesterases in vertebrates are divided into two main groups: Acetylcholinesterase (AChE) and pseudocholinesterase or Butyrylcholinesterase (BChE). This enzyme synthesized by liver is found in plasma, pancreas, brain and heart, and is a serine hydrolase capable of hydrolyzing esters such as acetylcholine, succinylcholine and miuvacoriuom, with a serum half-life of 8 to 12 days [10]. Cholinesterases are divided based on catalytic properties, substrate features and inhibition by specific inhibitors [11-14]. Acetyl cholinesterase is a serine hydrolase hydrolyzing the acetylcholine neurotransmitter at cholinergic synapses to terminate the neuronal signaling [15]. OPs covalently bond to the hydroxyl group of serine phosphorylation, inhibit the enzyme's active site and cause the accumulation of acetylcholine at synapses [1].

The inhibition of cholinergic muscarinic receptors pathway (mAChR) is the most important result caused by acetylcholinesterase inhibition. Cholinergic activities in autonomic nervous system are those that deal with general aspects of life in the brain such as various types of behaviors and functions including hunger, thirst, sweating, breathing, anger, communication and understanding. MAChR is also a kind of acetylcholine receptor responding to muscarin. AChE inhibition in MAChR leads to the accumulation of acetylcholine and eventually convulsion. High stimulation of mAChR disrupts the balance of glutamatergic and GABAergic activities and changes the concentration of calcium ions both inside and outside of the cells [1, 16-18].

The changes on calcium concentration affect on some other conditions such as ion concentration, hyperosmolarity and protein functions in the endoplasmic reticulum membrane [19-21]. This indicates that exposure to high levels of OPa harmfully causes prolonged effects on brain's structure and function [22]. Secondary damages to the nerves caused by OPs are memory loss, inability to concentrate, speech and behavioral problems [23]. Memory loss is usually occurs in 2-3 weeks after exposure. OPa cause a change on the activity of Ca^{2+} dependent calmodulin-kinase (CaMK2), which is responsible for the phosphorylation of Cytoskeletal proteins, alpha, beta tubulin and microtubules associated with triplet proteins and neurofilament (NF) involved in the pathogenicity of Ops [24, 25].

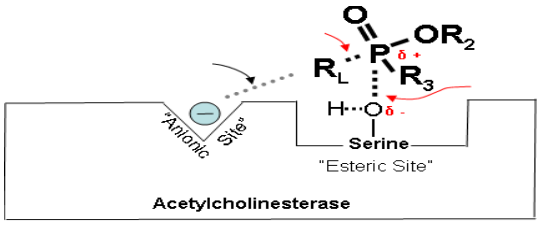

Figure 4. Inhibition of AChE active site [10].

Impairment of reproductive

Spermatogenesis and gametogenesis are regulated by the endocrine glands [26]. Some pesticides or their metabolites, such as DDT or parathyroid, act as an endocrine-disruption agent in animals or humans [27, 28]. The decrease in sperm concentration is the most common effect [29]. In a research on greenhouse workers, as an occupation involved with OPs, an increased level of dialkylphosphate led to a lower level of follicle-stimulating hormone (FSH), increased amount of testosterone and decreased level of the hormone inhibinB [30]. Another report showed that much contact with parathyroid pesticides is associated with increased levels of FSH and luteinizing hormone (LH) and also reduced levels of inhibinB [31]. High amount of organochlorine is also associated with changes in hormonal levels [32]. All these cases indicate that OPs effect on human reproductive system.

Impact on biological macromolecules

OPs lead to the formation of oxidized material (ROS) [33, 34]. The concentration of oxidized material can be increased considerably by different environmental toxins from industry, agriculture, accidental infections and smoking. Lipids, proteins and nucleic acids are sensitive to ROS. The first defense of the body against the effects of ROS is oxidant enzymes [35]. OPs cause oxidative stress which leads to the loss of mitochondrial energy (ATP), the induction of proteolytic enzymes and DNA fragmentation for apoptosis [36]. Insecticides also increase chromosomal abnormalities. Chlorpyrifos, MPT and MLT increase an

antioxidant enzymes including catalase activity (CAT) superoxide (SOP) and glutathione peroxidase in rat tissues and also cytochrome P450 [35]. Cytochrome P450 oxidase system is an important component of performance in relation to the metabolism of several substrates including drugs, xenopiotcs, environmental pollutants, and many other compounds. Cytochrome P450 oxidation changes phosphorotioate compounds to axon through oxidative disulforation [35-37]. Other vital systems such as the immune system [38], pancreas [39], liver [40], blood [41] and the reproductive system [42] are affected by organo-phosphate compounds.

The use of organophosphate insecticides can cause poisoning and certain related symptoms including meiosis, increased urination, diarrhea, excessive sweating, tears and saliva [43]. OPs have certain genotoxic, clastogenic and AlkilationL properties, so they have potential to cause mutations and clastogenic [44, 45]. OPs have multiple effects on enzymatic pathways contributing in the development of diabetes, such as cholinergic pathway that enables adrenergic receptors and induces Hyprglaysmy and finally gluconeogenesis, or the activation of the glucocorticoid receptor that activates the gluconeogenesis in liver, and also causes the disruption of pancreatic endocrine by oxidative stress.

The effect of organophosphate compounds on the environment

Many toxic compounds can persist in the environment with high resistance; therefore, they can influence the different populations of microorganisms and Macroorgani-sams [46]. Most of the reports are focused on the effects of OPs on protozoa (protists) in soil, which has a high level of reproduction as a part of the soil microorganisms and are very good sample for the measurement of toxic and biological assays [47]. OPs such as chlorpyrifos effect on reproduction, survival and fetal development in both vertebrates and invertebrates, leading to a decrease in the number of organisms in the environment [48-53].

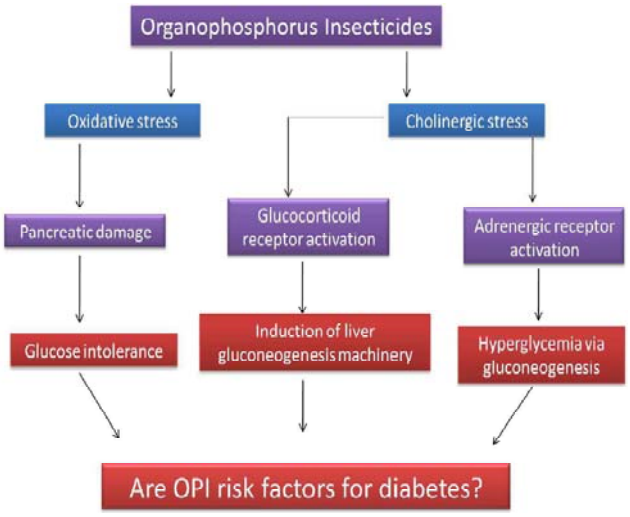

Figure 5. The proposal for the effects of organophosphates on hemostasis changes in glucose [53].

According to the communication between different organisms, if one of the creatures be infected by OPs, other creatures associated with it are also involved. For example, if the air or water or soil be contaminated with OPs, all organisms subjected to them will be infected. Eventually, the role of OPs on human life becomes clearer considering the chain of communication between these creatures and human [54].

Analysis of organophosphorus compounds

Biodegradation

Due to the effects of OPs, a major problem world widely, there is an urgent need to develop affordable and reliable approach to quickly disinfect the pollution caused by them in the environment [55-57]. Guidelines like the combus-tion and buried deep in the remote areas are of the earliest and most convenient methods to detoxify OPs. These methods release toxic gases into the atmosphere or leach the pesticides into the ground and surface water [58, 59]. Nonbiological methods are chemical degradation through photolysis reactions, hydrolysis, and oxidation and redoxion catalysis.

Formulated factors can act as a buffer or inhibitors in h drolysis or dehalogenation. Some pesticides have ability to be hydrolyzed in alkaline water and soil [60]. Optical analysis methodology, a combination of UV light with semiconductor solutions, is also a common method to dgrade OPs, the best example is combining semiconductor titanium dioxide (TiO2) with UV light. Compounds such as, UV-H2O2, UV-TiO2 and UV-H2O2-TiO2 are used for optical degradation of some OPs such as Diazinon [61-63].

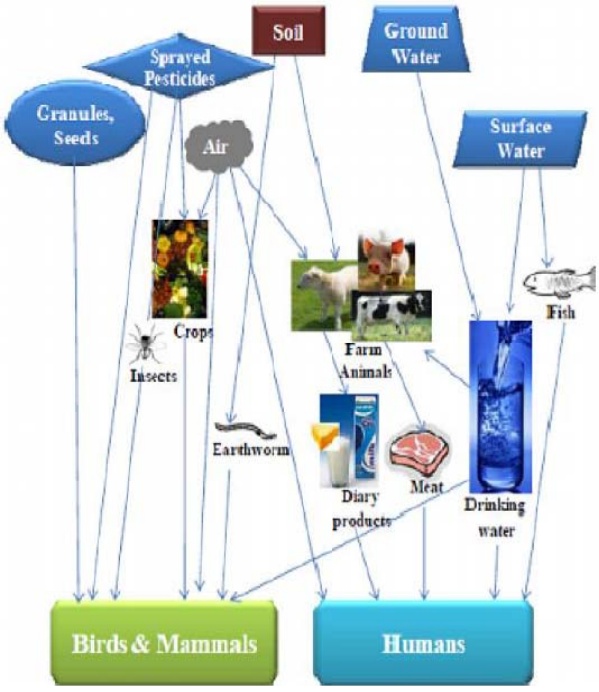

Figure 6. A view of the effect of organophosphorus compounds on the environment and on human and animal life [62].

Biodegradation of organophosphorus compounds

Most pesticides, such as organophosphate, carbamate, or pyrethroid are bio-degradable. These compounds hydrolyze spontaneously (especially in high pH) and by enzymes to less toxic materials [64, 65].

The biodegradation of pesticides by microbial enzymes in the soil is a key mechanism in preventing the accumulation of these chemicals in the environment. For example, several species of bacteria possessing the ability to hydrolyze pesticides, have been isolated from wastewater plants capable of hydrolyzing some organophosphorus compounds (Chlorpyrifos, Diazinon).

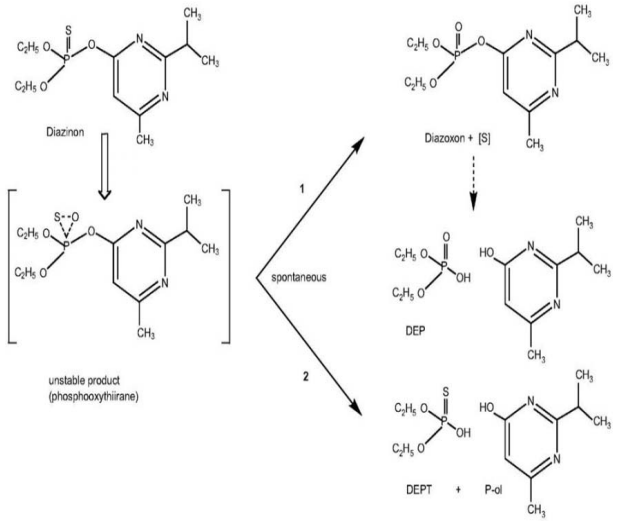

Figure 7. Decomposition of Diazinon [64].

Researchers have shown that a continual application of insecticides leads to an increased biodegradation rate, reduced half-life and ultimately the effectiveness [66-68]. Rapid degradation of pesticides effects remarkably on pest control, therefore, it can be very impressive and important [69]. For example, in an experiment using EPTC to control weed, the first two annual programs were reported well, however, the third application showed approximately 75% reduction [70]. The use of microorganisms for the biodegradation of certain chemicals may be affected by several things, such as:

1- An increase in the biodegradation activity of microorganisms in accordance with the specific gene expression.

2- An increase in the number of degrading microorganisms due to microbial growth or lateral gene transfer

3- The migration of some other degrading microorganisms to the desired location

4- The evolution of new essential enzymes to consume OPs as a source of chemical energy [71].

Many reports indicate the importance role of soil, microbial community and gene pool in the degradation rate of pesticides. The effectiveness will be lost in the absence of specific genes, unless in case of gene presence or migrating new microbes possessing the desired genes to the desired location [72-76]. Recently many detoxification strategies using engineered microorganisms capable of doing a lot of analysts are developed. Several continuous efforts are required to improve the detoxification process by the overexpression of hydrolyzing enzymes.

enzymes capable degradation of organophosphorus compounds

Organophosphates toxicity is related to the phosphoryl center, which can bind and inactivate cholinesterase enzymes and other biological enzymes. In phosphotriesters all four atoms bound to the phosphate are oxygen, although, in other subclasses it has been replaced with other atoms such as S, F, C, or N. The toxicity of OPs is reduced significantly by chemical and enzymatic hydrolysis. Pesticides-degrading enzymes are highly regarded. Based on International Union of Biochemistry, these enzymes are classified in the hydrolase subtypes. Esterases are classified in subgroup 1 of hydrolase. Various types of esterases are named based on a variety of ester bonds.

Carboxyl esterases (CbEs)

Carboxyl esterases are included within subgroup 3.1.1 of the International Union of biochemistry and have ability to hydrolyze carboxylesters. In 1953, Norman Aldridge classified esterases by the reaction with OPs to α and β subgroups. The subgroup unable to inhibit OPs is named α esterase, and the subgroup able to inhibit OPs is named β esterase [77]. OPs and carbamates show the most toxic effects by phosphorylation (or Karbamylation) of serine group in the active site of choline esterase. Phosphorylation Or Karbamylation of acetylcholinesterase and neurological esterase is associated with their toxic effects. However, the inhibition does not cause major effects in mammals (particularly in the liver and serum). The β esterase (AChE) may be defined as a detoxification enzyme of OPs and Karbamats. Each molecule of CbE is able to detoxify one pesticide molecule prior to creating any sign in the nervous system. It is very suitable detoxification. The OPs resistance in some beetles and birds is associated with the overexpression of β esterases, which inhibits the phosphorylation of the active centers in pesticides to make them inefficient [78, 79].

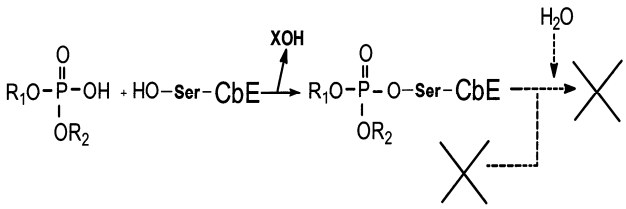

Figure 8. Organophosphates, with phosphorylation of the carboxyl esterase enzyme prevents the activation of the enzyme with water and enzyme activity recovery becomes impossible [77].

I. phosphatriesterase (PTEs)

Phosphatriesterases are major part of Organophosphates and include a central phosphate ester with three bonds [80, 81]. Phosphatriesterase enzymes are capable of hydrolyzing these compounds and are identified in various tissues in mammals, fish, birds, mollusks and bacteria [82]. These enzymes break the bonds between the phosphorus

atom and release groups down and produce some products with higher polarity, therefore, they would not be accumulated in fatty tissue and thus will easily be excreted from body by urine. Since this reaction inhibits the phosphorylation ability and reduces their toxicity, the hydrolysis by PTEs is considered as a detoxification reaction. One molecule of carboxyl esterase can hydrolase only one molecule of organophosphate, while phosphotriesterase could hydrolyze several organophosphates molecules, therefore, OPs detoxification by PTEs is much better. PTEs are usually named according to the substrate specificity [82, 83].

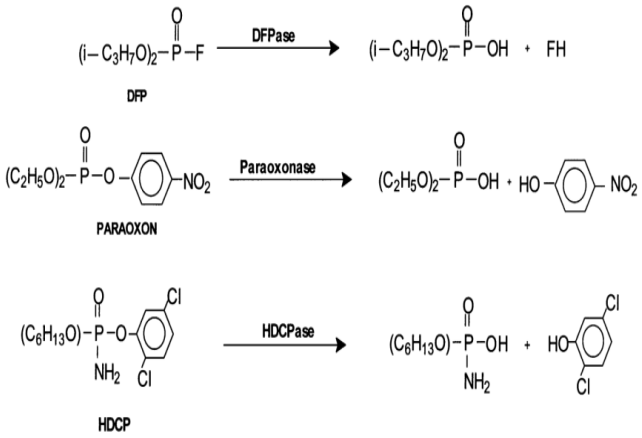

Figure 9. Some reactions catalyzed by Phosphotriesterase known [9].

Orghanophosphoruse hydrolase ((OPH (EC.3.1.8.1)

The gene encoding Orghanophosphoruse hydrolase (OPH, EC 3.1.8.1) was found in two strains from two different families with similar sequences, *Flavobacterum* sp. (ATCC 27551) and *Pseudomonas diminuta* MG. OPH has a strong hydrolysis for a range of insecticides such as phosphothioesters, and phosphorofluoridates including DFP and chemical weapons such as Sarin and Soman, and is the only enzyme that is able to hydrolyze P-S bond in OPs. Various forms of the enzymes associated with various divalent ions in the enzyme's structure illustrate different ability to hydrolyze OPs. For example, the activity of OPH (CO^{2+}) is approximately five to twenty times more than the activity of OPH (Zn^{2+}). This difference is due to the type of catalyst metal [84].

Organ phosphorus Anhydrides acid (3.1.8.2) (OPAA)

In 1946, DFPase was detected by Abraham Mazur in rabbit tissues as a DFP-hydrolyzing enzyme. It was then purified from *Alteromonas* sp, JD6.5.19 bacteria strain. This enzyme is a 60 kDa monomer metalloprotease, with Mn^{2+} in its natural form. Its activity is confirmed in the hydrolysis of the P-F bond presence in DFP, Sarin and Soman. The activity to hydrolyze P-O and P-CN is less and does not have the ability to hydrolyze P-S. It is also shown that OPAA from *Alteromonas* sp. hydrolyzes paraoxon with 2% rate of DFP. The OPAA acts on anhydrate phosphorus bonds in organophosphorus compounds (e.g. nerve agents) [85]. International Union of Biochemistry in 1992 named the enzymes capable of degrading P-F or P-CN bonds as OPAA [86]. OPAA is a single-chain polypeptide consisting of 517 amino acids with 58kDa, with pH range between 6.5-9.5 (with optimum of 7.5-8.5) and 65-10 temperature range (optimum at 55-40°C) and manganese (Mn^{2+}) [87].

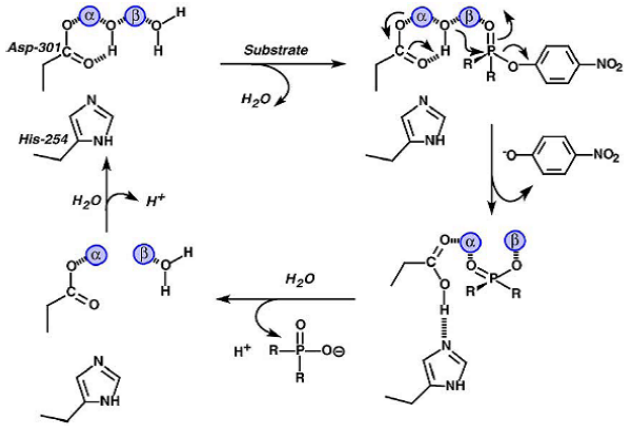

Figure 10. The decomposition mechanism of PTE in Paraoxon analysis [80].

Diisopropil flrophosphatase (DFPase)

DFPase is a 35 kDa protein with 314 amino acid subunits. It was firstly obtained from the nodes and brain of squid Loligo vulgaris by Francis hoskin in 1966. This enzyme is only found in Cephalopods and requires Calcium ions for its function [88]. Since these enzymes do not have a specific physiological substrate, is classified in the subgroup of phosphotriestrase (EC.3.1.8) more accurately is classified based on their substrate specificity into two subgroups of arylalkyl fluorophosphates (EC.3.1.8.1) and diisopropyl fluorophosphates (EC3.1.8.2) [89].
This enzyme is able to cut the P-F bond in fluorophosphates, soman and sarin with 0.1 rate of DFP. Hydrolysis activity of DFPase for Paraoxon is very low compared to substrate of P-F [90].

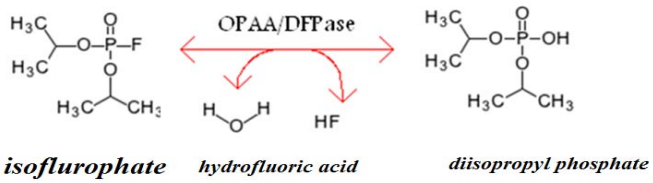

Figure 11. Proposed mechanism for the enzyme OPAA enzyme function and DFPASE [88].

paraoxonase (PON1)

PON1 is an enzyme found in mammalian body, and is responsible for the hydrolysis of oxidized Tioat produced

by the P450 system and is able to hydrolyze P-O, P-F and P-CN. Paraoxonase protein is a dimer protein with a molecular weight of 43000 to 45000. Paraoxonase gene is on chromosome 7. This enzyme possesses two active sites. The property is eligible for the hydrolysis of organophosphorus compounds [91-93].

Amino peptidase P

PepP is a metaloproteain in E. coli and the Mn2 + ion is required for its function. The highest degradation rate was observed when the substrate consists of methylisopropyl and methylisobutyl [94].

Phosphonate ester hydrolase (PEH)

H factors are produced by the degradation of G and V nerve agents using enzymes such as OPH and OPAA, which are toxic. In a study in 2001 by Elachvili et, a bacterial enzyme was found that converts H factor to methyl phosphonic acid (MPA) [95].

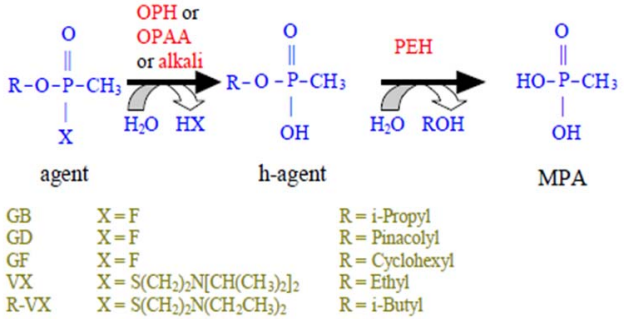

Figure 12. Decomposition of H generated by PEH [95].

Natural and native sources (microbes) containing organophosphate-degrading enzymes

In a study by Latifi et al., IRLM.1, IRLM.2, IRLM.3, IRLM.4 and IRLM.5 isolates were identified from chemical plant effluent and contaminated agricultural soils. These strains contain OPs-degrading enzymes and can consume Diazinon and Chlorpyrifos as a source of carbon and phosphorus [96]. A *Pseudomonas aeruginosa* strain isolated in North of Iran from contaminated agricultural soil showed a high ability to break down the Diazinon [97].

Commercial applications of enzymes

OPs- degrading enzymes has wide applications including; designing a bioreactor containing a consortium of bacteria or purified enzymes for the utilization of bio- pesticides [98-100], the treatment of poisoning with organophosphorus compounds [101, 102], with a nano protective cover to prevent immune reactions, designing biosensors to identify areas contaminated with Ops [103-108].

Strategies for enzyme engineering

The enzyme engineering strategies are applied to increase the strength and stability such as enzyme capsulation [109, 110]; changes in affinity of enzyme [111], changes in the specificity of the enzyme [112], a direct enzyme conjugation with quantum dots CDS [113], enzyme immobilization on nano-porous silica substrate [114, 115]; cell surface display [116], and secretory enzyme expression [117].

Table 1. Comparison of OPH, OPAA, and DFPase enzymes.

Enzyme	Sourse	Activity
DFPase	Squid (*Loligo vulgaris*)	DFP>GF=GB>GD>GA
OPH or PTE	Bacteria (*P. diminuta* and *Flavobacterium*)	GD>GF=DFP>GB>GA
OPAA	Bacteria (*Alteromonas* sp.)	DFP>GF=GB>GD>VX

Conclusion

Regarding to the fact that OPs have harmful effects to human health and nature, their use should be limited as far as possible or the analogues with lower toxicity should be applied such as organophosphorothioates, which is potent to be hydrolyzed in the mammals. In addition, developing the processes to detoxifyor degrade OPs is a serious global need and country planning.

Acknowledgment

The authors would like to thank all colleagues in the Applied Biotechnology Research Center (ABRC) of Baqiyatallah University of Medical Sciences for their kind contribution for this Review.

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