Environ Sci Pollut Res
DOI 10.1007/s11356-016-6143-1

Current and emerging strategies for organophosphate decontamination: special focus on hyperstable enzymes

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Received: 1 July 2015 / Accepted: 20 January 2016

Abstract Organophosphorus chemicals are highly toxic molecules mainly used as pesticides. Some of them are banned warfare nerve agents. These compounds are covalent inhibitors of acetylcholinesterase, a key enzyme in central and peripheral nervous systems. Numerous approaches, including chemical, physical, and biological decontamination, have been considered for developing decontamination methods against organophosphates (OPs). This work is an overview of both validated and emerging strategies for the protection against OP pollution with special attention to the use of decontaminating enzymes. Considerable efforts have been dedicated during the past decades to the development of efficient OP degrading biocatalysts. Among these, the promising biocatalyst *Sso*Pox isolated from the archaeon *Sulfolobus solfataricus* is emphasized in the light of recently published results. This hyperthermostable enzyme appears to be particularly attractive for external decontamination purposes with regard to both its catalytic and stability properties.

Responsible editor: Philippe Garrigues

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Keywords Bioremediation · Phosphotriesterase · Organophosphorus · Pesticide · Chemical warfare agent · SsoPox · Enzyme · Decontamination

Introduction

Man-made organophosphorus compounds (OPs) are highly-toxic chemicals that were produced as chemical warfare nerve agents (CWNAs) to harm, kill, or neutralize the opponent in a war strategy. The first massive production of OPs was developed by German scientists before and during the 2nd world war, with several molecules, dubbed agents G (for Germany), such as tabun (in 1936), sarin (in 1937), and soman (in 1944; Szinicz 2005). After the 2nd world war, other toxic molecules, dubbed agents V (for Victory, Venomous, or Viscuous), were developed by other nations, such as VX (USA), RVX (Russia), or CVX (China; Fig. 1). Massive stocks of these compounds had been accumulated during the cold war (Gupta 2009). In 1993, the international convention, signed by some 200 countries, called for the arrested development of these chemical weapons, and planned for the destruction of existing stocks before 2007 (Organisation for the Prohibition of Chemical Weapons 2005; Gupta 2009). Large stocks, however, still exist today, partly because of the lack of low-cost, rapid, environmental friendly and safe solutions to destroy these chemicals. Additionally, such nerve agents (e.g., sarin) have been used during terrorist attacks in Matsumoto and Tokyo subway (Japan) in 1994 and 1995. Furthermore, organophosphate-based pesticides also constitute a serious threat as they are both highly toxic and widespread and could be fraudulently used for terrorist attacks or asymmetric conflicts.

OP compounds, and particularly V-agent derivatives, were also commercialized as insecticides, during the

Published online: 02 February 2016

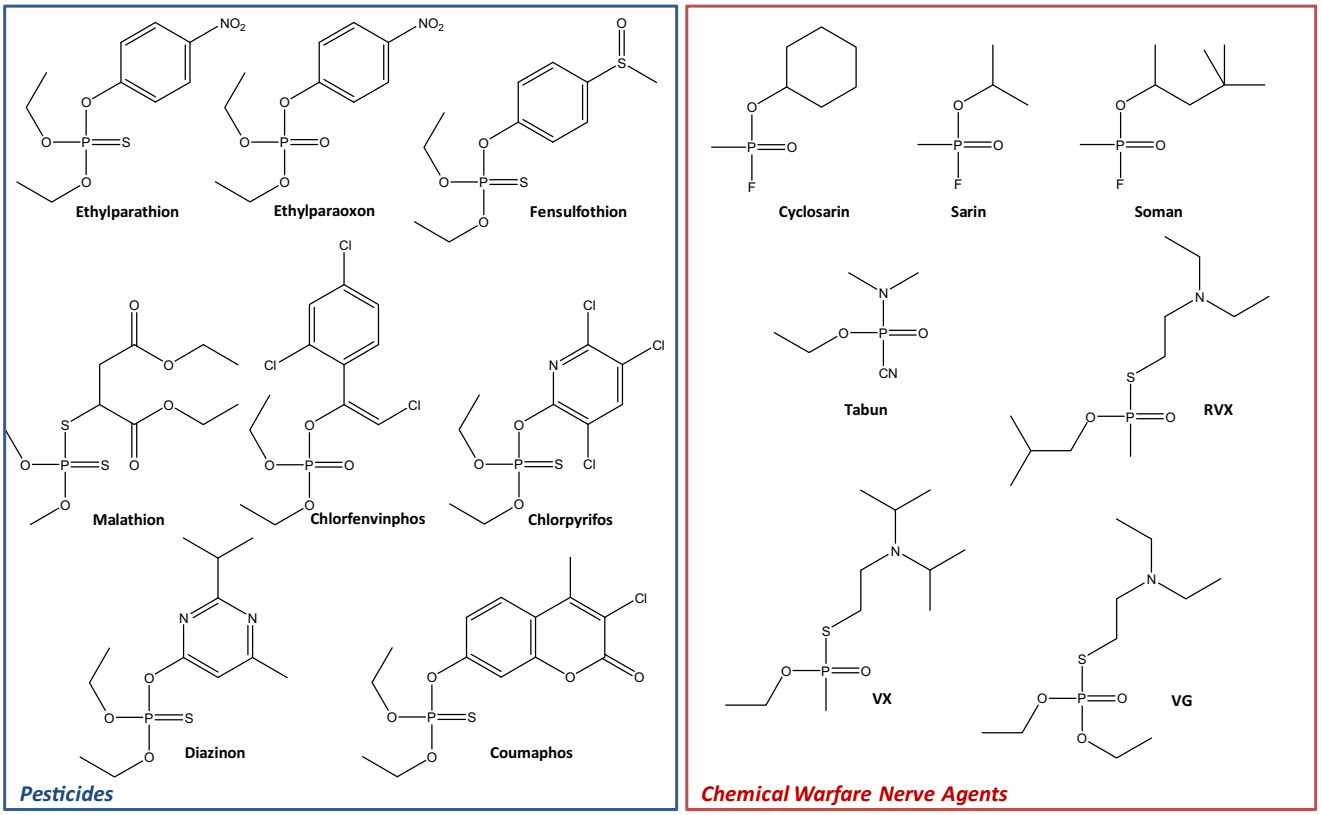

Fig. 1 Chemical structures of main organophosphate pesticides and chemical warfare nerve agents

1950s. They progressively replaced the insecticide Dichlorodiphenyltrichloroethane (DDT), an organochlorine banned in the 1970s. Being less persistent in the environment than DDT, the use of OPs have, nevertheless, been restricted in Europe and USA. However, they remain massively used in other parts of the world in intensive agriculture. The most commonly used OP insecticides are malathion, ethyl- or methylparathion, and chlorpyrifos (Fig. 1). OPs comprise major environmental (soils and water) pollutants (Jaipieam et al. 2009), and their use is estimated to lead to 3 million intoxications, yielding to 300,000 deaths in the world every year, the main part of these being ascribable to suicides due to deliberate ingestion of pesticides (Gunnell et al. 2007; Patel et al. 2012).

The extreme toxicity of OPs stems from their ability to irreversibly inhibit acetylcholinesterase (AChE), a key enzyme in the nervous system. Indeed, AChE terminates the degradation of the neurotransmitter acetylcholine in synaptic clefts and neuromuscular junctions. When AChE is inhibited, acetylcholine accumulates and saturates the cholinergic receptors. Acute intoxication by OPs results in apparition of various symptoms, abbreviated SLUDGEM, for salivation, lacrimation, urination, diaphoresis, gastrointestinal upset, emesis, and miosis (Zwiener and Ginsburg 1988; Lessenger and Reese 1999). Mechanistically, OPs react with the catalytic serine (Ser 200) of Human AChE, via phosphylation, forming a covalent phosphoenzyme (Beauregard et al. 1981). This OP-AChE intermediate can subsequently, depending on the OP nature, undergo an aging process (i.e., dealkylation occurs on the adduct). This process prevents reactivation of the phosphoenzyme. The aging kinetics is a very important parameter to account for while treating OP poisoning.

Due to the health threat presented by OPs both from acute and chronic intoxications, special attention has been dedicated to the development of decontamination strategies. Ideally, a decontamination solution would offer high efficiency, broad spectrum of action, smooth utilization compatible with skin or material treatment, no environmental impact, no secondary contamination, and low price. Hereafter is presented an overview of both current and emerging decontamination methods either physical, chemical, or biological. Their respective characteristics are summarized, and further developments and future perspectives are discussed.

Existing methods for decontamination of OP intoxications

Current methods of decontaminating pesticides and CWNAs can be separated into three categories:

(i) Physical decontamination: It encompasses all passive methods aimed at removing the contaminating agent

from any given surface, be it biotic or abiotic. It relies on mechanical action to absorb, remove, or flush the agent away, but does not usually destroy it.

(ii) Chemical decontamination: It refers to any given method that allows neutralization of the agent by chemical reactions, such as: hydrolysis, oxidation, or reduction.

(iii) Enzymatic decontamination: This method relates to the use of enzymes that are capable of hydrolyzing pesticides or CWNAs.

A possibly ideal decontamination device would use several methods that belong to the aforementioned categories, allowing for a surface cleaning combined with a rapid, efficient neutralization and/or destruction of the contaminating agent, thus, maximizing the effects and reducing the health and environmental hazards.

Large-scale decontamination

Large-scale decontamination is performed on surfaces after a massive OP exposure. The objectives are both to eliminate the contamination in situ as well as to reduce the potential of cross contamination, for example between the victim and the treating personnel. Currently available techniques are well suited for material decontamination; however, they often are aggressive and lack the ability to be fully safe for treating the victims. The solutions presented here are a non-exhaustive panel of available solutions.

– Hypochlorite

  Decontamination with a sodium hypochlorite solution may be used at the concentration of 0.5 % for personnel and 5 % for equipment (Tuorinsky et al. 2009). This solution proves to be useful both at removing the nerve agent from the surface and neutralizing it by oxidation. Be it for material and personnel, this method is still quite aggressive, due to its strong corroding potential towards eyes, skin, open wounds, and materials.

– Sodium hydroxide

  Sodium hydroxide is used to perform alkaline hydrolysis of OPs by a nucleophilic attack of the hydroxide ion on the phosphorus atom of OPs. It allows for the degradation of large amounts of nerve agents into their corresponding, non-toxic phosphonic acid.
  Combination of both aforementioned products, called super tropical bleach with 93 % calcium hypochlorite and 7 % sodium hydroxide, is used with high efficiency for material decontamination, with hypochlorite ion acting as a catalyst in the hydrolysis reaction (Singh et al. 2010). However, this method is highly corrosive and may neither be used for sensitive material nor personnel decontamination.

– DS2

  DS2 or "Decontamination solution 2" is used for material decontamination. It contains 70 % diethylenetriamine (DETA), 28 % 2-methoxyethanol, and 2 % sodium hydroxide. The active ingredient, 2-methoxyethanol, is highly effective against sulfur mustard and acts by elimination reaction, thus, forming divinyl sulfide. The mode of action against CWNAs is somewhat different as 2-methoxyethanol acts as a nucleophile and reacts with G- and V-agents (Singh et al. 2010). However, it is toxic and aggressive to treated surfaces as it even tends to degrade paints (Fielding 1964; Firmin 2003).

– BX-24 and BX-29

  These decontamination solutions, used in NATO forces, act by oxidation and hydrolysis. Personnel decontamination solution is composed of amphoteric compounds, amides, and a surfactant. It is advertised to be a safe decontamination solution. The BX-24 is a non-corrosive product designed for material decontamination.

– Decontamination formulation DF-200

  This product is used as foam in order to decontaminate toxic chemical warfare material. An enhancement over the previous DF-100 product, DF-200 is composed of nucleophilic compounds and oxidizing products, a bleaching activator, a sorbent, and an inorganic base, dissolved in water. It is in three distinct parts to be mixed together prior to use, with part one being a proprietary mix of quaternary ammonium compounds and surfactants, part two is composed of 8 % hydrogen peroxide, and part three is composed of glycerol diacetate, which catalyzes the oxidation process (Tucker and Comstock 2004; Tucker and Engler 2005; Tucker 2014). It is active against G- and V-agents with a 99 % decontamination of the treated surface after 30 min (Tucker 2014). The fact that this product is divided into three parts might cause logistical difficulties on the onset of a massive event.

Personal-scale decontamination

The kinetics of nerve agent penetration in the body is so fast that decontamination and treatment have to be performed on site and as soon as possible, at the individual level. Indeed, transportation time to proper medical facilities without prior application of topical solutions would greatly reduce the

chances of a patient's survival and would threaten medical personnel by spreading secondary contaminations. Currently available methods dealing with nerve agent exposure rely on external decontamination followed by medical treatment of the personnel exposed.

Ready-to-use kits for personal decontamination are primarily used by armies around the world. They have to comply with operational requirements and find the best trade-off in terms of size, weight, easiness of use, and of course efficiency.

– M291 SDK (Skin Decontamination Kit)

  Adopted by US Army in 1989, the M291 SDK is a ready-to-use, non-woven fiber pad, filled with an absorbent and a resin. The absorbent is a high-contact surface carbonaceous compound, in order to remove the agent from the skin. The resin itself is composed of two ion exchange resins, an anionic and a cationic one, able to neutralize the contaminant through hydrolysis (Khan et al. 2013). This technology was in use by the US Army until its progressive replacement by the Reactive Skin Decontamination Lotion (RSDL) around 2007. Its efficacy is very low on VX and very limited against soman as compared to other decontamination methods, such as 1 % soapy water and 0.5 % bleach (Braue Jr. et al. 2010a, b). It is not suitable for eye and wound decontamination.

– RSDL (Reactive Skin Decontamination Lotion)

  The RSDL is the current solution provided to soldiers and civilian security personnel in order to deal with topical exposure to OPs. It consists of an individually packed sponge, impregnated with a solution containing Diacetylmonoxime (DAM) and Dekon 139—a proprietary mix—dissolved in polyethylene glycol monomethyl ether (Bannard et al. 1991). This formulation allows for desorption of the agent from the skin and its chemical degradation via a nucleophilic reaction. The time required to achieve decontamination is less than 3 min for G- and V-agents (Elsinghorst et al. 2015). Its efficacy outperforms the one of M291 towards agents such as VX and soman (Braue Jr. et al. 2010a, b). The protective ratio (defined as the ratio between the LD_{50} of treated versus untreated group) of RSDL on guinea pigs is about 60 times higher than the one of M291 against VX (66.4 and 1.1, respectively; Braue Jr. et al. 2010a). Regarding soman, the protective ratio of RSDL still is five times better than the one of M291 (14 and 2.7, respectively; Braue Jr. et al. 2010b). The RSDL is efficient at decontaminating the skin as long as it is applied within minutes post-exposure. Like the M291 SDK, RSDL is not compatible with eye and wound cleaning.

– Fuller's earth

  Used by several NATO countries, pads impregnated with Fuller's earth purely rely on a physical and passive absorbance of the contaminating OP. It is mainly composed of fine aluminum silicate powder that confers a high-surface area, allowing for a good topical absorbance of contaminants (Taysse et al. 2007). The major drawback of using such a pad comes from the generation of contaminated dusts presenting an inhalation hazard.

  There is a current lack of non-corrosive solutions that would prove to be adequate both for sensitive material and personnel decontamination. Indeed, oxidative and corrosive solutions for decontamination are harmful and destructive to most military and civilian equipment and basic supplies. They may eliminate the threat of CWNAs but will leave behind a chemical contamination that is disastrous. Moreover, it should be noted that personnel decontamination using surfactant containing solutions (i.e., soapy water) represents a risk of spreading the agent over a wider skin surface. Even though it helps solubilize the chemicals, it may negatively affect the very aim of decontamination. It is, thus, of uttermost importance to opt for methods enabling sequestration and/or swift destruction of said nerve agents.

Stockpile destruction

In order to comply the Chemical Weapon Convention, the means of disposal were studied that could lead to destruction of stockpiles without harmful effect on environment and associated health hazards, as previous dumping at sea and burying method were problematic (US National Research Council 1996). It encompasses large hydrolysis systems and incineration used by western countries. Russia uses another method for destruction of G-agents, relying on neutralization followed by bitumination (Pearson and Magee 2002).

– Incineration

  The destruction by two-stage incineration is a process in use since the 1980s for the destruction of CWNA ammunitions, bulk agents, effluents, and equipment associated with stockpile disposal (US National Research Council 1994). First stage allows for the pulverization of the liquid agents and their heating to 1,480 °C. The subsequent gases serve as a fuel for processing by the second stage, an afterburner maintained at a lower, 1,100 °C temperature (English II 1974; Pearson and Magee 2002). The incinerator is equipped with a pollution abatement system that mixes the gases with sodium hydroxide and water to

remove acid gases and particles to reach safe standards (Pearson and Magee 2002).

– Hydrolysis

OPs are hydrolyzed using sodium hydroxide. However, the way hydrolysis is performed depends on the nature of the agent. While G-agents can be hydrolyzed safely at ambient temperature, VX hydrolysis has to be performed at 90 °C, temperature at which P–S hydrolysis is favored over P–O bond (Yang 1999; Pearson and Magee 2002; Kim et al. 2011).

– Neutralization–bitumination

Mainly used by Russia, neutralization–bitumination is a two-stage method that allows for the destruction of G-nerve agents. CWNAs are first mixed with 80 % monoethanolamine for 1 h at 110 °C (neutralization), followed by a reaction with bitumen and calcium hydroxide for 1 h at 200 °C (bitumination). Russian VX (RVX) is disposed of by a reaction with potassium isobutylate dissolved in isobutanol and *N*-methylpyrrolidinone. This solution is heated at 95 °C for 30 min before being added in bitumen (Pearson and Magee 2002).

Emerging chemical and physical alternatives for OPs decontamination

Cyclodextrins (CDs)

CDs are cyclic oligosaccharides composed of six to eight D-glucopyranoside units. They form a hydrophobic cavity able to host hydrophobic and apolar molecules (Sambrook and Notman 2013). This biomimetic catalysis capacity allows CDs to scavenge OPs.

Native CDs (α-CD, β-CD, and γ-CD) were first demonstrated to inactivate CWNAs. Among these, β-CD was shown to be the most efficient (Désiré and Saint-André 1986; Désiré and Saint-André 1987). Then, starting from β-CD as a precursor, substituted CDs such as iodosobenzoate-β-CD (IBA-β-CD) were developed and showed the ability to hydrolyze pesticides and CWNAs (Hoskin et al. 1999). The importance of the presence of an α-nucleophile such as IBA was underlined (Masurier et al. 2005; Wille et al. 2009; Müller et al. 2011; Rougier et al. 2011). Other derivatives were synthesized such as O-benzyl-β-CD, fully methylated β and α-CD (TRIMEB and TRIMEA, respectively), partially methylated β-CD (DIMEB), depicting a better solubility in water and organic solvents. CDs are promising compounds which could be used for skin, mucosa, wound, and also for material decontamination (Letort et al. 2015). Notwithstanding these benefits, some limitations have to be pointed out that circumvent the use of CDs, as a high-molecular weight (1, 134 $g.mol^{-1}$ for β-CD) and a low turnover. Additionally, none of the current CDs are able to degrade VX and possibly any OP containing a P–S bond due to the remarkable stability of such a linkage (Wille et al. 2009; Kalakuntla et al. 2013). Furthermore, *p*-nitrophenol (degradation product of paraoxon) inhibits the degradation process efficiency of paraoxon by β-CD (Masurier et al. 2005; Estour et al. 2013). Presumably, other OPs degradation products could lead to the same effect. Moreover, CDs rate for P–F degradation is very low (Hoskin et al. 1999).

Photochemical methods

Photochemical methods are based on light radiation to degrade OPs and organic substances in general. These methodologies have been well-reviewed elsewhere by Reddy and Kim (2015). Photochemical methods are part of advanced oxidation processes (AOPs), which use the high reactivity of HO radicals to degrade pollutants (Hossain et al. 2013). Briefly, photochemical-based techniques include five classes used for OPs degradation in soil, water, or air:

– Photolysis

Photolytic degradation of targeted compound consists in the absorption of radiation in both direct and indirect ways, leading to the destruction of the compound. Several examples of OP photolysis (diazinon, methylparathion, or quinalphos) have been reported to date in both direct (Wan et al. 1994; Sinderhauf and Schwack 2003; Gonçalves et al. 2006; Segal-Rosenheimer and Dubowski 2010; Muñoz et al. 2011) and indirect manner (Lam et al. 2003; Garbin et al. 2007).

– Photolysis with an oxidant

The association of classical photolysis with chemical oxidants like H_2O_2 and O_3 enhances the efficiency of the process and seems to prevent formation of unfavorable products (Wu and Linden 2010).

– Photo-Fenton method

This method involves photo-Fenton process, relying on the photoreduction of dissolved Fe(III) complexes into Fe(II) ions, followed by the Fenton reaction and the oxidation of organic compounds (Kim and Vogelpohl 1998; Ikehata and El-Din 2006).

– Photocatalysis

It refers to the photo-excitation of a semiconductor such as titanium dioxide (TiO_2), zinc oxide (ZnO), or tungsten trioxide (WO_3). Semiconductors are intermediates to reduction or oxidation of the OPs. This technique shows certain limits like electronic recombination (Balkaya 1999; Konstantinou et al. 2001; Jonidi-Jafari et al. 2004; Li et al. 2005; Evgenidou et al. 2006; Chen et al. 2011).

– Photosensitized-induced process

This process uses sensitizers able to absorb light radiation, then transfering the excess energy to a targeted compound. This technique is useful for OPs with a low absorption efficiency (Kamiya and Kameyama 2001; Nowakowska et al. 2005).

Photochemical methods have been mainly used for water decontamination (Reddy and Kim 2015). A few studies on air and solid surface decontamination of CWNAs have been reported to date (Zuo et al. 2007; Kim et al. 2007). Photochemical methods are easy to handle and rather inexpensive. However, the efficiency of those methods depends on numerous variables such as effluent flow rate, types, and concentrations of contaminants (like inorganic ions).

Others

Other techniques for OP decontamination exist. These methods remain marginal and would require further development. Hereafter is a non-exhaustive list of these techniques.

– Metal–Organic Framework (MOF)

MOFs are crystalline materials, composed of metal ions or clusters linked together by polydentate organic linkers. Based on metal nodes, MOF could be tailored to work as an artificial enzyme (Lee et al. 2009). For example, it could be used for CWNAs degradation through hydrolysis by adsorbed water using Cu-BTC (HKUST-1, $Cu_3(C_9H_3O_6)_2$) or NU-1000 ($Zr_6(\mu_3\text{-}O)_4(\mu_3\text{-}OH)_4(H_2O)_4(OH)_4$) (DeCoste and Peterson 2014; Mondloch et al. 2015).

– Degradation by non-thermal plasma (NTP)

NTP (also called dielectric barrier discharge plasma) is able to destroy a broad spectrum of OPs as well as other chemicals and biological pathogens (Kim et al. 2007). When OPs are exposed to plasma, their chemical bonds are effectively broken; subsequently, generated products seem harmless (Bai et al. 2010). This technique is mainly used for wastewater decontamination (Hu et al. 2013).

– Gamma irradiation process

Gamma irradiation process is used for degradation of numerous pollutants. It is also part of AOPs such as photochemical methods. Gamma irradiation is used to degrade OPs such as chlorpyrifos, diazinon, or malathion in water. This technique is mainly used for water decontamination (Basfar et al. 2007; Mohamed et al. 2009; Hossain et al. 2013).

Organophosphate neutralizing enzymes

Organophosphate hydrolase (OPH)

OPHs belong to the aryltriphosphate dialkylphosphohydrolase (EC 3.1.8.1) and are part of the amidohydrolase superfamily (Schomburg and Stephan 1998; Seibert and Raushel 2005). They are encoded by the OP degradation (*opd*) genes and have been initially found in *Brevundimonas diminuta* MG (previously *Pseudomonas diminuta*) and *Sphingobium fuliginis* ATCC 27551 (previously *Flavobacterium sp.*; Sethunathan and Yoshida 1973; Serdar et al. 1982; Mulbry et al. 1986; Harper et al. 1988). Another enzyme, encoded by a gene closely related to *opd*, namely *opd*A, was isolated from *Agrobacterium radiobacter* P230 (Horne et al. 2002; Horne et al. 2003). Extensive efforts have been devoted to the characterization and engineering of these enzymes (Theriot and Grunden 2010; Wales and Reeves 2012). Relevant works are summarized hereafter.

– OPH from *Brevundimonas diminuta*

B. diminuta carrying the plasmid pCMS1 was found to hydrolyze the pesticide parathion (Serdar et al. 1982). From this plasmid, the gene *opd* coding for a 35-kDa phosphotriesterase was cloned and sequenced (McDaniel et al. 1988). This enzyme was purified and characterized against a wide panel of OP-based insecticides (Dumas et al. 1989). The catalytic efficiency (k_{cat}/K_M) against paraoxon was found to be particularly high: 4.0×10^7 M^{-1} s^{-1}. Efficacy against sarin and soman was further highlighted, albeit with lower k_{cat}/K_M values as compared to paraoxon, 8.0×10^4 M^{-1} s^{-1} and 9.3 x 10^3 M^{-1} s^{-1}, respectively (Dumas et al. 1990). The 3D-structure of the apoenzyme was shown to consist of an $(\beta/\alpha)_8$ fold (Benning et al. 1994). The holoenzyme reconstituted with cadmium and a complex with the substrate analog diethyl 4-methylbenzylphosphate were also obtained (Benning et al. 1995; Vanhooke et al. 1996). Protein engineering strategies were further considered to increase OPH activity (Iyer and Iken 2015). Variants at positions W131,

F132, and F306 with enhanced activity against diisopropylfluorophosphate (DFP) were obtained demonstrating for the first time that mutagenesis was relevant to increase the hydrolysis of P–F linkages (Watkins et al. 1997). Three hydrophobic binding pockets were identified in the structure and 14 residues forming these subsites were targeted for mutagenesis purposes (Chen-Goodspeed et al. 2001a). The role of G60 residue on stereoselectivity was emphasized as G60A substitution drastically altered R_P-enantiomers recognition leading to a 13000-fold greater k_{cat}/K_M in favor of methyl phenyl *p*-nitrophenyl phosphate S_P- compared to R_P-enantiomer. Substitution H257Y was then associated with a reversal of chiral specificity with an increase by up to 500-fold regarding R_P-enantiomers for a variant harboring 4 substitutions I106G/F132G/H257Y/S308G (Chen-Goodspeed et al. 2001b). DNA shuffling coupled with a bacterial cell surface display based screening, led to the isolation of variant 22A11 (A14T/A80V/K185R/H257Y/I274N) and a 25-fold increase in methylparathion hydrolysis (Cho et al. 2002). A similar strategy led to the variant B3561 (A14T/L17P/A80V/V116I/K185R/A203T/I274N/P342S) that exhibited a 725-fold increase in k_{cat}/K_M for chlorpyrifos and a 39-fold improvement in paraoxon hydrolysis (Cho et al. 2004). K185R and I274N substitutions were shown to increase overall hydrolysis rate by being involved in the formation of hydrogen bonds with surrounding residues leading to beneficial structural changes (Cho et al. 2006). Directed evolution of the phosphotriesterase from *B. diminuta* was also investigated and resulted in the selection of variant S5 (K185R/D208G/R319S) with a 20-fold enhancement in functional expression in *E. coli* (Roodveldt and Tawfik 2005). The degradation of V-type nerve agents was recently investigated through computationally focused library screening and two-site mutagenesis (Cherny et al. 2013; Bigley et al. 2013). The first study led to various improved variants, among these C23 (K77A/A80V/F132E/T173N/G208D/H254G/I274N) was particularly relevant with a k_{cat}/K_M value for the toxic S_p isomer of VX of 8.3 x 10^4 M^{-1} s^{-1} (Cherny et al. 2013). In the second study, 12 active-site residues were targeted and led to a 26-fold improvement for the hydrolysis of the VX analogue DEVX as compared to the wild-type protein and a 230-fold improvement for the racemic nerve agent VX with catalytic efficiency up to 7 x 10^4 M^{-1} s^{-1} (Bigley et al. 2013). Very recently, PTE was engineered into a functional trimer showing enhanced activity when displayed onto semiconductor quantumdot (Breger et al. 2015). Altogether, these results underline that phosphotriesterase from *B. diminuta* is a good candidate for catalytic decontamination of organophosphorus chemicals that can be enhanced with enzyme engineering strategies.

– OPH from *Sphingobium fuliginis*.

S. fuliginis (ATCC 27551) was isolated from paddy water and shown to degrade both diazinon and parathion (Sethunathan and Yoshida 1973). An enzymatic extract partially purified from *S. fuliginis* was further shown to possess a phosphotriesterase activity for the degradation of various OPs (Brown 1980). The production of parathion hydrolase in *S. fuliginis* strain ATCC 2751 was associated to a 43-kb plasmid harboring a gene closely related with the *opd* sequence from *B. diminuta* (Mulbry et al. 1986), the encoded proteins sharing 99 % sequence identity. The recombinant expression of OPH from *S. fuliginis* was investigated (Rowland et al. 1991). *Streptomyces lividans* was targeted for this purpose as it expresses the enzyme as a secreted soluble enzyme at a milligram scale (Steiert et al. 1989). The production yield was further optimized by investigating the effect of signal sequences (Rowland et al. 1992). Preparation of OP-decontaminating solutions were considered and the enzyme was found to be stable for long term storage after freeze-drying in the presence of trehalose and its activity was enhanced with 0.01% sodium carboxyl polyoxyethylene tridecylether (Sode and Nakamura 1997). The influence of cobalt and zinc on both activity and expression of the enzyme was evaluated using *opd-lacZ* fusions (Manavathi et al. 2005).

The capacity of the native enzyme to discriminate enantiomers was considered using various OPs and found to be limited (Ohuchi et al. 1997). Protein engineering was thus considered to enhance its efficiency (Iyer and Iken 2015). Variant H254G/H257W/L303T was able to catalyze the hydrolysis of a chromogenic analogue of the most toxic stereoisomer of soman nearly 3 orders of magnitude faster than the native enzyme (Hill et al. 2003). Further mutagenesis enhanced the hydrolysis of VX and pesticides (Gopal et al. 2000). Variant L136Y displayed a 33% increase in VX hydrolysis rate. Rate hydrolysis of mutants W131F and F132Y was enhanced by 4-fold and 5.8-fold with demeton-S methyl and DFP respectively. Further optimization lead to 25-fold increased in k_{cat}/K_M compared to the wild-type with demeton-S methyl for variant with nine mutations (A80V/I106V/F132D/K185R/D208G/H257W/I274N/S308L/R319S) and up to 18-fold increase against malathion for variant harboring 10 amino-acid substitutions (G60V/A80V/I106V/F132D/K185R/D208G/H257W/I274N/F306V/R319S) (Schofield and DiNovo 2010). Protein engineering was also considered for enhancing CWNAs degradation. Position 254 was proved to be crucial for VX hydrolysis (Nakayama et al. 2014), and catalytic efficiency of variant L271/Y309A was enhanced up to 150-fold compared to the wild-type enzyme (Jeong et al. 2014).

– OPH from *Agrobacterium radiobacter* (OpdA)

The bacterial strain *Agrobacterium radiobacter* P230 was found capable of hydrolyzing OP-based pesticides. The gene, namely *opdA*, involved in OP hydrolysis was thus cloned and sequenced and was found closely related to the *opd* gene from *Flavobacterium* sp. strain ATCC 27551 (Horne et al. 2002).

OpdA shares 91% identity with OPH from *Flavobacterium* sp. strain ATCC 27551. The structure of OpdA was solved and complexes with ethylene glycol and dimethyl thiophosphate revealed similarities between OpdA mechanism and other previously reported metallophosphoesterases (Jackson et al. 2005). This enzyme showed 10-fold enhancement compared to homologous phosphotriesterase from *Flavobacterium* sp. strain ATCC 27551 for the degradation of dimethyl organophosphate based insecticides. Substitutions Y257H and F272L were mainly responsible for this difference (Horne et al. 2006).

Protein engineering was used to enhance the degradation of OP-based pesticides. Rational design enabled to identify two successive residues, namely F131 and W132, involved in stereospecificity. Variant W131H/F132A displayed 480-fold and 8-fold enhancement in catalytic efficiency for stereoisomers *Z* and *E* of chlorfenvinphos respectively (Jackson et al. 2009). Combinatorial active site mutagenesis was also applied to OpdA and led to the selection of variant S308L/Y309A resulting in a 5000-fold increase in k_{cat}/K_M for the hydrolysis of malathion (Naqvi et al. 2014).

The potential of OpdA for degrading CWNAs was also considered. The hydrolysis of both G- and V-type nerve agents by OpdA was investigated. The enzyme hydrolyzed cyclosarin and soman with catalytic efficiencies of 3.9 x 10^2 M^{-1} s^{-1} and 5.8 x 10^2 M^{-1} s^{-1}, respectively. The activities towards V-agents were lower by two orders of magnitude when compared to agents from G-series. Regarding VX, a k_{cat}/K_M value of 4.5 M^{-1} s^{-1} was measured (Wille et al. 2012). Three OpdA variants were investigated and shown k_{cat}/K_M values up to 1.7 x 10^5 M^{-1} s^{-1} for tabun. Conversely, soman was poorly hydrolyzed (Dawson et al. 2008).

– Examples of applications of OPH for OP decontamination

Insofar as OPH are efficient biocatalysts for OP hydrolysis, they have been considered for decontamination purposes. In order to promote the use of these enzymes and to address their stability limitations, immobilization strategies were envisaged. Trityl agarose was used for the immobilization of a phosphotriesterase in a fixed bed reactor maintaining its ability to hydrolyze organophosphate pesticides (Caldwell and Raushel 1991a). The thermostability of the immobilized enzyme was moreover enhanced compared to the free form with a 6-fold increase in half-life at 55°C. A process for the immobilization onto nylon was also described (Caldwell and Raushel 1991b). The K_M was increased by 5-6 times compared to the soluble enzyme but reduced the maximum rate against paraoxon to less than 10%. Long term storage was also enhanced for the immobilized enzyme. The enzyme was immobilized into a decontaminating sponge (Havens and Rase 1993), and covalently linked within polyurethane foams (LeJeune and Russell 1996). The stability of the enzyme was tremendously enhanced and enzymatic foams became attractive for large scale nerve agents decontamination (LeJeune et al. 1997; LeJeune et al. 1998; LeJeune and Russell 1999). However, limitations of the technology were due to insufficient activity of OPH for the most toxic organophosphorus chemicals (Donarski et al. 1989). Surface-expression coupled with immobilization was further considered to develop low-cost decontamination systems (Richins et al. 1997; Chen et al. 2000; Richins et al. 2000; Cho et al. 2002; Mansee et al. 2005). Recently, covalent immobilization of OpdA onto polyester textiles was reported offering various perspectives for environmental decontamination (Gao et al. 2014). Other examples of OPH immobilization on amyloid fibril nanoscaffold or amphiphilic block copolymer were also described (Raynes et al. 2011; Suthiwangcharoen and Nagarajan 2014) as well as an original approach consisting in the immobilization of OPH onto carbon nanotube paper (Mechrez et al. 2014).

DFPase from *Loligo vulgaris*

A special attention was raised to the Diisopropylfluorophosphate fluorohydrolase (DFPase, EC 3.1.8.2) from *Loligo vulgaris*. This squid-type enzyme was initally found to efficiently hydrolyze DFP and tabun (Hoskin 1971; Hoskin and Roush 1982). Its catalytic mechanism was considered and the 3D-structure was solved and described as a 6-fold β propeller (Scharff et al. 2001a; Scharff et al. 2001b). Kinetics studies and site-directed mutagenesis were performed to decipher the reaction mechanism of the enzyme and identify the essential residues in the active site (Hartleib and Rüterjans 2001a; Scharff et al. 2001a; Scharff et al. 2001b; Katsemi et al. 2005). Residue H287 was shown to act as a general base catalyst whereas two other histidine residues, namely H181 and H274, appeared to have a stabilizing effect. Roles of the two high-affinity Ca^{2+}-binding sites were also described as being strongly involved in the overall stabilization of the DFPase structure and probably in the catalysis (Hartleib et al. 2001). The heterelogous production of the enzyme was considered using an *E. coli* expression system and yielded to large amounts of active and soluble protein that could be further purified using an His-tag and appeared to be stable for 1 year at 4 °C (Hartleib and Rüterjans 2001b).

Organophosphate acid anhydrolase (OPAA) and related prolidases

A moderately halophilic bacterial isolate identified as a species of *Alteromonas* was found able to degrade several OPs (DeFrank and Cheng 1991). From this extract, the main enzyme, OPAA-2, was purified to homogeneity and found to be a 60-kDa polypeptide. This enzyme was shown to hydrolyze DFP ($k_{cat}/K_M = 7.7$ x 10^4 M^{-1} s^{-1}) as well as phosphofluoridate nerve agents including sarin ($k_{cat}/K_M = 2.8$ x 10^5 M^{-1} s^{-1}), soman ($k_{cat}/K_M = 6.1 \times 10^4$ M^{-1} s^{-1})

or cyclosarin ($k_{cat}/K_M = 9.6 \times 10^5$ M^{-1} s^{-1}) and was poorly active with paraoxon (Cheng et al. 1996; Theriot and Grunden 2010). A special attention was thus paid to *Alteromonas* strains that were screened for their OP-hydrolyzing activity (DeFrank et al. 1993; Cheng et al. 1993). Many genes encoding OPAAs were reported and the enzymes were shown to be closely related to prolidases (EC 3.4.13.9). Their promiscuous ability to hydrolyze dipeptides with C-terminal proline (Xaa-Pro) was then underlined (Cheng et al. 1996; Cheng et al. 1997). Nevertheless, OPAAs are particularly efficient for hydrolyzing OP compounds and are also able to degrade G-type CWNAs. Their large-scale production has moreover been demonstrated (Cheng et al. 1998; Cheng et al. 1999; Cheng and Defrank 2000). Both substrate and stereochemical specificity of OPAA from *Alteromonas* sp. JDS6.5 were analyzed and a preference for S_p enantiomers was demonstrated (Hill et al. 2000). The X-ray structure of the enzyme revealed two distinct domains, N- and C-terminus. The C-terminus domain contains Mn^{2+} divalent cations and displayed a "pita-bread" fold (Vyas et al. 2010). This work highlighted relationships between prolidases and OPAAs that were completed by the 3D structure of OPAA from the marine bacterium *Alteromonas macleodii* (Štěpánková et al. 2013). Prolidases from hyperthermophilic sources were also attractive and *Pyrococcus* sp. strains were investigated. Enzymes from *Pyrococcus furiosus* or *Pyrococcus horikoshii* (Maher et al. 2004; Theriot et al. 2009; Theriot et al. 2010), appeared to be good candidates for the development of decontamination solutions and were further considered for random mutagenesis investigations. Improved variants against nerve agents and Xaa-Pro dipeptides over a broad range of temperature were described (Theriot et al. 2010; Theriot et al. 2011). The dual activity prolidase/OPAA was also observed with prolidase from human origin (Wang et al. 2004; Wang et al. 2006; diTargiani et al. 2010; Costante et al. 2012). This is particularly interesting as human enzymes are really appealing for the development of catalytic bioscavengers.

Paraoxonase

Another class of enzyme from human origin, namely paraoxonase (PON, EC 3.1.8.1.), has found considerable interest. Since the earliest report describing the ability of a mammalian serum esterase to degrade paraoxon (Aldridge 1953) and nerve agents (Broomfield and Ford 1991; Davies et al. 1996), numerous data were collected regarding the potential of PON for OP bioscavenging (Rochu et al. 2007). PON is capable of hydrolyzing a wide spectrum of OP pesticides (Mackness et al. 1991; Furlong et al. 1991; Li et al. 1995). PON from rabbit and human serum were purified to homogeneity and were shown to be similar, though differing in the amino terminus sequence (Gan et al. 1991; Furlong et al. 1991). However, human PON remains difficult to purify from plasma or heterologously express in high yield. Baculovirus and adenovirus infections of *Trichoplusia ni* larvae allowed to produce large quantities of functional recombinant HuPON1 (Otto et al. 2010; Hodgins et al. 2013). Enzyme engineering was deeply investigated to generate improved variants. Site-directed mutagenesis and group-selective labeling modifications were applied to decipher the role of molecular determinants of catalysis (Josse et al. 1999a; Josse et al. 1999b). Supplementary essential residues were also identified with a DFPase-like homology model (Yeung et al. 2004). Complete description of PON1's active site was achieved by solving the 3D structure of a recombinant PON1 variant (rePON1-G2E6) expressed in *E. coli* and obtained through directed evolution (Harel et al. 2004; Aharoni et al. 2004). Mammalian paraoxonase PON1 was found to be more efficient for the detoxification of soman and cyclosarin than OPH from *P. diminuta* and DFPase from *Lolio vulgaris*. Variants obtained from PON1 were further improved by about 10-fold against cyclosarin and soman as well as DFP, parathiol, or chlorpyrifios-oxon with up to 380-fold increase (Amitai et al. 2006). An engineered recombinant human PON1 ($rHuPON1_{K192}$) was also generated and highly purified. The stereospecificity of both recombinant and wild-type PON1 were evaluated using fluorogenic surrogates of nerve agents and were shown to be in favor of R_p isomers (Ashani et al. 2010). Directed evolution experiments were, thus, utilized to increase the efficiency of PON1 towards G-agents (Gupta et al. 2011). Rational and random mutagenesis were combined using a high-throughput methodology based on the utilization of fluorogenic analogs in emulsion compartments and a low-throughput plate screening. The activity against the coumarin analog of S_p cyclosarin was enhanced by $\approx 10^5$-fold for variant 4E9 (L69G/S111T/H115W/H134R/F222S/T332) as compared to the wild-type like variant rePON1-G3C9 reaching catalytic efficiency $>10^5$ M^{-1} s^{-1}. Another work led to the isolation of significantly improved variants for degrading G-Agents (Goldsmith et al. 2012). Among these, variant VIID2 (L55I/L69V/H115A/H134R/H197/R/F222M/I291L/T332S) was of special interest. Although significant enhancements for the decontamination of G-agents have been achieved, the hydrolysis of V-type agents still remains critical. Computational experiments were applied to understand the way PONs hydrolyze VX and drew new perspectives for their engineering (Peterson et al. 2011). Double-substituted variants of human PON1 (H115W/Y71A and H115W/F347W) were constructed and showed up to 2-fold increase in catalytic efficiency as compared to the wild-type enzyme (Kirby et al. 2013). Noteworthy, PON were also deeply studied for evolutionary biology considerations. Original strategies including back-to-ancestor/consensus mutations or neutral-genetic drift were employed to evolve PON in non-conventional manners (Amitai et al. 2007; Khersonsky et al. 2009; Alcolombri et al. 2011; Bar-Rogovsky et al. 2013). Although rarely investigated, PON3 paraoxonase was also purified (Draganov et al.

2000; Reddy et al. 2001) and considered for directed evolution purposes. The catalytic efficiency of recombinant variants of PON3 against paraoxon was enhanced by up to 240-fold (Aharoni et al. 2004). Both activity and stability of PON3 were shown to be tunable through combination of family shuffling and phylogeny-based mutagenesis (Khersonsky et al. 2009). Of note, another enzyme, namely, the Senescence marker protein 30 (SMP30), has been showed to display a 3D structure closely related to PON1, although containing a single metal-binding site. SMP30 is a lactonase able to hydrolyze various aldolactones and is also promiscuous towards OPs sush as DFP (Kondo et al. 2004; Scott and Bahnson 2011).

Methylparathion hydrolase

Methylparathion hydrolase (MPH, EC 3.1.8.1) is an aryldialkylphosphatase belonging to metallo-β-lactamase family. Gene *mpd* coding for MPH was first identified in *Plesiomonas* sp. strain M6 (Zhongli et al. 2001). The gene *mpd* was then sequenced, effectively expressed in *E. coli* and further purified as a monomer (Fu et al. 2004). Another closely related gene was identified in *Pseudomonas* sp. strain WBC-3 (Liu et al. 2005). The encoded enzyme was purified and crystallized and the 3D structure revealed a dimeric enzyme each subunit containing a mixed hybrid binuclear zinc center in which the more solvent-exposed β-metal cation was replaced by cadmium (Dong et al. 2005). Interestingly, MPH was shown to be homologous to other metallo-β-lactamases but did not display any similarity with phosphotriesterases. Recombinant MPH expressed in *E. coli* was characterized and was shown to be active on methylparathion as well as other OP pesticides including malathion or dichlorvos (Yang et al. 2008). Another MPH, dubbed OPHC2, was identified in *Pseudomonas pseudoalcaligenes* strain C2-1 (Ningfeng et al. 2004) and *Stenotrophomonas* sp. strain SMSP-1 (Shen et al. 2010). The former was characterized and its 3D structure was solved (Gotthard et al. 2013a; Gotthard et al. 2013b). Recently, an OPHC2 analogue, namely *Po*OPH from *Pseudomonas oleovorans*, was discovered (Luo et al. 2014). This enzyme displays lactonase and arylesterase activities as well as a promiscuous OPH activity. Variant $PoOPH_{M2}$ (H250I/I263W) was designed and found improved by 6,962- and 106-fold against methylparathion and ethylparaoxon, respectively. Random mutagenesis of MPH from *Pseudomonas stutzeri* further led to a 5-fold increase in the hydrolysis of chlorpyrifos (Xie et al. 2014). Additional *mpd* genes harbored by seven strains isolated from methylparathion contaminated sites were subcloned and expressed in *E. coli* (Zhang et al. 2006). Phylogenetic analysis supported by GC-content comparisons between *mpd* genes and respective host's chromosome suggested that horizontal gene transfer may have occurred, playing an important role for bacterial adaptation to methylparathion contamination.

Phosphotriesterase-Like Lactonase

Among the enzymes able to degrade OPs, phosphotriesterase-like lactonases (PLLs) constitute a promising protein family closely related to bacterial phosphotriesterases (Afriat et al. 2006; Draganov 2010). PLLs are natural lactonases catalyzing the hydrolysis of *N*-acyl-homoserine lactones (AHLs) involved in the *quorum* sensing system of bacterial species, and additionally, exhibit a promiscuous OPH activity. Regarding both the predominant metabolic role of lactones in bacterial communication and the relatively recent occurrence of OPs, widely used as pesticides during the last decades, it has been assumed that the phosphotriesterase activity of PLL has emerged from a lactonase template harboring a promiscuous activity for OP hydrolysis. Interestingly, modern phosphotriesterase have probably evolved from a lactonase ancestor that lost its capacity to hydrolyze AHL to the benefit of higher OP-degradation rates. Natural evolution has led to the selection of enzymes with phosphotriesterase activity close to catalytic perfection to the detriment of the natural lactonase activity. Afriat-Jurnou and coworkers have, for example, demonstrated that ancestral reconstruction, starting from OPH from *B. diminuta* restore its lactonase catalytic ability, providing an experimental evidence of the evolutionary linkage between these enzymatic activities (Afriat-Jurnou et al. 2012). PLLs have been isolated from various hosts including hyperthermophile bacteria (Xiang et al. 2009; Zhang et al. 2012), or archaea (Merone et al. 2005; Hiblot et al. 2012a; Gotthard et al. 2013b; Bzdrenga et al. 2014; Kallnik et al. 2014). These enzymes display remarkable stability to temperature, pH, detergent, solvent, or storage, offering good perspectives for utilizations in external decontamination of OPs. The enzyme *Sso*Pox from the archeon *Sulfolobus solfataricus* is particularly interesting and is one of the most promising PLL for OP countermeasures. Major results and perspectives are detailed hereafter.

*Sso*Pox: a highly stable, OP-degrading capable enzyme

An archaeon-sourced enzyme with tremendous stability

The archea *Sulfolobus solfataricus* was discovered within the Vesuvio's sulfatare and found to possess a gene homologous to the *B. diminuta* PTE. The encoded enzyme was expressed and its phosphotriesterase ability, albeit low, was highlighted. Among the hydrolyzed substrates was paraoxon, hence, was it called *Sso*Pox: *Sulfolobus solftataricus* Paraoxonase (Merone et al. 2005). It was also found to efficiently hydrolyze various lactones, including *N*-acyl homoserine lactones, known to be involved in bacterial communication (*quorum* sensing). Actually, the lactonase activity of *Sso*Pox was found to be two orders of magnitude higher than its phosphotriesterase activity, underlining that it is most probably a natural

lactonase, whose capacity to hydrolyze OPs chemicals stemmed from a promiscuous enzymatic template.

Due to its hypertermophilic origin the enzyme exhibits an incredible stability to temperature, being active from 10 to 100 °C, with an impressive denaturation temperature of 106 °C as well as a tremendous resistance to the denaturing effect of urea. Furthermore, *Sso*Pox was active from pH 5.0 to pH 9.0 confirming the unusual properties of this enzyme. *Sso*Pox was crystallized and its 3D structure was solved in both *apo* and complex forms (Elias et al. 2007; Elias et al. 2008). The structure was reported as a distorted $(\beta/\alpha)_8$ barrel-fold and was found to be close to mesophilic OPH from both *B. diminuta* and *A. radiobacter*.

The major structural discrepancies between *Sso*Pox and OPHs consist in the shortening of loop 7 as well as both extremities of the polypeptide chain. The arrangement of loop 8 is also variable between the structures and extra loops are observed within *Sso*Pox structure (Fig. 2). These conformational changes are probably involved in the overall enzyme stability by either rigidifying the structure or favoring dimer formation. An investigation of the structural determinants of *Sso*Pox as compared to its mesophilic counterparts was also performed (Vecchio et al. 2009). Surface salt bridge network and a tight quaternary structure were shown to be involved in complex networks and optimization of dimer subunit interfaces, respectively. From this observation came the idea to benefit from the outstanding structural stability of this enzyme, in order to transfer the efficient *B. diminuta* PTE active site within *Sso*Pox scaffold.

Engineering *Sso*Pox to enhance promiscuous phosphotriesterase activity

Thus, protein engineering strategies have been considered in order to increase its potential for bioremediation. Indeed, protein stability has been proved to promote evolvability by minimizing the effect of destabilizing mutations and buffering their deleterious damages (Tokuriki and Tawfik 2009). Hyperthermophile scaffolds are usually good candidates for evolutionary considerations as their tremendous stability may confer an enhanced tolerance to mutation-induced stability changes. Protein polymorphism may thus be generated without drastic effect on protein fitness. Moreover, substrate promiscuity has been identified as a potential origin of functional divergences that could be used as starting point in evolutionary trajectories (Khersonsky et al. 2006).

The rationale was that mesophilic phosphotriesterases probably emerged from PLLs, so their respective sequences and structures were compared and led to the identification of residues probably involved in the enhancement of OP-degradation. Mutational databases listing potential beneficial substitutions for phosphotriesterase activity were designed (Chabriere et al. 2014; Chabriere et al. 2015). These databases combined with *in silico* analyses were used as starting point for engineering

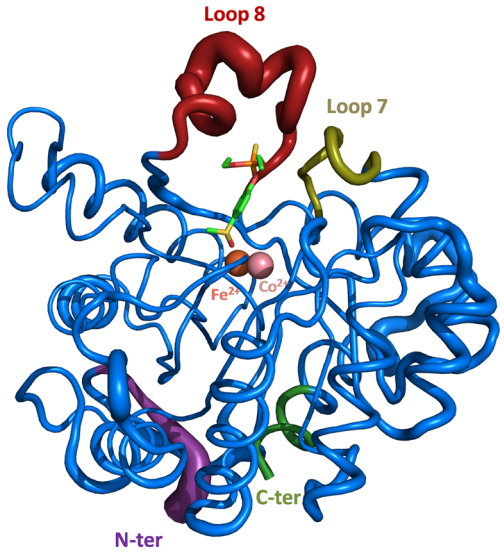

Fig. 2 Overall 3D structure of *Sso*Pox in complex with fensulfothion (PDB ID: 3uf9). Protein backbone is showed as *cartoon*; N- and C-ter are highlighted in *purple* and *green*, respectively. Loop-7 and loop-8 that govern substrate promiscuity are emphasized in *yellow* and *red*. Divalent active-site cations are shown as *spheres*

*Sso*Pox toward OP based pesticide degradation (Fig. 3). Variants with up to 2000-fold increased activity against certain pesticides and enlarged substrate scope were obtained (unpublished results). Mutations R223H and Y97W were first shown to impact phosphotriesterase activity (Elias et al. 2008). Residue W263 was also deeply investigated (Hiblot et al. 2012b). This residue is located in the active site and was shown to be involved in enzyme conformational flexibility, mediating substrate promiscuity. Saturation mutagenesis was performed at this position using NNS-degenerated primers. Two subsets of mutations were distinguished as regard to either their lactonase or phosphotriesterase activity. Within this latter, variants W263F, W263L and W263M were particularly attractive. Catalytic parameters of *Sso*Pox variants towards OPs are reported in Table 1. *Sso*Pox and its variants were shown to be active on a wide range of OPs including pesticides (*e.g.* paraoxon, parathion, malathion) and chemical warfare agent analogs (IMP-, PinP-, and CMP-coumarin). The stimulation of *Sso*Pox activity by anionic detergent at ambient temperature was also demonstrated and is of prime interest for external decontamination purpose as it could help spreading the enzyme on soiled surfaces (Hiblot et al. 2012b). Future engineering experiments on *Sso*Pox are in progress in order to provide a stable, proficient catalyst capable of detoxifying a broad spectrum of toxic OPs.

Concluding remarks

Decontamination methods against OP poisoning have been exhaustively studied during the past decades. Efficient physico-chemical methods including hypochlorite and

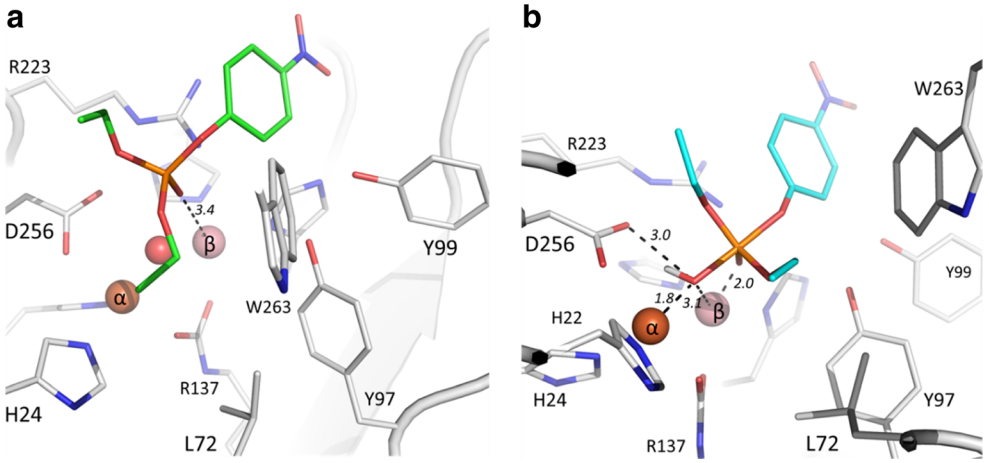

Fig. 3 Molecular docking of paraoxon to *Sso*Pox structure. The docking of paraoxon (ground state, GS; green sticks; **a**) and of its corresponding putative oxyanionic pentavalent hydrolytic transition state (TS; cyan sticks; **b**) was performed in *Sso*Pox (PDB ID: 2vc5). Metal cations and active-site water molecules (in *red*) are shown as *spheres*. Distances are indicated in Ångstroms. Briefly, the molecular docking was performed using torsion and charge parameters generated using ADT 4.2 (http://autodock.scripps.edu/resources/adt) and the Gasteiger method. A single-negative charge was attributed to the oxyanion atom of the pentavalent paraoxon hydrolysis intermediate. Hydrogen atom positions and the atomic partial charges (Gasteiger method) of the receptor protein models were added using ADT 4.2. A +2 charge was attributed to the two-cation ions that comprise the active site of *Sso*Pox. The docking simulations were performed using Autodock 4.0 (Morris et al. 1998; Huey et al. 2007) and the Lamarckian genetic algorithm. Forty runs of genetic algorithm were performed for each couple ligand-receptor pair using the default parameters. The output ligand configurations were clustered and selected by their binding energy scores and their chemical relevance

Table 1 Catalytic parameters of SsoPox wild-type and variants towards organophosphate pesticides and nerve agents with respective reactions conditions (Elias et al. 2008; Hiblot et al. 2012b; Hiblot et al. 2013)

Substrate	Enzyme	Condition	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1}s^{-1}$)
Ethylparaoxon	WT	70 °C	3.98	3,270	1.22×10^3
		25 °C	12.59	24,250	5.19×10^2
		SDS 0.1 %, 25 °C	40.72	12,340	3.30×10^3
		SDS 0.01%, 25 °C	24.59	3,830	6.42×10^3
		DOC 0.1 %, 25 °C	6.30	570	1.10×10^4
		DOC 0.05 %, 25 °C	4.72	270	1.22×10^3
		DOC 0.01 %, 25 °C	10.51	730	1.72×10^4
	W263F	25 °C	8.47	700	1.21×10^4
		SDS 0.1 %, 25 °C	117.70	2,462	4.78×10^4
		SDS 0.01 %, 25 °C	85.85	1,168	7.35×10^4
	W263M	25 °C	6.82	931	7.33×10^3
	W263L	25 °C	ND	ND	2.37×10^3
	W263I	25 °C	ND	ND	1.21×10^3
	W263V	25 °C	ND	ND	8.83×10^3
	W263T	25 °C	ND	ND	1.06×10^3
Methylparaoxon	WT	25 °C	2.71	2,142	1.27×10^3
Methylparathion	WT	25 °C	1.1×10^{-3}	121	9.09
Malathion	WT	25 °C	8.9×10^{-4}	160	5.56
CMP-coumarin	WT	25 °C	ND	ND	8.13×10^3
	WT	SDS 0.01 %, 25 °C	25.47	137	1.86×10^5
	W263F	25 °C	9.41	114	8.23×10^4
	W263F	SDS 0.01 %, 25 °C	8.64	60	1.44×10^5
IMP-coumarin	WT	25 °C	ND	ND	1.67×10^3
	W263F	25 °C	8.39	95	8.85×10^4
PinP-coumarin	W263F	25 °C	0.11	16	7.08×10^3

sodium hydroxide have emerged for the efficient external decontamination of OPs or stockpile elimination and have already been used for this purpose. These methods usually involve harsh chemical conditions and are not compatible with the decontamination of personnel or sensitive materials. Other methods were developed such as RSDL or Fuller's earth. Whereas RSDL is efficient at decontaminating the skin, it is not compatible with eye and wound cleaning and would be hardly adaptable to a large number of contaminated people. Fuller's earth shows good topical absorbance of contaminants but do not degrade OPs, possibly resulting in large amounts of secondary pollution. Moreover, toxic dusts are generated during the decontamination process, complicating the use of the method at a large scale. Alternatively, photochemical methods are under development for the mineralization of OP chemical in environmental media but would not be relevant for people decontamination. Methods that can be used for both material and personel are under intensive investigations. CDs were proved to be efficient bioscavengers but because of their stoichiometric action, they may not be cost-effective in a case of large scale pollution. Investigations also focus on another promising solution, the use of OP-degrading enzymes. Indeed, the use of enzymes is appealing because they offer a non-corrosive, safe and catalytic way for decontaminating OPs. Protein engineering has been deeply applied for increasing the catalytic efficiencies of recombinant enzymes. Whereas the stability and the production costs of enzymes may be an issue, the discovery of highly thermostable OPs-degrading enzymes such as *Sso*Pox, and their engineering for higher activity is expected to turn them into competitive and economically-attractive OP-biodecontaminants.

Acknowledgments P.J. and J.B. are Ph.D. students granted by Direction Générale de l'Armement (DGA).

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