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Review

Organophosphate-degrading metallohydrolases: Structure and function of potent catalysts for applications in bioremediation

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ABSTRACT

Organophosphate compounds (OPs) have been employed in the agricultural industry as pesticides and insecticides for several decades. Many of the methods used currently for the detoxification of OPs are harmful and possess serious environmental consequences. Therefore, utilizing enzymes for the detection and decontamination of OPs is gaining increasing attention as an efficient and clean bioremediation strategy. Microbial enzymes, such as OP hydrolases, OP acid anhydrolases or methyl parathion hydrolase (MPH), are potent agents for OP decontamination. Their biochemical properties and biotechnological applications are discussed in this review, including a discussion on methods that may be employed to immobilize such enzymes, and essential steps to generate reusable and affordable biocatalytic systems for use in bioremediation and biorestoration.

1. Introduction

Organophosphate[2] pesticides (OPs) are important in agriculture, accounting for approximately 40% of the total global pesticide usage (Fig. 1) [1–3]. OPs are also produced as chemical warfare agents (CWA); 200,000 tons of these agents are thought to be

Abbreviations: BChE, butyrylcholinesterase; CB, carbon black; CBD, cellulose-binding domain; CNT, carbon nanotube; DFP, diisopropylfluorophosphate; GpdQ, glycerophosphodiesterase; HFB, hollow fiber bioreactor; MBL, metallo-β-lactamase; MPH, methyl parathion hydrolase; OP, organophosphate; OPAA, OP acid anhydrolase; OPH, OP-degrading hydrolase; PEG, polyethylene glycol; PNP, p-nitrophenol; PON, paraoxonase; PTE, phosphotriesterase; QD, quantum dots.

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[2] The term organophosphate is often used to denote a class of pesticides or nerve agents that are triesters of phosphoric, phosphonic and phosphinic acid and sulfur-containing compounds. While there are formal nomenclature conventions for these compounds they are usually referred to by their common names in the literature and this convention is followed here.

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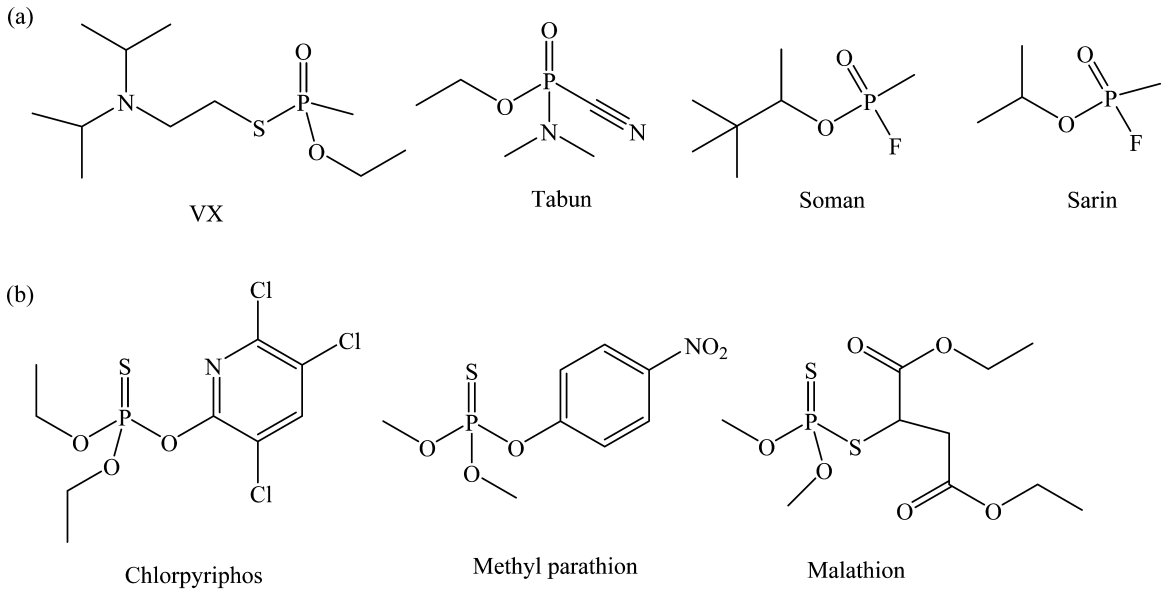

Fig. 1. Structures of OPs representing nerve agents and pesticides.

stored throughout the world [4,5]. These compounds are generally toxic, primarily due to their ability to irreversibly inhibit acetylcholinesterase [6]. OPs are partially water soluble and can easily enter the soil and ground water and, being mutagenic, they have been linked to a number of nervous and immune system-related diseases [7–23]. In both developing and developed countries OPs are also a common cause of poisoning by occupational exposure or deliberate self-harm and environmental contamination [1,2,8–16,24–39].

Disposing of OPs can be problematic. Stockpiles dumped in landfills can leach into the surrounding soil while chemical degradation can result in the production of toxic by-products [40]. Incineration of OPs is an approved but costly process that can also result in toxic emissions [41–43]. An efficient "green and clean" approach to degrade OPs is thus much desired, and enzymes expressed in some soil-dwelling microorganisms may be an ideal tool for this purpose. The history of OP-degrading enzymes dates back to 1946 when hydrolysis of diisopropylfluorophosphate (DFP) was observed in mammalian tissue extracts [44]. In 1973 it was discovered that the soil bacterium *Flavobacterium sp.* (strain ATCC 27551) is able to thrive in an environment where the OP diazinon is the sole carbon source [45]. Several other microorganisms, including *Agrobacterium radiobacter*, *Enterobacter aerogenes* and *Pseudomonas diminuta* have since been shown to possess the enzymatic machinery to degrade OPs [43,46–48]. Common to these organisms is that they have evolved an enzymatic activity that is very effective in hydrolyzing the OP triesters into di- and monoesters, and ultimately phosphate; the products of these hydrolytic reactions are generally far less harmful than their parent OP compound.

In this review several OP-hydrolyzing enzymes will be discussed with a view to describing their general physicochemical features and how they can be utilized in the context of bioremediation, *i.e.* the degradation of harmful OP compounds into less or non-toxic products. We realize that we have been selective in our focus and apologize in advance to authors whose significant contributions to the field may not have been covered in detail. For instance, insects are one of the main targets of OP pesticides and it is thus not surprising that they have developed some resistance, involving in particular a family of esterases [49]. However, since these enzymes do not require metal ions for their function they will not be discussed further here. Also, we like to stress that the purpose of the article is to describe enzymatic systems in context of their potential for applications in bioremediation, not their mechanistic details (which are outlined in a series of primary literature as indicated in relevant sections).

2. Paraoxonase (PON) and OP acid anhydrolase (OPAA)

In 1946 Mazur, studying rabbit tissue samples, discovered the first example of an enzyme capable of degrading an OP, DFP [44]. Due to its ability to hydrolyze the OP paraoxon (Fig. 1) this OP acid anhydrolase (OPAA) was given the name paraoxonase (PON) [50–52]. However, it was subsequently found that this enzyme is rather promiscuous acting on a broad spectrum of diverse substrates, including a range of OPs and lactones [53,54]. While this broad substrate range complicates the assessment of the precise biological function(s) of PON, there is strong evidence that links the enzyme to atherosclerosis [55–57].

Based on sequence homology PON enzymes are divided into three groups, PON1–PON3 [58]. PON1 is the best studied group in this family of enzymes and has been identified in a variety of higher organisms, including human, rabbit, bovine, dogs, cats and horses [59]. PON1 appears to be the only family member with efficient OP hydrolase activity [53]; the enzyme is likely to act as a lactonase *in vivo* [54] but is capable of degrading a variety of OPs including the oxon metabolites of parathion, diazinon and chlorpyriphos, as well as the nerve agents Sarin and Soman [52]. In directed evolution experiments it was demonstrated that the substrate preference of PON1 can easily be modified, underlining this enzyme's potential in the detoxification of OPs, in particular for victims suffering from poisoning [60]. The enzyme consists of about 350 amino acids. The crystal structure of rabbit PON1 was solved to 2.2 Å resolution (Fig. 2), showing a six-bladed β-propeller overall structure [60]. Importantly, the active site contains two Ca^{2+} ions, one with a structural role, the second required for catalysis [58]. In the proposed model for the reaction mechanism the substrate coordinates to the catalytic calcium ion (labeled Ca1) prior to a nucleophilic attack by a hydroxide ion that does not directly coordinate to this Ca^{2+} but is activated *via* hydrogen bonding interactions involving two conserved histidine residues in the active site (Fig. 2) [60].

OPAAs are frequently categorized in two classes, "squid-type" and "Mazur-type" [61,62]. The former prefers DFP to Soman as substrate and requires Ca^{2+} ions for activity [63]. The Ca^{2+}-dependent PON1 described in the previous section therefore classifies as a