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REVIEW

Organophosphorus degrading enzymes: Molecular basis and perspectives for enzymatic bioremediation of agrochemicals

Enzimas degradantes de organofosforados: Base molecular e perspectivas para biorremediação enzimática de agroquímicos

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ABSTRACT

Many organophosphorus compounds (OP) are used until today in agriculture as pesticides and, unfortunately, they are used as chemical warfare agents (or nerve agents) as well. Organophosphorus pesticides and nerve agents are extremely toxic molecules, since they act as Acetylcholinesterase (AChE) inhibitors. The most worrying effect of the exposure to these compounds is the acute cholinergic toxicity, which is the loss of muscle coordination. Once one is contaminated, the intoxication process begins through the binding of the OP in the active site of the AChE enzyme inactivating it. Current treatments for people exposed to low doses of OP can be performed with atropine, oximes and benzodiazepines. Important remediation processes involve the employment of bioremediation techniques using different degrading enzymes, such as the Phosphotriesterase from *Agrobacterium radiobacter* and SMP-30. Due to the high number of intoxications annually, it is crucial to search for more potent and effective treatment methods, and in this line, the techniques involving bioremediation seem to be quite promising for this purpose.

Index terms: Enzymatic biodegradation; organophosphorus pesticides; warfare nerve agents; oximes.

RESUMO

Muitos compostos organofosforados (OP) são utilizados até hoje na agricultura como pesticidas e, infelizmente, como agentes de guerra química (ou agentes dos nervos) também. Os pesticidas organofosforados e os agentes dos nervos são moléculas extremamente tóxicas, uma vez que atuam como inibidores da enzima Acetilcolinesterase (AChE). O efeito mais preocupante da exposição a estes compostos é a toxicidade colinérgica aguda, ou seja, a perda de coordenação muscular. Uma vez que o indivíduo se contamina, o processo de intoxicação começa através da ligação do OP no sítio ativo da enzima AChE inativando-a. Os tratamentos atuais para pessoas expostas a baixas doses de OP podem ser realizados com atropina, oximas e benzodiazepínicos. Processos de remediação importantes envolvem o emprego de técnicas de biorremediação utilizando diferentes enzimas degradantes, como a Fosfotriesterase da *Agrobacterium radiobacter* e SMP-30. Devido ao elevado número de intoxicações anualmente, é crucial buscar métodos de tratamento mais potentes e eficazes, e nesta linha, as técnicas envolvendo biorremediação parecem ser bastante promissoras para este propósito.

Termos para indexação: Biodegradação enzimática; pesticidas organofosforados; agentes dos nervos de guerra; oximas.

INTRODUCTION

Organophosphorus (OP) insecticides began to be synthesized and used on a large scale in the 1940s, during the World War II to protect soldiers from pests that transmit diseases like malaria in tropical and subtropical regions of Asia and Africa. Boosted by military incentive, the researches resulted in the development of many pesticides that are intensively used to date (Perezgasga et al., 2012). In parallel, during the Second Great War, a new class of highly toxic OP compounds was developed and used as chemical warfare agents. The extreme toxicity of these compounds is related to their strong affinity with acetylcholinesterase (AChE) enzyme, causing its "irreversible" inhibition (Quinn, 1987).

Drugs that inactivate AChE enzymes are named anticholinesterase (anti-ChE) agents. These compounds cause the accumulation of the acetylcholine (ACh) neurotransmitter in the vicinity of cholinergic nerve

endings, producing an equivalent effect of an excessive stimulation of cholinergic receptors throughout the central and peripheral nervous system (Bunya et al., 2016).

All pesticides are toxic. However, their use use is not obligatory to be dangerous. Toxicity is inherent to the compound and its hazard refers to the risk of poisoning when the product is used. In this way, the risk associated with the compound depends not only on its toxicity, but also on the product exposure like: route of absorption, dose and duration of exposure (Delfino; Ribeiro; Figueroa-Villar, 2009). Poisonings due to pesticides may be: (1) acute, when they manifest immediately after the absorption of the compound in a sufficient amount to induce the harmful symptoms; or (2) chronic, when they manifest after a more prolonged period of exposure to the compound. Acute intoxications occurs mostly in professionals who work in urban pest control or agricultural crops (Perezgasga et al., 2012).

Another form of contamination is the accumulation of pesticides in the biotic and abiotic segments of the ecosystems due to the large use of agrochemicals for food production. OPs accumulated in nature lead to several environmental impacts and to the contamination of living organisms (Barr et al., 2005).

Considering the OP chemical structure, the predominant valences of the phosphorus element are +3 and +5. The largest part of these compounds that has agrochemical and industrial applications is pentavalent. The nature of the phosphorus-related substituents plays an important role in determining the toxicity of the agents (Balali-Mood; Abdollahi, 2014). With all exposed previously, it becomes increasingly important the search for new methods of remediation for the intoxication caused by these toxic agents.

ORGANOPHOSPHORUS COMPOUNDS

Agrochemicals: organophosphorus pesticides

Any organic molecule that contains phosphorus is an organophosphorus compound. Figure 1 presents some examples of such molecules. OPs are usually esters or thiol derivatives of phosphoric, phosphinic, or phosphoramidic acids (Perezgasga et al., 2012).

The use of these compounds is extremely versatile, being employed in industries as antioxidants, stabilizers for plastic, industrial oils, etc. As discussed in the introductory section of this article, they have a huge importance as agrochemicals as well (insecticides, fungicides, herbicides) (Balali-Mood; Abdollahi, 2014).

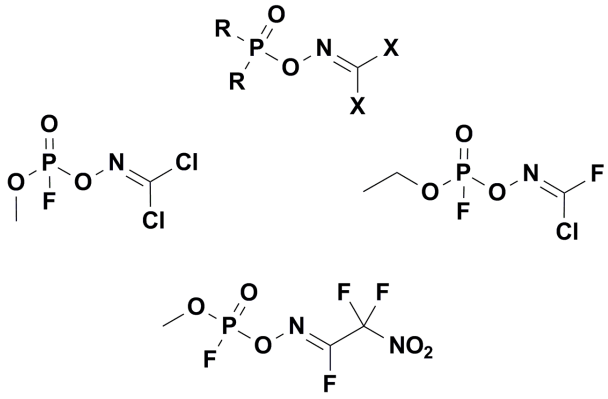

Figure 1: Examples of some OP compounds structures (Mcconnell et al., 1999).

Currently, more than 100 types of pesticides and herbicides are used in agriculture in different regions of the world. The use of each type of pesticide depends on their cost, the regulation of each country and the climate of each region. In many countries, some highly toxic OP pesticides, like parathion, were banned decades ago, but, unfortunately, they are still in clandestine use. Overall, OP pesticides are responsible for about 38% of all pesticides employed globally (Singh; Walker, 2006). In the last years, although some new non-OP and inorganic pesticides were discovered, their expensive prices do not encourage farmers to change their traditional methods that include the harmful OP compounds (Balali-Mood; Abdollahi, 2014).

Along with the so-called chemical weapons, pesticides are the only compounds intentionally produced in order to be toxic and introduced directly into the environment (Perezgasga et al., 2012). With the goal of controlling pests, pesticides are often spread out in agricultural crops without any environmental control. Since the OP toxicity is not completely specific to a determined target organism, the indiscriminate use of these substances may be a great risk for many organisms health. Indeed, just a short percentage of pesticides really reach the target organisms. The remains escape into water or dissipate in the soil and air, favoring the contamination of many species like mammals and birds. The latter appears to be the most sensitive class of animals directly affected by these toxic substances (Perezgasga et al., 2012). Furthermore, the continued use of pesticides has already raised great concern about the impacts caused on human health, making it a public health case.

It is known that one of the major public health problems in the world is the pesticide poisoning. Every year, more than 5 billion pounds of pesticides are spent

worldwide, according to a 2009 study by the German company Kleffmann at the request of the Brazilian Agribusiness Association (Abag). In 2001, just in the United States, about 73 million pounds of OP pesticides were employed (World Health Organization, 2003).

The World Health Organization (WHO) estimates that 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths. In emerging countries, wherein the use of these OP is frequent due to the hot weather conditions, the number of deaths may be higher than in colder countries. The main three forms of exposure to pesticides are: (1) during their application in crops, (2) through drainage in water supply and (3) through consumption of contaminated food (World Health Organization, 2003).

Because of the great risk of humans and nature contamination, on the Food Quality Protection Act (1996), the US Environmental Protection Agency (www.epa.gov) put 40 OP in the top priority group, with crucial restrictions regarding the employment of harmful OP like chlorpyrifos, azinphos-methyl and methyl parathion (see structures in Figure 2) (Perezgasga et al., 2012).

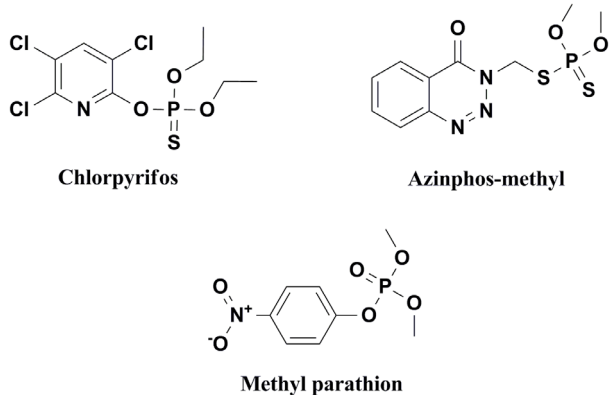

Figure 2: Structure of chlorpyrifos, azinphos-methyl and methyl parathion (Perezgasga et al., 2012).

Mainly because of the Food Quality Protection Act, OP compounds have been restricted to agricultural sectors in the US. Despite the fact that chlorpyrifos remained an extensive OP insecticide, its overall employment was reduced by around 50% since 2000 (Iyer; Iken; Leon, 2015).

Stricter regulations worldwide have also significantly decreased the percentage of OP agents in overall use, limiting contamination levels. However, in Asiatic and Western Pacific countries, exposure and suicidal ingestion are still a constant issue in countries which are deeply dependent on agriculture, and therefore, provide broader access to pesticides including OP (Iyer; Iken; Leon, 2015). In China, a drop in relative OPs usage is balanced by a growing demand for pesticides that now exceeds that of all other countries around the world, increasing risks of chronic exposure for farmland workers. In India, the main problems are still the access to highly toxic OPs, the large pesticide use, and precarious safety awareness in agricultural populations (Kumar et al., 2010).

The most worrying effect of exposure to OPs is the acute cholinergic toxicity, a delayed ataxia, known as organophosphorus ester-induced delayed neurotoxicity (OPIDN), which is the loss of muscle coordination. It happens due to the inhibition of AChE activity, which can take place within minutes or hours after exposure; usually, these effects may disappear after days or weeks with an appropriate treatment. Plasma activity or erythrocyte AChE is used to monitor acute exposure to these substances (Balali-Mood; Abdollahi, 2014).

Toxicology and interaction of OPs with Acetylcholinesterase

OP insecticides and nerve agents have been developed to act as acetylcholinesterase (AChE) inhibitors. OP compounds are significantly lipophilic and promptly move to the nervous system, wherein the intoxication process begins through the binding and subsequent inactivation of AChE. Governmental strategies to combat the predominance of OP poisoning are usually based on a restriction system, by regulating the acquisition and use of certain OP insecticides, encouraging the substitution of the most dangerous products by less toxic substances (Tse; Comba; Alaee, 2004).

The OP chemical structure is directly linked to specific activity and application of each agent. The basis for understanding the toxicity level of each compound is to be thoroughly familiar with its chemical structure. The nature of the substituents attached to the phosphorus atom and the susceptibility to hydrolysis is a determining factor for understanding their action (Balali-Mood; Abdollahi, 2014).

The main toxicological action associated with OPs in humans is their ability to inhibit esterase enzymes. In the 1930s, the toxicity of OP in humans and insects was discovered, and decades later, it was found that OP compounds act as inhibitors of the acetylcholinesterase (AChE) enzyme in both humans and insects, i.e., they have the same principle of action (Bunya et al., 2016).

As a mechanism of action, the presynaptic nerve releases the acetylcholine (ACh) neurotransmitter and sends it through the synapse to bind to the AChE receptor. After the binding, the hydrolysis of ACh is catalyzed

by AChE giving rise to acetic acid and choline, which causes the interruption of nerve impulse transmission. The catalytic mechanism of AChE involves three important chemical steps: (1) the formation of an intermediate acyl-tetrahedral enzyme, (2) a nucleophilic substitution and (3) acid-base reactions (Balali-Mood; Abdollahi, 2014).

AChE is not selective and its catalytic activity is not limited to the hydrolysis of ACh, but various reactions involving aryl esters, anilides, thioesters, amides and other ACh-like acyl compounds may inhibit the AChE enzyme, avoiding normal breakdown of the ACh neurotransmitter. Thus, ACh concentrations increase at the neuromuscular junctions and result in an involuntary contraction of all muscles, causing respiratory and cardiac arrests, which may lead to death (Balali-Mood; Abdollahi, 2014).

In case of OP poisoning, there is an accumulation of the ACh neurotransmitter in the nerve endings, as already said before (Giacoppo et al., 2015). The inhibition mechanism involves a nucleophilic attack of Ser203 from the AChE catalytic triad to the phosphorus atom in the OP. The phosphorylated enzyme resulting from this process is stable and, depending on the R groups of the neurotoxic agent, may become irreversibly inhibited. Thus, the OP inhibition mechanism is different from acylated AChE, which is rapidly converted into to acetic acid and the enzyme is regenerated. The loss of the enzyme catalytic activity is due to the fact that the phosphorylated serine residue in the catalytic triad is no longer able to hydrolyze ACh (Balali-Mood; Abdollahi, 2014).

The phosphoryl-enzyme complex undergoes one of the two following processes: (1) reactivation, which is the hydrolysis of the phosphoryl-enzyme complex triggered by the use of an appropriate nucleophile; or (2) cleavage of the OP-C bond of the phosphorylated enzyme, which subsequently releases an alkyl-carbene (Eddleston et al., 2005).

In many cases, the reactivation of aged AChE (see Figure 3) is not possible, and the inhibition of the enzyme becomes irreversible. In this case firstly the complex at the enzyme active site is negatively charged, which naturally makes nucleophilic attacks more difficult. In addition, structurally, the enzyme adopts a conformation that protects the aged complex from nucleophilic attacks. The non-covalent interactions of the OP and the oxyanion orifice also contribute to the irreversibility of the aged enzyme (Balali-Mood; Abdollahi, 2014). Figure 3 shows the mechanism of aging of the neuro agent Soman-AChE complex as an example of interaction of OP with AChE.

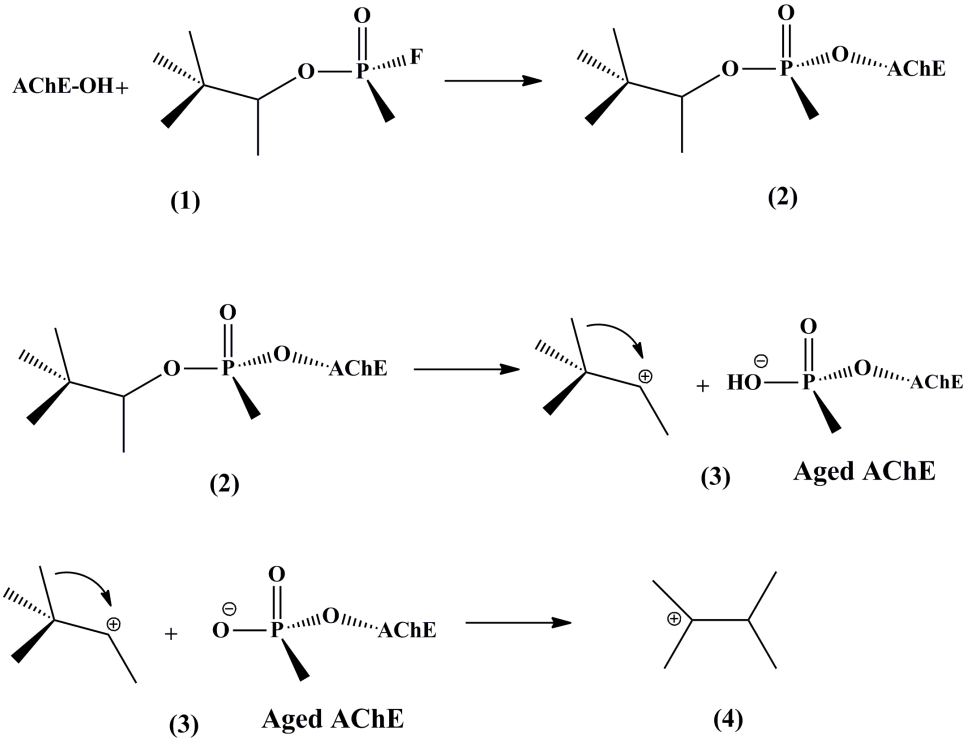

Figure 3: Mechanism of aging of the Soman-AChE complex (Eddleston et al., 2005).

A significant group of metabolic reactions lead to the detoxification of OPs. The chain of metabolic reactions generally involves the cleavage of one of the phosphorus bonds and the formation of negatively charged molecules increasing the solubility of the agents, which facilitates their excretion and reduces their half-life within the body. Worryingly, pesticide ingestion is a common suicidal method employed in rural areas. Current treatments for people exposed to low doses of OPs can be performed with atropine, oximes, and benzodiazepines with limited success (Perezgasga et al., 2012). Important remediation processes also involve the usage of bioremediation techniques using different degrading enzymes, as we describe in next sections.

REACTIVATION PROCESSES: OXIMES

Oximes are organic molecules with the general formula RR'C=N-OH. They can be synthetized through some reaction routes that include many functional groups. The most used method for the synthesis involves the addition of hydroxylamine (NH_2OH) to aldehydes and ketones in order to form aldoximes and ketoximes (Damljanović; Vukićević; Vukićević, 2006). This reaction is initiated by the nucleophilic attack of hydroxylamine on carbonyl, followed by dehydration of the compound, and thus obtaining oximes (Araújo; Gonsalves, 2015). Figure 4 illustrates this reaction.

The interest in oximes became more significant after 1905, when Lev Tschugaeff described the high selectivity and sensitivity of dimethylglyoxime in the gravimetric determination of Ni(II). Another application of oximes that have been stimulating the interest of researchers is their use in the synthesis of natural, industrial and pharmaceutical products (Vessally; Abdoli, 2016).

For many years, oximes have been studied for the treatment of the OP poisoning (Patocka et al., 2005), like the intoxication caused by pesticides. After OP exposure, the spontaneous reactivation rate of most AChE-OP complexes is insignificant, and the aging reaction prevails (Hornberg; Tunemalm; Ekstrom, 2007). Therefore, when AChE is inhibited, this enzyme is not able to hydrolyze the ACh neurotransmitter, causing an intoxication frame, the so-called Cholinergic Syndrome (Albuquerque et al., 2006), as described previously.

In order to treat the effects of the Cholinergic Syndrome, anticholinergic drugs such as atropine, and an AChE reactivator (usually oximes) may be used (Kuca et al., 2005). For chemical weapons, particularly in the case of tabun poisoning, the treatment with just atropine is not able to prevent convulsions and brain damages induced by the OP. In this case, for a most successful treatment, it is necessary to combine atropine with an oxime (Kassa; Kunesova, 2006). Oximes act as a nucleophile by dephosphorylating the enzyme and restoring its activity. The dephosphorylation reaction of Ser203 is highly dependent on the chemical structure of the OP and the reactivator (oxime). The administration of oximes must be performed prior to the aging reaction of AChE, since after the dealkylation reaction in the enzyme-inhibitor complex, it is not possible to reactivate the enzyme (Stojiljkovic; Jokanovic, 2006).

Currently, many oximes used in AChE reactivation are known, but there is not a single molecule effective against all existing OP. The most common oxime is the pralidoxime (Kuca et al., 2004). However, depending on the dose, the use of pralidoxime in the reactivation process may not be efficient, and can be even harmful (Eddleston et al., 2009). In one hand, individuals intoxicated with a relatively low dose of OP insecticides show clinical improvement following the administration of a small dose of pralidoxime; on the other hand, for highly intoxicated individuals, there is no concrete evidence to encourage the use of this oxime, and more effective oximes with a better risk/benefit ratio should be used (Eyer, 2003).

Other oximes, such as trimedoxime and obidoxime, are extremely important in the reactivation of the AChE inhibited by less toxic OP insecticides. For OP with higher toxicity level, such as phosphonates, HI-6 oxime showed to be more efficient. Instead, in the case of nerve agents,

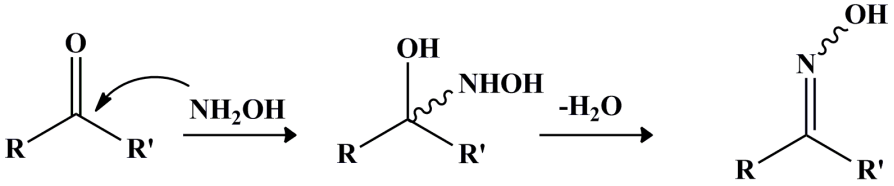

Figure 4: Obtaining oximes from ketones or aldehydes (R=Alkyl radical and R'=Alkyl radical or hydrogen) (Araújo; Gonsalves, 2015).

like tabun and VX poisoning, another oxime named HLö-7 proved to be slightly more effective than HI-6 (Stojiljkovic; Jokanovic, 2006).

A newly developed oxime, still more promising in the reactivation of tabun-inhibited AChE, is K203. Although there is not an universal oxime for the AChE inhibition yet, K203 remains the most effective one. A recent study show that a low concentration of 10^{-5} M of K203 may reactivate 13.5% of the inhibited enzymes (Kuca et al., 2015), which guarantees the life of the intoxicated organism. Figure 5 shows the structures of the oximes cited in this section.

ENZYMATIC BIODEGRADATION

Phosphotriesterase from *Agrobacterium radiobacter*

Phosphotriesterases are enzymes that can hydrolyze several phosphotriesters compounds, as OP pesticides and nerve agents (Ely et al., 2011). Several OP-degrading enzymes have already been studied; however, the OP hydrolases most studied are the degrading enzyme from *Pseudomonas diminuta* (OPH) and the one from *Agrobacterium radiobacter* (OpdA) (Horne et al., 2006). These enzymes take part of a large family that require

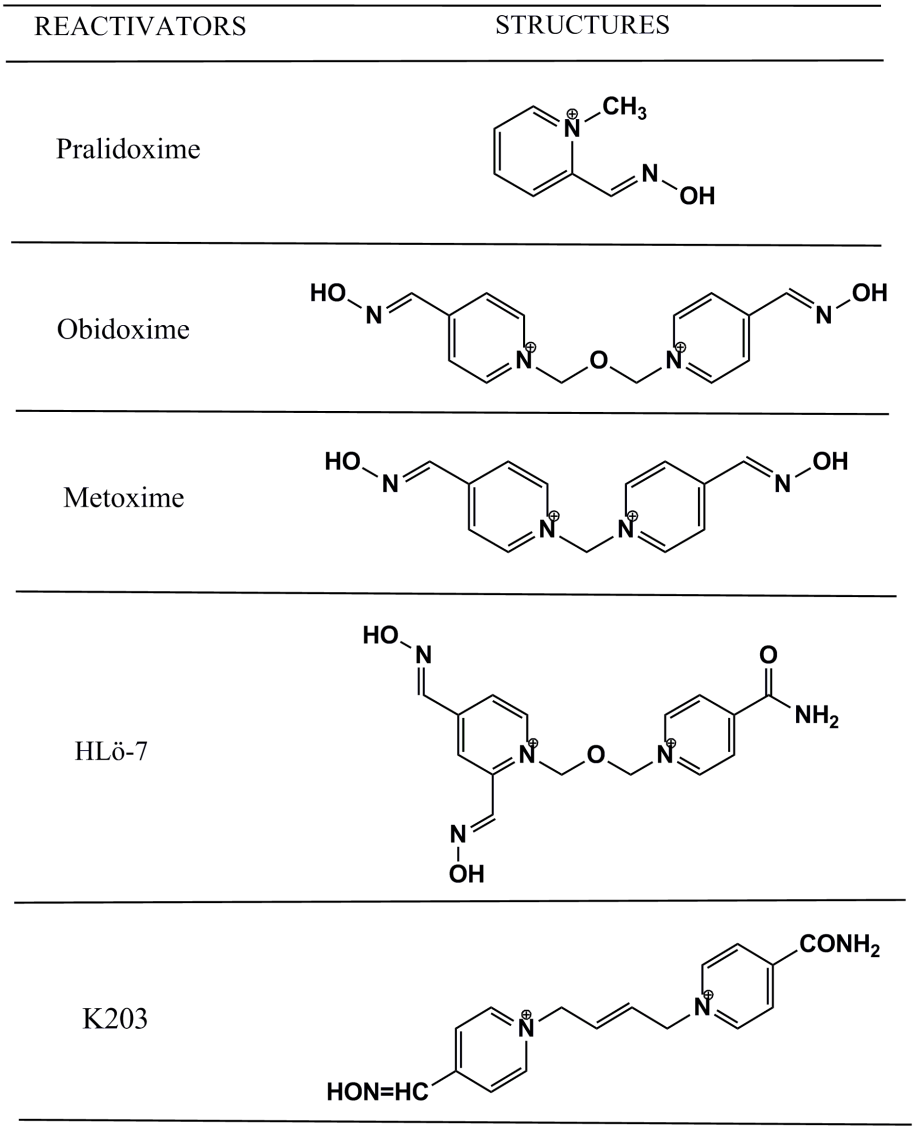

Figure 5: Chemical structures of AChE reactivators: oximes (Kuca et al., 2015; Kuca et al., 2004).

two metal ions like Zn^{2+} (OPH) or Co^{2+} (OpdA) in α and β sites for the hydrolytic reaction process; both enzymes have a binuclear metal center and are promising in studies of OPS degradation (Jacquet et al., 2016; Schenk et al., 2012).

According to structural studies, the two metal ions coordinated in both (OpdA and OPH) enzymes interact with a carboxylated lysine (Lys169) residue and a hydroxide ion or water molecule (see Figure 6) (Ely et al., 2010; Jackson et al., 2005).

Homology studies show that OpdA enzyme shares 90% of the OPH identity; however, there are some differences in substrates specificity and kinetic behavior between them (Jacquet et al., 2016). The most important amino acids sequence differences between OpdA and OPH consist in: (1) different residues in the active site as Arg254/His254, Tyr257/His257 and Phe272/Leu272, respectively in OpdA and OPH (Pedroso et al., 2014), and (2) 20 additional amino acids at the C-terminus of OpdA, which seem to be irrelevant for catalysis since are located reasonably far away from the active site (Ely et al., 2010; Horne et al., 2006).

The knowledge about the number of interaction residues around the substrate is essential to investigate how the hydrolysis mechanism takes place. Spectroscopic techniques are very advantageous in the search for active site structures, an example of those techniques is the Magnetic Circular Dichroism (MCD), which has been successfully employed to study the OP hydrolysis mechanism in OpdA enzyme (Ely et al., 2011).

Diverse structural techniques were employed in order to understand the chemical mechanisms of the OP hydrolysis catalyzed by OpdA. After many years of effort, scientists discovered that the hydrolysis efficiency depends mainly on the metal ion in the enzyme, the pH of the environment and the substrate (Ely et al., 2011). For the initial phase of the OP-degrading mechanism in OpdA, a new model describes how the substrate can be prepared for a nucleophilic attack (see Figure 7) (Schenk et al., 2012).

In OpdA, two of the three amino acid residues nearby the active site, Arg254 and Tyr257, have an important role in modulating the catalyzed reaction due to a complex hydrogen bond network. These hydrogen bonds are less significant in OPH enzyme. In this way, whether a mutation occurs in OpdA, it generally leads to the break of these hydrogen bonds, and the enzyme may become more similar to OPH. Thereby, the functional differences between OpdA and OPH may be explained already by the different amino acids in the active site. Furthermore, most researches with OPH were performed using Zn^{2+}, Cd^{2+} or Mn^{2+} as metal ions, while in OpdA was performed mainly using Co^{2+} (Schenk et al., 2012), and as we emphasized before, this ionic difference is crucial for the enzyme efficiency.

OpdA enzyme has a large substrate specificity, being able to break P-O, P-CN and P-F bonds in OP agents, thus having a very high catalytic activity (k_{cat} of ~3000 s^{-1}) (Gao et al., 2014). Due to this fact, OpdA is a good alternative for bioremediation applications in OP (Ghanem; Raushel, 2005).

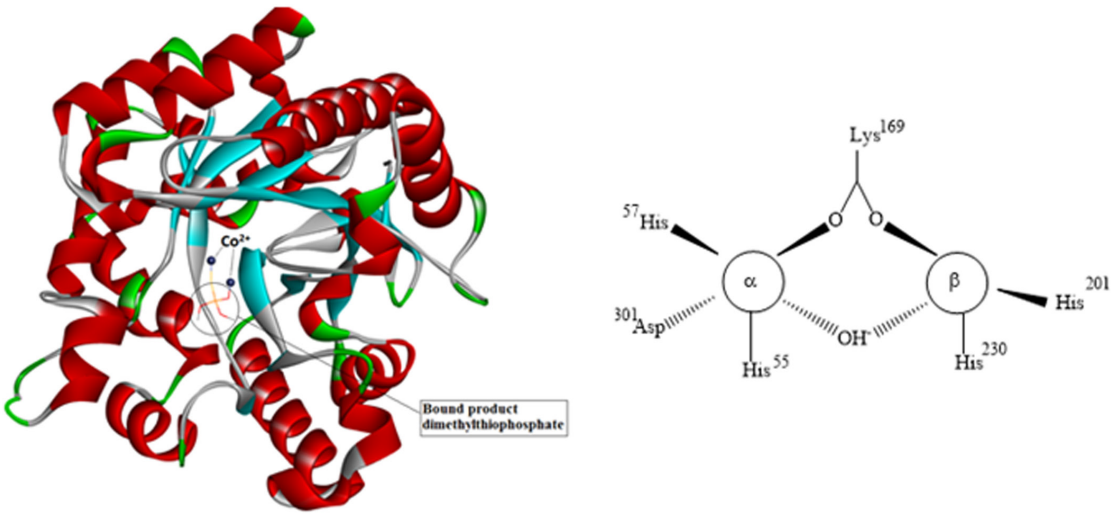

Figure 6: Crystalline structure of OpdA from *Agrobacterium radiobacter* with bound product dimethylthiophosphate (PDB ID: 2D2G), and representation of its active site (Jackson et al., 2005).

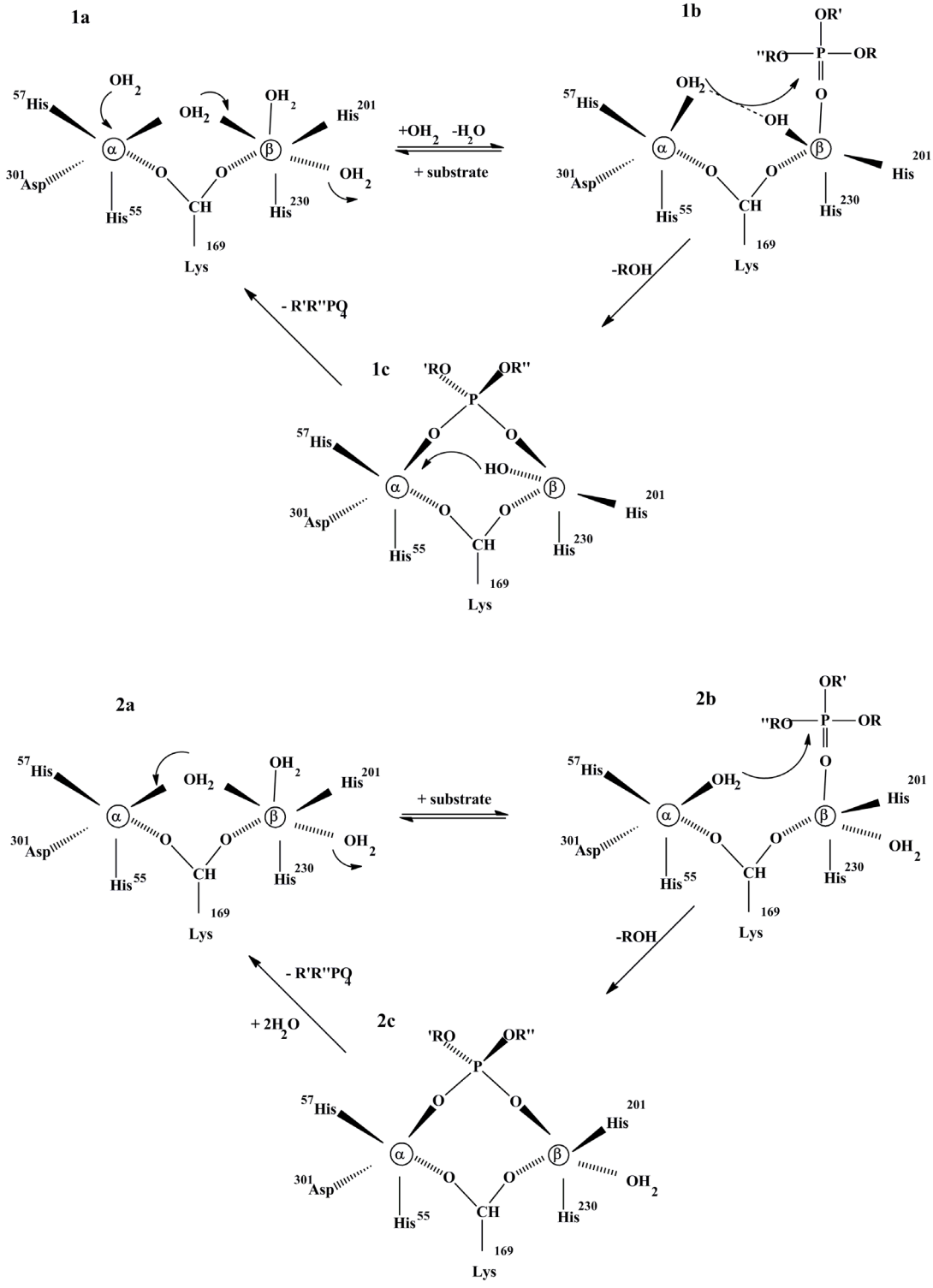

Figure 7: Reaction mechanisms proposed for OpdA. Draft 1 is proposed for the di-Co^{2+} derivative of OpdA at high pH. Draft 2 is proposed for the di-Co^{2+} derivative of OpdA at low pH (Raushel, 2002; Schenk et al., 2012).

Senescence Marker Protein 30 (SMP-30)

In 1989, Little et al. enunciated an enzyme found in rat liver capable of hydrolyzing four different OP compounds in the following order of efficiency: sarin, soman, tabun and diisopropyl phosphorofluoridate (DFP), being sarin the most hydrolyzed molecule (Little et al., 1989).

In the same way, Billecke et al. were able to isolate a liver cytosol enzyme from mice with similar catalytic properties to that quoted by Little et al. (Billecke et al., 1999). The authors were also capable of determining the amino acid sequence of a 7 kDa fragment of that enzyme, 58 of 60 residues corresponding to the Senescence Marker Protein 30 (SMP-30) sequence enunciated by Fujita et al. (Fujita; Shirasawa; Maruyama, 1996). Despite those findings, Billecke et al. were not successful in determining sufficient amounts of the rat SMP-30 amino acid sequence to assist in the complete understanding of the hydrolyzing activity of OP.

At first, SMP-30 was identified as a protein whose expression decreases in an androgen independent manner with aging, but its functions and substrates were nuclear (Fujita; Uchida; Maruyama, 1992). Several works have been connecting the SMP-30 with an anti-aging function, as well as a role in calcium homeostasis in mammalian cells and protection against cellular apoptosis (Son et al., 2008). Kondo and Ishigami reported several diseases linked to SMP-30 deficiency in rats, such as glucose metabolism disorder, liver fat accumulation, kidney tubule damage, insulin release disorder in pancreatic cells, and other maladies (Kondo; Ishigami, 2016).

Recent studies have revealed a physiological role of SMP-30, zinc-dependent gluconolactonase activity, which is the penultimate step in vitamin-C biosynthesis in non-primate mammals, wherein L-gulonate is converted to L-gulonolactone by SMP-30. Primates are not able to synthesize vitamin C due to the non-production of gulonolactone oxidase, whose enzyme is responsible for the last step in the conversion of gulonolactone to ascorbate (Linster; Van Schaftingen, 2007).

The large amino acid sequence homology between SMP-30 and bacterial gluconolactonases suggested the former folds into a six-bladed β-propeller structure (Chen et al., 2008). A recent obtained Human SMP-30 crystal structure proved its six-bladed β-propeller structure (Figure 8) (Chakraborti; Bahnson, 2010).

In the literature, for most solved β-propeller proteins, the active site is located in its central tunnel. There is a flexible region, a short loop, between residues 121-127, which is positioned at the top of the central tunnel. This arrangement acts as a tip of the active site and it may have a gatekeeper function or may present interactions that are responsible for the specificity of the enzyme substrates (Chakraborti; Bahnson, 2010).

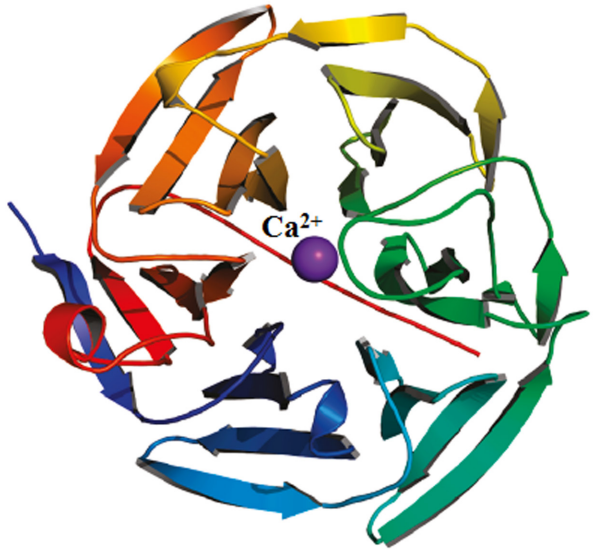

Figure 8: Crystalline structure of human SMP-30 with Ca^{2+} bounded (PDB ID: 3G4E). The ribbon structure of SMP-30 displays the six-bladed β-propeller fold with each blade displayed in a rainbow color (Chakraborti; Bahnson, 2010).

Studies have shown that the metals of choice for the SMP-30 glucolactonase and DFPase (OP degrading enzyme) activities are respectively Zn^{2+} and Mg^{2+}, besides using Ca^{2+} for the regulation of cellular calcium and homeostasis. In contrast to other analogous calcium-dependent enzymes, studies have put the role of Ca^{2+} cation in SMP-30 in check, and data suggest that rat SMP-30 is not a calcium binding protein. In addition, it has been suggested that rat SMP-30 does not have calcium-dependent gluconolactonase activity (Chen et al., 2008).

Chakraborti et al. reported the crystalline structure of *Hss*SMP-30, which may be bound to both Ca^{2+} and Zn^{2+}. Their study, using gluconolactone as substrate, also evidenced the preference of Zn^{2+}, with a decreasing catalytic activity with Mn^{2+}, Mg^{2+} and Ca^{2+}. The comparison of the dissociation constants of the metals with their free cell concentrations indicates that SMP-30 can convert itself to calcium dependent lactonase under stress conditions, where there is a high concentration of free calcium (Chakraborti; Bahnson, 2010).

In the literature, there is a shortage of information regarding the possible mechanisms of OP degradation by SMP-30. Belinskaya et al. have reported in their work a model of *Hss*SMP-30 with Zn^{2+} being the bound metal in the active site, coordinated to three residues, Glu18, Asn154 and Asp204, two water molecules and one DFP molecule. The mechanism for the possible nucleophilic attack on enzymes homologous to SMP-30, such as squid DFPase, is not the same of SMP-30. Indeed, the residue Asp204, which is equivalent to Asp229 in the homologous enzyme, cannot become a nucleophile due to residue Ser271 that would receive its proton instead. This residue is replaced by Thr246 in SMP-30 (Belinskaya et al., 2012).

CONCLUSIONS

The search for remediation techniques of the intoxication caused by OP compounds, such as chemical weapons and pesticides, has aroused the interest of researchers. Although the use of these compounds is restricted, they are still considered the most powerful weapons in chemical wars, and the OP pesticides are responsible for hundreds of thousands of intoxications annually, resulting in a number of dead extremely high. Thus, some possibilities described in many studies, such as the employment of degrading enzymes (bioremediation) and oximes, have been shown to be highly promising. Unfortunately, to date no antidote has been found to be capable of reactivating the AChE inhibited by all more potent OP. However, the understanding of how each antidote works in organisms for the reactivation process of the inhibited AChE is of paramount importance, since once one knows the role of each antidote in the intoxicated organism, strategic measures can be performed more efficiently. For bioremediation, it is extremely important a deeper understanding on the means of action of these enzymes, for instance, through the reaction mechanism unveiling.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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