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REVIEW ARTICLE

Insights in detection and analysis of organophosphates using organophosphorus acid anhydrolases (OPAA) enzyme-based biosensors

Tanmay Vyas, Vinay Singh, Prashant Kodgire, and Abhijeet Joshi

Department of Biosciences and Biomedical Engineering, Indian Institute of Technology Indore, Indore, India

ABSTRACT

The human population is dependent on agriculture for its food requirements and survival. Several insecticides and pesticides have found their use for improvements in agricultural yields. Organophosphates (OP) are one of the many compounds used as insecticides and pesticides. OPs have also been used to develop G and V-series chemicals which act as highly toxic nerve agents that can severely influence the normal function of the nervous system in all living beings. Thus, OP compounds utilized as insecticides/pesticides and nerve agents are hazardous to the environment, lethal for humans and other non-target animals. To avoid their toxicity, approaches to detect and neutralize them have become essential. A variety of analytical procedures such as electrochemical processes and chromatography methods, namely liquid and gas chromatography, have been employed to detect OPs. Though these techniques are sensitive and highly accurate they suffer from drawbacks, for instance: their bulky nature and expensive instrumentation, the difficulty of operation, long detection times, and they can yield unpredictable results with variable sample complexities. With the advent of several types of biosensors, the assay of OP compounds has become simpler, faster, cost-effective with improved sensitivity, and provides the capability for onsite detection. OP biosensor assays typically utilize several enzymes with the capability to hydrolyze/degrade OP compounds, such as organophosphate hydrolase (OPH) and organophosphate acid hydrolase (OPAA). This review focuses on discussing various aspects of OPAA as biological recognition unit in terms of its: structure, properties, activity enhancement methods, and utilization for developing OPAA-based biosensing technologies for insecticides, pesticides, and nerve agents.

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Introduction

In this populous world, food production, yields, quality, and security have remained a great challenge. About 1.8 billion people work in the agriculture sector worldwide and agriculture remains the main source of livelihood for many developing countries [1]. To improve the agricultural produce, more than 5.6 billion pounds of pesticides are spent on agriculture globally annually [2]. Chemical pesticide compounds also influence the environment by leaching into the soil, surface water, and groundwater, affecting soil fertility by killing the beneficial microbes. The use of pesticides has been so extensive that it causes various harmful effects to the majority of the population worldwide. In Asia, after China, India is the second biggest pesticide producer. In India, 76% of the pesticides used are insecticides, 13% fungicides, and 10% herbicides. Nearly 45% of the pesticides are used for cotton crops, following that, are for paddy and wheat [3].

Almost all pesticides are composed of complex mixtures of active ingredients and excipients which increase the efficiency of the pesticide cost-effectively. It contains a variety of compounds that are kept confidential by the companies selling these products [4]. They are typically classified into three categories: assignment, method of impact, and chemical nature as described in Figure 1(A). There are many chemical compounds such as organophosphates, pyrethroids, carbamates, and organochlorines, which are used widely as insecticides, herbicides, fungicides, rodenticides, growth regulators, nematicides, etc., all classified as pesticides. Organochlorine (OC) insecticides were also used to control diseases like malaria and typhus [5]. Organophosphates (OP) compounds, also known as phosphate esters, have gained popularity among these

CONTACT Abhijeet Joshi abhijeet.joshi@iiti.ac.in Department of Biosciences and Biomedical Engineering, Indian Institute of Technology Indore, Khandwa Road, Indore 453552, India; Prashant Kodgire pkodgire@iiti.ac.in Department of Biosciences and Biomedical Engineering, Indian Institute of Technology Indore, Khandwa Road, Indore 453552, India

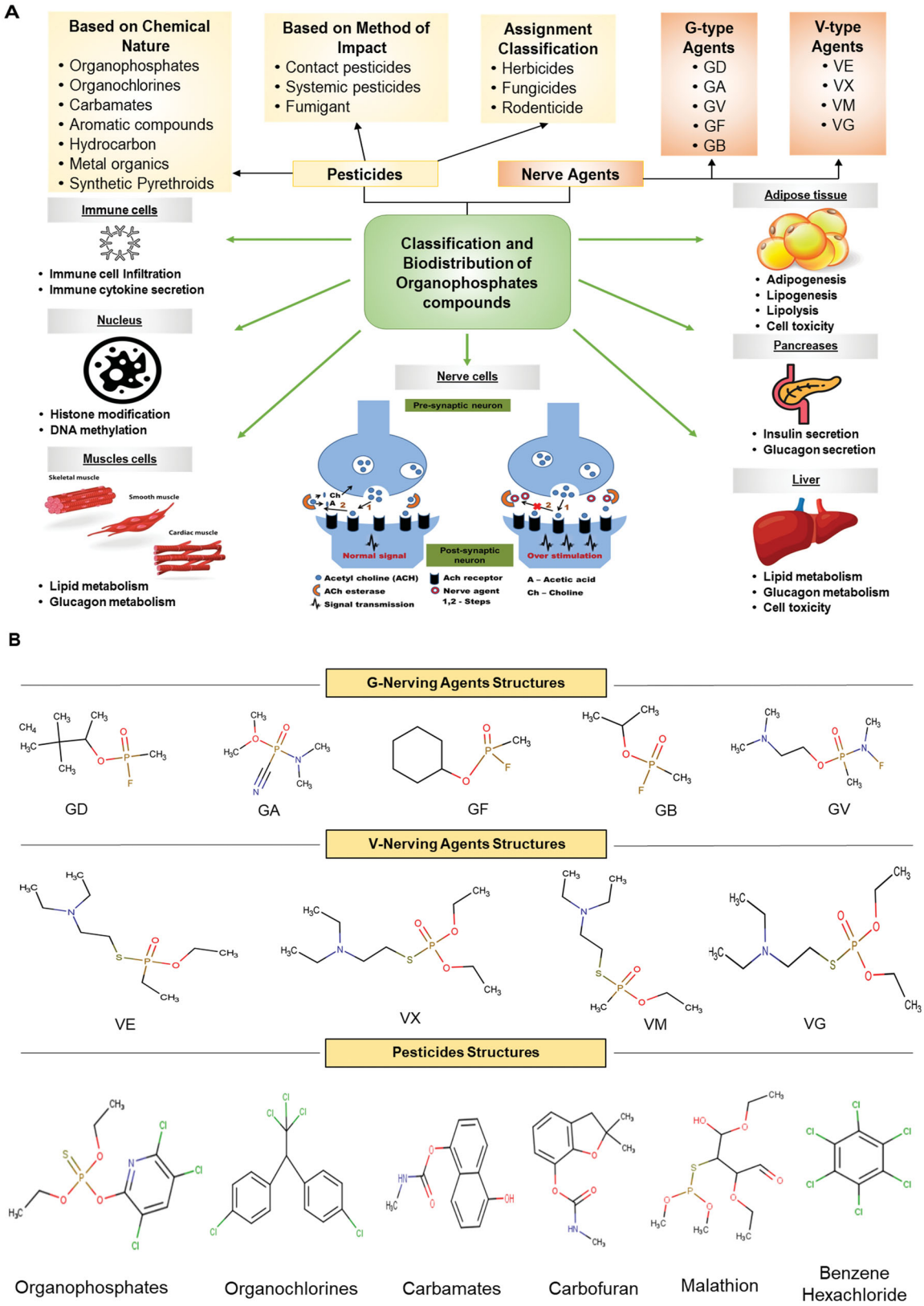

Figure 1. (A) Classification, bio-distribution, regulatory mechanisms and action of OP compounds, such as: pesticides and nerve agents on different cells, tissues, and organs in the body. (B) Chemical structures of OP compounds including nerve agents and pesticides.

chemicals owing to their efficiency and low-cost synthetic methods. They are directly connected to phosphorus by aryl or alkyl groups or carriers such as sulfur and oxygen. They contain one of the aromatic, halogen, aliphatic, or heterogeneous groups that are connected to the phosphorus as mentioned in Figure 1(B). Its continuous exposure affects health. It may transverse the respiratory epithelial tissues through aerosols, and dermal membranes through direct contact, and become accumulated as depots in fat tissues [6]. From where it releases into the blood to reach the nervous system for interaction with the acetylcholinesterase (AChE) enzyme, which is the main enzyme involved in the hydrolysis of acetylcholine. OPs also target enzymes such as butyrylcholinesterase (BChE), esterases, and lipases that are vital for the proper condition of the physiological activities of a human body [7]. Both enzymes are also present prominently in neurons for the execution of neurological functions.

Apart from pesticides, some of the OPs are phosphorothioates and phosphonofluoride, which are universally described as chemical warfare agents and classified as nerve agents. A series of organic esters of phosphoric acid derivatives are nerve agents also known as OP-based nerve gases [8]. They are highly toxic compounds that bind to the enzymes AChE and BChE in the bloodstream very quickly and inhibit their normal activity. Based upon alkyl phosphonic acid esters, nerve agents are mainly classified into 2 types (G- and V-series nerving agents) outlined in Figure 1(A) [8]. The G-series agents are fluorinated OP compounds, except tabun which is a cyanide derivative. The V-series agents are sulfur-containing organophosphates. Both G- and V-series are colorless, transparent, viscous liquids with high boiling points. These can change into aerosols when sprayed or during a blast from a bomb [9]. Nerve agents, which have been used as chemical weapons in many military operations, can be delivered by guided missiles, mines, free rockets, battery and mortar shells, spray tanks, and aerial bombs.

Some OPs undergo non-oxidative changes to form lethal compounds like insecticides [9]. After absorption, these can accumulate in the liver, fatty tissues, and other organs in the body causing several health issues depicted in Figure 1(A). OP pesticides are highly neurotoxic and can act on acetylcholinesterase preventing the break-down of acetylcholine at the neuromuscular junction. The lethal dose of organic compounds at LD50 mg/kg is approximately 80–300 mg/kg for diazinon, 2–10 mg/kg for parathion, 0.5 mg/kg for paraoxon, 0.3 mg/kg for DFP, 0.01 mg/kg for all three sarin, soman, and tabun, 0.001 mg/kg of VX [10]. The nerve agents also inhibit the normal action of acetylcholinesterase both in the central nervous system and peripheral nervous system. The metabolism of OPs results in the formation of alkylphosphates and alkylthiophosphates. Some degradation products of OP pesticides are diazinon oxon, dibutylphosphate [11], 2,4-dichlorophenol, diethylphosphate, dimethylphosphate, dimethylthiophosphate, paraoxan methyl, and phosmet oxon [12].

Poisoning with quickly acting OP pesticides can cause respiratory arrest in as little as 30 min, and many people die before receiving medical help. The degradation of nerve agents in humans leads to methyl phosphonic acid (MPA) as the end product, as in Figure 2, which is hazardous to health. Nerve agent catastrophe treatment has been achieved by applying five principles: (i) supportive care, particularly respiratory and antidote strategies; (ii) decontamination; (iii) anticholinergic, (iv) oxime therapy; and (v) anticonvulsant therapy [13]. Atropine, oxime, glycopyrrolate, antimuscarinic, and pralidoxime are some listed drugs for the treatment of poisoning due to nerve agents [13].

Since OPs pose a threat to health, their detection is necessary. Most of the traditional approaches for sensing pesticides and OPs have several drawback, such as time taken, cost, and complex requirements. AChE and BChE have been used to detect OP compounds. Organophosphate acid anhydrolase (OPAA) is a promising enzyme with phosphotriesterase activity and has the potential to catalyze the reaction with a broad range of OP compounds. OPAA can be found and isolated from several sources, such as bacteria, archaea, squid, and higher mammals. The enzyme diisopropyl fluorophosphatase (DFPase) or organophosphorus acid anhydrolase (OPAA) from *Loligo vulgaris* or *Alteromonas* sp. JD6.5, respectively, are the most potential enzymes for cleaving organophosphorus compounds [10]. In this review, we discuss a detailed account of the OPAA enzyme for its utilization as a biological recognition unit for OPs and several detection approaches. OPAA has the potential to detect many OP compounds and its activity has been enhanced with several approaches, such as increasing the catalytic efficiency of OPAA by mutations and using other materials that can improve stability and activity, for example, sterically stabilized liposomes, spore-based display systems, functionalized mesoporous silica, and metal–organic framework, etc. [6]. Coupling of OPAA with many different transducing mechanisms, such as electrochemical, colorimetric, and fluorometric methods focused on developing biosensors with enhanced speed, selectivity, and sensitivity of

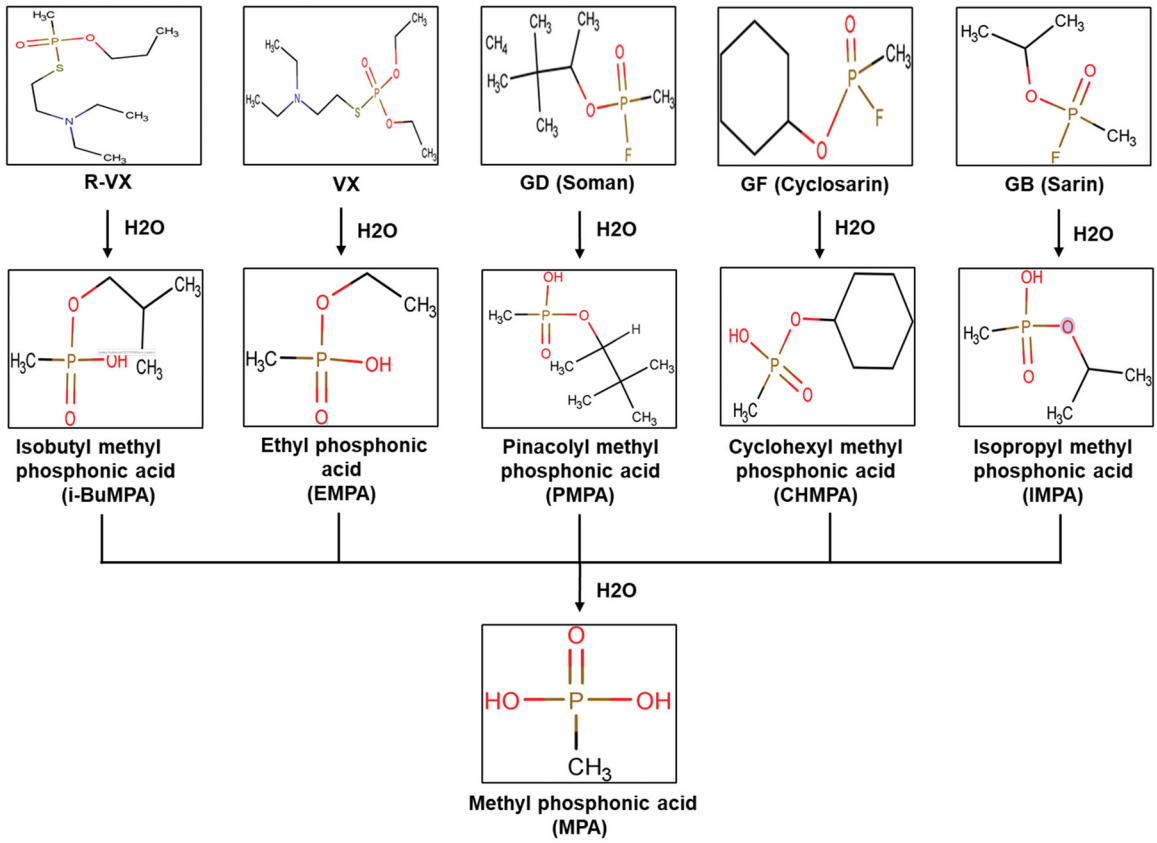

Figure 2. The degradation pathway of nerve agents into their precursors in presence of water leads to the formation of methyl phosphonic acid.

detection are also covered. The review encompasses OPAA enzyme and its role in sensing OPs compounds with different approaches, along with a perspective toward future studies on OP detection utilizing OPAA based on different transducing mechanisms.

OP degrading enzymes

The continuous use of organophosphates in agriculture and as chemical warfare agents has caused its accumulation in the environment. Using enzymes that can degrade both pesticides and nerve agents are emerging aspects of research for improving the reliability and safety of the environment. These enzymes are engineered and used with other supporting materials to increase stability, activity, shelf-life, and tolerance [14]. All these enzymes hydrolyze OPs with different specificities but undergo a similar mechanism of action i.e., disposal of OPs with the aid of water molecules, divalent ions, and volatile amino acids accommodating the active site of the enzyme through a nucleophilic attack on the complex phosphorous center. In Table 1, all types of degrading enzymes that have the potential for the degradation and hydrolysis of OPs (together with their reaction details are described) like *Agrobacterium radiobacter* P230 organophosphate-degrading like enzyme are promiscuous not only in the processes of its catalysis but also in the metals it uses to catalyze these reactions.

Three different binuclear metal centers were investigated in the study: Cd^{2+}, Mn^{2+}, and Zn^{2+}-Fe^{2+}. These metal centers are used by this enzyme to hydrolyze trimethyl- and dimethyl-phosphates, etc. [24]. Organophosphate hydrolase (OPH), one of the numerous enzymes capable of hydrolyzing OP compounds, received attention due to its capacity to hydrolyze a wide range of OP neurotoxins, including conventional insecticides and chemical warfare weapons such as sarin and soman. The hydrolysis of OP compounds is catalyzed by diisopropylfluorophosphatase (DFPase), identified from the European squid *Loligo vulgaris*. DFPase has a six-bladed ß-propeller structure with two calcium ions, one for catalysis and another for structural stability [25]. SsoPox, a hyperthermophilic PLL isolated

Table 1. Organophosphates degrading enzymes and their source microorganism and substrate specificity details.

Enzyme	Source	Broad class of substrates	Specific substrates	Kcat/km $M^{-1}s^{-1}$	Notes	Reference
OpdA (organophosphate hydrolase)	*Agrobacterium radiobacter*	OPs, G-type nerve agents	Methyl-paraoxon	1.1×10^4	Ability to hydrolyze a wide range of OP pesticides and G-type nerve agents. Commercially used as bioremedier	[15]
PTE (Phosphotriesterases)	*Brevundimonas diminuta*	OPs, Sarin, Cyclosarin, VX	DEVX	1.2×10^3	High catalytic activity for pesticides but limited activity toward nerve agents	[16]
DFPase (Diisopropylfluorophosphatase)	*Loligo vulgaris*	DFP, G-type nerve Agents	DMVX Malathion DFP	1.9×10^3 3.0×10 5.6×10^4	Stable at high pH and temperature. Extremely specific for P-F bond hydrolysis	[17]
PON1 (Paraoxonase)	Human liver	OPs, G- and V-type nerve agents	GB GF Paraoxon	4.2×10^4 7.2×10^5 0.6×10^4	Has activity for VX, a promising therapeutic agent, etc.	[18]
SsoPox	*Sulfolobus solfataricus*	OPs	Paraoxon	4×10^3	Has a melting temperature of 106 °C	[19,20]
OPAA (Organophosphorous Acid Anhydrolase)	*Alteromonas* sp.	G-agents- Soman Cyclosarin	Paraoxon	9.36×10^2	Shows high activity towaeurotoxnds that can cleave P–F, P–O, P–CN, and P–S bonds	[21,22]
OPAA (Organophosphorous Acid Anhydrolase) OPH (Organophosphorous hydrolase)	*Alteromonas* sp. *Pseudomonas diminuta*	G-agents, sarin and soman	DFP, Paraoxon, Demeton-S DFP, Paraoxon, Demeton-S	2.7×10^5 7.75×10^3 8.0 2.4×10^6 9.6×10^7 1.2×10^3	OPAA in combination with OPH was used to enable the construction of an array of biosensors able to discriminaeurotoxinins	[23]

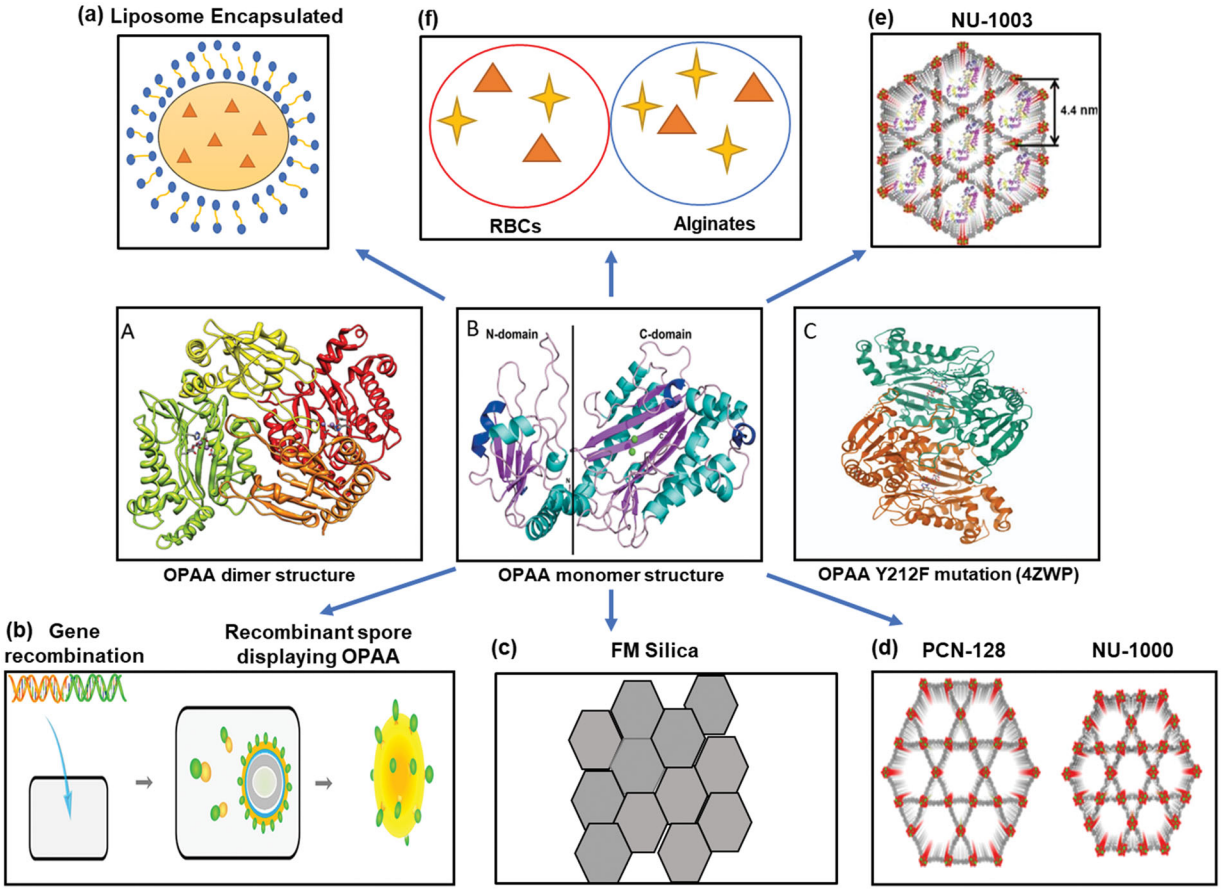

Figure 3. Structure of OPAA (A) OPAA Dimer structure in which yellow color indicated N-domain and green indicated C-terminal in one protomer and Mn^{+2} as a sphere and metal-ligand as a stick and N-terminal and C-terminal domain in orange and red respectively shown in second protomer [29]. (B) shows monomer structure from *A. macleodii* having 2 domains N and C make up the OPAA structure [21]. (C) Structure of OPAA Y212F mutation (4ZWP) developed for the enhancement of activity [30]. And the process for enhancement of OPAA activity by modification of its structure or encapsulation in. (a) Liposomes; (b) spore-based display system: after the gene recombination, expression and assembly of fusion proteins occur on forespore. (c) Functionalized mesoporous silica; (d) MOFs: PCN-128 and NU-1000; € NU-1003 MOF; (f) micro/nanoparticles of sodium alginate along with indicators and encapsulation in RBCs.

from the archaeon *Sulfolobus solfataricus,* was discovered, the folded structure of SsoPox is an (α/β) 8 barrel with an active site located in the C-terminal section [19].

The calcium-dependent paraoxonase enzyme generated in the human liver can hydrolyze aryl esters, lactones, and OPs. Human paraoxonase-1 (PON1) easily hydrolyze paraoxon, a commercial OP pesticide; but can only hydrolyze the less-lethal enantiomers of G- and V-type nerve agents [18]. Phosphotriesterase (PTE), also known as OPH, is a zinc-dependent bacterial enzyme that corresponds to the amido-hydrolase family which can hydrolyze OPs [16]. The Opd (organophosphorus degrading) gene from *Flavobacterium* sp. has a genomic structure containing the entire istAB operon, the tnpA and tnpR genes, and orf243, a metabolite utilization gene [26].

Organophosphorus acid anhydrolases (OPAA)

***History*:** OPAA is an enzyme group capable of catalyzing the hydrolysis of OP compounds. In 1946, during the crude preparations of rabbit tissue, Abraham Mazur initially recognized the presence of an enzyme that degraded the acetylcholine esterase (AChE) inhibitor diisopropyl fluorophosphate (DFP) [27]. In 1991, DeFrank and Cheng discovered and clarified the OPAA enzyme of the JD 6.5 strain of halophilic *Alteromonas* [28]. These enzymes have been identified from several sources, from bacteria and archaea across to higher animals. Two types of DFPases are known to degrade chemical warfare agents. One of them is isolated by squids having a molecular weight of 30–40 kDa (squid DFPase), and another is derived from *Alteromona* bacteria (Mazur DFPase or OPAA) having a molecular weight of 40–96 kDa. Early studies have revealed that

OPAA only catalyzes G-nerve agents but not V nerve agents.

***Structure*:** A metalloprotease monomer OPAA-2 from *Alteromonas* sp. (JD6.5) in parent form in Figure 3(B) has a molecular weight of 60 kDa and Mn^{2+} ion as a cofactor. This enzyme is a 517 amino acid chain polypeptide. However, only the first 440 residues could contain the electron density of the three separate molecules in the asymmetrical unit of the two OPAA structures [31]. There has been no influence on enzyme function on the final 77 residues of OPAA.

In Figure 3(B), an OPAA structure embraced a minimum N-terminal domain (residues 1–160) and a broad C-terminal domain (residues 161–440). Their architecture and shape vary overall as both OPAA areas consist of mixed β-sheets with approximately identical β-strand counts (six on the N-domain and five on the C-domain). The catalytic core is placed on the concave surface of the deeply curved plate in an oval shallow groove on the C-terminal area [21]. OPAA's substrate binding area has three pockets: a minor pocket, a major pocket, and a pocket left by the group. The side pocket of which the N-terminal domain of the contrary subunit in the course of dimer has D45′ cap or coverage and its residues are Y212, V342, and H343. The huge residue pocket includes L225, H226, H332, and R418, and the other unit is capped by W89. Residues F292 and L366 from the left group ZWP mutant are produced by substituting a polar amino acid (Tyr) with a nonpolar amino acid (Phe), in position 212 (Y212F) in Figure 3(C), leading to increased enzyme binding hydrophobicity [30].

OPAA is a proline dipeptidase since it can chop down the P–F bond in G-type nerve agents into the bargain of dipeptide C-terminal protein residue (Xaa-Pro) [31], and this is the main advantage of OPAA over AChE and BChE enzyme as it has specificity for P–F bonds mainly, hydrolyze G-nerving agents, dispropylphosphoflouridate, other fluoride-containing compounds, and highly selective for them. However, OPAA demonstrates certain drawbacks also, as compared to AChE and BChE enzymes, as it does not have the potential to cleave some bonds like P–S, P–CN, etc., some of which are present in many insecticides [32]. OPAA shows activity in oligomeric form, which is a tetramer, a dimer of dimers, and functional studies define the catalytic activity of all OPAAs in stereoisomer-dependent phosphate esters. The high temperatures and the long-term storage of the enzyme show that it is unstable under harsh conditions and the loss of action in organic solvents. Unprotected OPAA enzymes are also vulnerable to deactivation by other enzymes. These enzymes are widely employed for the treatment of poisoning with OPs [15]. OPAA from various sources exhibit differences in molecular weight, sensitivity to inhibitors, substrate specificity, and activation by metals. To demonstrate the molecular structure, specificity, sensitivity, and true nature of substrate of OPAA, some scientists performed purification and characterization assays. Functional studies have shown that OPAA from *Alteromonas undina* exhibits high enzyme activity and a diverse array of substrates discussed in Table 2. According to previous research, disulfide bond reducing agents like dithiothreitol (DTT) and β-mercaptoethanol help in stimulating and stabilizing the enzyme [31]. OPAA's natural activity is to break the proline peptides formed during collagen-like protein degradation. Prolidase is the second residue, having a definite action for dipeptides with proline [21]. OPAA is highly active over a temperature from 10 °C to 65 °C and pH from 6.5 to 9.5 with an optimum pH of 8.5. Various metal ions affect the hydrolyzing activity of organophosphorus acid anhydrolase against DFP, metal ions like Ni^{2+}, Zn^{2+}, and Fe^{2+} inhibit enzyme activity [34].

Table 2. Substrate specificity of OPAAs against various OPs compounds obtained from different microorganisms species.

	Specific activity (U mg^{-1})			
Substrate	*Alteromonas sp. JD6.5*	*Alteromonas undina*	*Alteromonas haloplanktis*	*Pvrococcus ftuiosus*
DIP	1320	1403	691	0.73
Leu-Pro	639	810	9SS	15S2
Gly-Pro	13.5	ND	ND	347
Sarin	611	426	308	–
Soman	3145	2826	1667	–
Cyclosarin	1654	1775	323	–
Paraoxon	2.8	5.6	–	–
Mipafox	<1.0	5.2	–	–
Tabun	–	711.0	–	–
Sarin	–	221.2	–	–
NPMPP[a]	35.1	161.5	–	–
NPEPP[b]	28.3	265.2	–	–
Reference	[28]	[31]	[23]	[33]

[a]p-Nitrophenyhnethylphenylphosphinate.
[b]p-Nitrophenylethylphenylphosphinate.

Enhancement of catalytic efficiency of OPAA

OPAA is an important enzyme to detect and catalytically hydrolyze a wide variety of OP compounds. Hence, efforts have been continuously made by researchers to determine its optimum performance conditions and improved activity in terms of a wide range of substrates, high catalytic efficiency to neutralize large quantities of OP compounds, etc. [21]. G-class OP agents, like sarin and soman, form the substrate for the wild-type OPAA while V-class agent, like VX, is not a substrate for it. Approaches, such as change in the amino acid sequence, active sites, and developing mutants, have been developed to increase the catalytic efficiency of enzymes. Enhancements of OPAA catalytic efficiency can be conducted in various approaches specifically through mutation, carrier-based techniques, spore-based display, metal–organic frameworks, and engineering for specific targets lead to better efficiency.

Site-specific mutations

Heidari et al. showed the mutations indicate that the catalyst efficiency of the overall enzyme will be affected by 212, 342, and 215 amino acids at the OPAA binding site. A native and three mutant forms of the OPAA enzyme were studied with the docking process toward parathion, paraoxon, and R-VX compounds. The amino acid substitution was conducted at the small pocket (Y 212 and V 342) and I 215, between the small and large pockets. The three mutant forms obtained were 4ZWP, 4ZWU, and Mut6 [35]. The 4ZWP, 4ZWU, and Mut6 forms showed a decrease in the catalytic activity toward parathion and paraoxon. However, the docking prediction results for R-VX showed higher catalytic activity of 4ZWP and 4ZWU. It was concluded that the 212 and 342 amino acid positions were important and substitution at these sites, based on the substrate structure, can change the catalytic efficiency of the enzyme. When compared to the results of Daczkowski et al. [36], it was revealed that making small-pocket mutation led to a 30-fold increased effect on the enzyme activity than on large group pockets [35].

According to Yang *et al.,* one variation (22A11) with up to 25-fold enhanced methyl parathion hydrolysis was created using directed evolution [15]. Surprisingly, this variation destroyed all other substrates tested 2- to 10-fold quicker (paraoxon, parathion, and coumaphos). Because only one mutation (H257Y) lies directly in the active site, site-directed mutagenesis and saturation mutagenesis were employed to determine how the other distal substitutions (A14T, A80V, K185R, H257Y, and I274N) affected substrate selectivity and activity. According to sequential site-directed mutagenesis, K185R and I274N are the most important substitutions, resulting in an improvement in the hydrolysis of methyl parathion and total hydrolysis rate up to 2–10-fold faster [15].

OPAA toward V-nerve agents

The wild-type OPAA has a poor catalytic efficiency for VR (Russian VX), a V-series OP agent. However, Daczkowski et al. through a novel approach of protein engineering, increased the catalytic efficiency of OPAA to 30-fold toward racemic VR [36]. They used a 440-amino acid divergent of OPAA from *Alteromonas sp*. JD6.5 to change the activity of OPAA on VR. Both the enantiomers of VX (Sp and Rp) have an approximately 13-fold toxicity difference between them. Hence, a mutation to increase the catalytic efficiency of the OPAA and both the enantiomers of VR was required. Small pocket modifications i.e., three consecutive amino acid changes were made within the OPAA substrate-binding site. OPAA F (Y21F), showed a five times increase in the catalytic action than the WT enzyme. Y212F and Y212V mutations further increased the activity of the mutant enzyme (OPAA-FL) toward VR. OPAA-FL showed 10 times higher efficiency than the wild-type enzyme. Following this, modification of the third and fourth amino acid resulted in OPAA-FLY and OPAA-FLYD, which reduced the racemic catalytic efficiency by 2-fold but allowed the mutant OPAA to catalyze both the VR enantiomers with the same efficiency. This approach aimed to provide a useful genetic backdrop for further improvement in the OPPA VR activity [36].

OPAA toward G-nerve agents

Substitution of amino acids at OPAA Y212 (FL), V342 (FI), and I215 (FY) was conducted to check its effect on sarin, soman, and GP [37]. As chemical warfare weapons, Sarin, GP, and Soman are classified as G-series nerve agents. The Rp-stereoisomers are less toxic than Sp-stereoisomers in event of both sarin and soman. The Sp-configuration can inactivate acetylcholinesterase 10^3–10^4 times faster than the Rp-configuration. Bae et al., in their work, concluded that the mutants FY and FI showed a very small shift in the catalytic regulation in comparison with wild type [37]. Study shows the catalytic activity in presence of sarin nerve agent in OPAA mutant genotype (FL/FI) increase by 7-fold (85×10^5) than wild-type (WT) genotype (13.3×10^5). In the presence of soman, it increases by 5-fold (748×10^5) than WT (161×10^5) whereas GP influences enzymatic activity with 10-fold (1290×10^5) than WT (129×10^5) &

VR increases it by 2-fold (11894×10^5) whereas WT shows 548×10^5 enzymatic activity [37].

Catalytic enhancement of OPAA using immobilization

Enzymes are one of the most important biomacromolecules for life and they are used for many applications, e.g., in industries, medical fields, etc., but due to their fragile nature in the: presence of varying temperatures, pH, low tolerances to organic solvents, and metal ions, etc., there is a need to protect them. Immobilization of enzymes in certain platforms could serve a great deal in protecting them under adverse conditions and lead to an improved lifespan and stability [38]. Certain carriers, such as liposomes, metal-organic frameworks (MOFs) and spore-based systems described below, have displayed a protection of OPAA in harsh conditions (Figure 3).

OPAA stabilized liposomes

A new approach to encapsulate the OPAA enzyme in the sterically stabilized liposomes (SL) was conducted by Petrikovics et al. [39]. The encapsulated OPAA in Figure 3(a) was used to degrade DFP. Its stability in blood was approximately 2 days at 37 °C and it degraded the DFP to fluoride and diisopropyl phosphate. DFP hydrolysis by SL was detected with an increase in fluoride ion concentration using a selective electrode of fluoride ion. OPAA can be used as a free enzyme but encapsulating them into liposomes can decrease the immunological reactions when used in blood and helps it to stay for a little longer in the blood than in the free state [39]. It not only antagonizes and treats OP poisoning but prevents a delay in organophosphate toxicity. This approach can be used to prevent poisoning cases by OP-insecticides used in agriculture and OP nerve agents in military applications. The OPAA within the liposomes was administered either alone or in association with pralidoxime (2-PAM) or/and atropine. Using 2-PAM and/or atropine with SL increased the protection against DFP, especially when used together with 2-PAM and atropine along with SL. This study showed that a better carrier system with a longer half-life and less macrophage recognition can be coupled to obtain an increase in the use of catalytic enzymes for chemical intoxications.

According to Pei *et al*., the OPAA–DFP enzyme–substrate system's *in vitro* efficiency is significantly lower than OPH–paraoxon system; consequently, the latter could be employed as a prospective technique to pursue more potent antidotes [40]. With the OPH-stabilized liposome or the OPH–carrier RBC systems, the LD_{50} of paraoxon was 920 mg/kg. This means that the encapsulated OPH increased the paraoxon's LD_{50} value to 0.85 mg/kg when it was not antagonized by almost 1000 times.

Spore-based display system

Recombinant spores can be used for many practical applications including immobilization of enzymes leading to improved catalytic activity and stability [41]. Immobilization of enzymes in a given area on spores can help in repeated utilization in Figure 3(b) and maintenance of catalytic activity. In a study, OPAA was immobilized on recombinant spores of *Bacillus subtilis*. In this system, recombinant spores were produced steadily, without interference in the normal sporulation, with greater activity and improved resistance in diverse unfavorable circumstances, OP substrates were degraded by the spored enzyme. The fusion OPAA showed resistance toward: ethyl ether, low temperature, proteinase K, alkaline, and acidic environments. The resistance toward ethyl ether and acidic conditions (pH 4.0) was 2.8 times and 3.2 times greater than the free OPAA. Although there were some drawbacks, like the spore displayed, OPAA did not show much resistance to high temperature, methanol, and trypsin. It was assumed that this hindrance was due to the natural effects of the enzyme because of the protein interaction in the coating layer [41].

Functionalized mesoporous silica (FMS)

OPAA was encapsulated in NH_2-FMS by electrostatic interactions in Figure 3(c). It can be released from the support whenever needed for the response. It was observed that at pH 9 $NaHCO_3$–Na_2CO_3, OPAA was released and was gradually released at pH 7.4. The released OPAA retained its natural conformational organization and the same enzyme activity as that before encapsulation for the substrate soman and diisopropyl fluorophosphate (DFP). This confirmation might not be favorably constructive toward stability and activity improvement [42]. However, there are some challenges to this method, such as denaturation or decrease in enzyme loading, and leaching of enzymes [43].

Metal-organic frameworks

The metal-organic frameworks have gained popularity for enzyme immobilization. Metal-organic frameworks are comprised of metal nodes and organic ligands, interconnected with a conjunction, shown in Figure 3(d). Instead of their free form, immobilized enzymes have greater thermic and functional steadiness [44]. Various reports have shown that entrapment of enzymes mesoporous MOFs (mesoMOFs), which is

generally a cage-like structure, and have increased loading capacity of enzymes along with greater stability when compared to the enzymes integrated into mesoporous silica. There are different types of immobilization procedures for enzymes, such as *in situ* synthesis, covalent linkage, surface immobilization, and pore entrapment [38]. Pore entrapment or cage encapsulation is a method in which the enzyme is encapsulated into the cage or pores of the mesoporous MOFs by diffusion. This approach protects the enzyme from degradation in adverse conditions to a significant extent. It does so by separating the protein molecules in the pores of MOFs. Furthermore, if the enzymes are placed in a 3D setting of mesoporous MOFs, ingress to substrates becomes easier and enzyme accumulation and protein unfolding are also reduced [45]. Li et al. for the first time, introduced OPAA encapsulated in a water-soluble zirconium metal-organic framework, a framework with crystal structure network topology that has large hexagonal and small triangular channels that would be an ideal encapsulation platform for enzymes [44]. The enzyme activity of OPAA within the PCN-128y was observed toward DFP that was used as a sample substrate. The initial DFP hydrolysis rate of OPAA@PCN-128y was lower. The activity varied at different temperatures: both free OPAA and OPAA@PCN-128y showed optimum activity after incubation at 45 °C, the incubation of free OPAA at 55 °C resulted in the depletion of enzymatic activity and thereby the loss of DFP conversion. However, OPAA@PCN-128y resulted in a 90% conversion at 55 °C. At 70 °C, the free OPAA was denatured whereas OPAA@PCN-128y exhibited high stability and conversion of approximately 70%. These results suggested that immobilization of OPAA showed greater thermal and long-term stabilities [44]. Even though OPAA@PCN-128y showed better activity than free OPAA, the initial catalytic turnover rates were much lower than the free enzyme. This is due to the sluggish spread of reactants and products across intra-MOFs. The reactant must spread across the entire MOF-enzyme crystal to employ all the enzymes contained. The reactant concentration is usually larger at the ingression of the pores and the MOF surface in micron pore crystals rather than in the inside of the crystal. As such, the enzymes within the MOF crystal are not accessible, resulting in a lower total reaction rate than the superficial surface of the MOF enzyme support. The reduction of the size and/or increase of the cavity aperture of the MOF-enzyme crystals may reduce the internal diffusion and reaction restrictions [38].

Li et al. created the biggest aperture (4.6 nm) known as a zirconium MOF, the hydrostable zirconium NU-1003 MOF [44] (Figure 3(e)**)**. In addition to that, the size was set from 300 to 1000 nm for NU-1003 crystals. However, OPAA@NU-1003–7000 nm and OPAA@NU-1003–1000 nm (Figure 3(e)**)** displayed a higher hydrolytic rate similar to OPAA free of charge than OPAA@NU-1003–1000 nm exhibited. When OPAA@NU-1003–300 nm has been applied, DFP hydrolysis has achieved 100%. Thus, for the hydrolysis not only of the DFP but also for soman, the nanosized OPAA@NU-1003 (300 nm) demonstrated enhanced initial turnover. In comparison with the micro-sized NU-1003, the catalytic efficiency of OPAA when encapsulated in NU-1003 was remarkably boosted and even eclipsed the free OPAA in a buffer. There is still a need to perform more kinetics studies to do further mutations to improve efficiency. Furthermore, enhancement of careers is needed to improve their efficiency and binding affinity with OPAA. Some new strains are to be discovered which are more efficient to produce a high yield of OPAA enzyme and which are even less hazardous. More efforts is required to narrow the detection limit, quick response time and the techniques should be time-efficient, reproducible, and stable enough.

OPAA-based sensing of OPs

Among the various sensing techniques, mass spectrophotometry and optical biosensors and chemo-sensors are the most used interventions for the detection of OPs. Even though mass spectrophotometric methods provide high sensitivity and specificity, skilled technicians are required to operate them which is costly in nature. A device that is small, portable and which can be used on-site has become highly desirable considering the limitations of laboratory-based sophisticated techniques. Biosensors are analytical devices that can transform biological processes into responses in the form of transduced signals, such as color, fluorescence, or electrical signal [46]. Compared to traditional methods, biosensors provide portability, ease of use, improved stability and sensitivity and are inexpensive in nature. Biosensors and chemo-sensors can also be used for *in situ* analysis and rapid detection of OP compounds. Biosensors can provide better selectivity but must be used carefully as environmental conditions, such as temperature, pH, and humidity could affect its performance [47]. Various surveying of sensing procedures is summarized in Table 3 which uses OPAA to detect OP compounds using different transduction processes and the resultant efficacy achieved by these methods is described schematically in Figure 4. Instrumental analysis by electrochemistry or optical

Table 3. Various OPAA based sensing approaches, their details of biosensing components along with their analytical and sensing parameters.

Enzyme	Substrate	Microorganism	Cell type	Transducer	Detection limit	Linear range	Sensitivity	Response time	Reference
OPAA-FL	Ethyl paraoxon	*Alteromonas* sp. JD 6.5	*E. coli*	Colorimetric	40 µM	0.01–1 mM	1.38 a.u./mM	–	[36]
OPAA-FL	Ethyl paraoxon	*Alteromonas* sp. JD 6.5	*E. coli*	Fluorometric	56 µM	0.1–0.5 mM	95%mM	3 min	[36]
OPAA-FL (Alginate beads)	Ethyl paraoxon	*Alteromonas* sp. JD 6.5	*E. coli*	Colorimetric	60 µM	0.025–1 mM	1.79 a.u./mM	–	[36]
OPAA (RBC)	Paraoxon	*Pseudomonas diminuta*	*E. coli*	Colorimetric	10 µM of p-nitrophenol		≥0.1 U/ml of blood	~20 min	[40]
OPAA	DFP	ND	ND	Fluorometric	1 µM	–	–	–	[28]
OPAA based biosensing chip	DFP	*Alteromonas* sp. JD 6.5		Potentiometric	100 µM	250–3000 µM	0.61 mV/decade	200s	[48]
OPAA Fluoride sensor in textiles	DFP	–	–	Potentiometric	200 µM	250–2500 µM	25.1 µV/µM	–	[49]
OPAA in silica gel	DFP	*Alteromonas* sp. JD 6.5	*E. coli*	Amperometric	25 µM with glass electrode (batch mode)	20–500 mM (Stability: 5 months)	–	–	[23]
OPAA on chip					20 µM with pH-FET flow injection (stop-flow)	12.5–500 mM (Stability: 22 days)	–	100–250s	

spectroscopy *via* absorption, fluorescence, or luminescence is used in nanosensors for OP analysis. These nanosensors have various advantages over conventional sensors, including: unique sensing processes, better loading capacity, higher sensitivity, and a speedier beginning of the action. Some are due to intrinsic nanomaterial properties, for example, porosity, electric conductance, surface plasmon resonance (SPR) excitation, fluorescence, or luminescence, while others are due to a combination of chemical and biochemical functionalization aimed at generating OP-specific signals in response to OP adsorption or hydrolysis [50]. OPH enzyme linked with reporter CNF fluorophore, a study by Viveros et al. revealed the direct detection of OP neurotoxins. Any response occurring in the 7–10 pH range could be measured using CNF as an indicator [51].

Spectroscopic biosensors

Various spectroscopic approaches have been employed to investigate OPAA-DFP interactions, namely circular dichroism (CD), fluorescence spectroscopy, and infrared reflection absorption spectroscopy (IRRAS). The CD spectroscopic analysis of OPAA showed some peaks characteristic of α-helix chains which were regular (23%) and distorted (16%). Upon interaction with DFP, changes in the shape of the peaks were not apparent when the DFP concentration was below 1.1×10^{-3} M while the secondary structure was almost totally lost when the concentration increased to 1.1×10^{-3} M. Hydrolytic reaction results in decreasing α-helix chains and increasing β chains. The liberated fluoride ions did not show significant changes in the secondary structures of OPAA [28]. Both CD and fluorescence spectroscopy results suggested changes in the secondary structure of OPAA during catalytic reaction with DFP in addition to liberated fluoride ions which may change the polarity of the microenvironment of tryptophan moieties. The IRRAS spectra of the OPAA monolayer at the air − water interface in the absence and the presence of DFP showed that at higher concentrations, the fingerprint spectra were completely lost at 1.1×10^{-3} M of DFP. The CD and IRRAS spectra indicate that a definite loss of secondary structure was observed at 1.1×10^{-3} M of DFP. A color spot test and kits are used to identify field levels of nerve agents such as GB (sarin), GA (tabun), GC (soman), and VX (O-ethyl-S-diisopropyl amino methyl phosphonothiolate). But they have some disadvantages like being less specific and not easy to differentiate, etc.

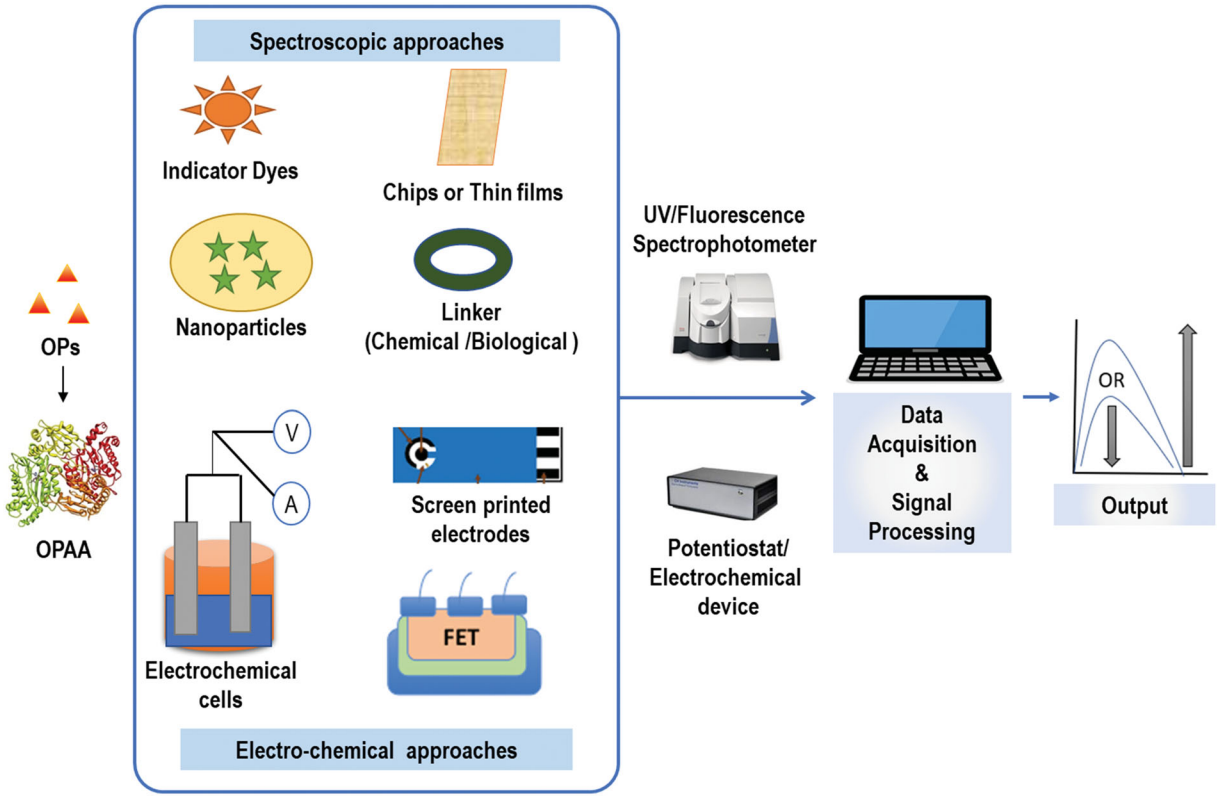

Figure 4. Schematic diagram of OPAA-based detection system with different spectroscopic and electrochemical approaches for detection of OPs.

In an attempt to check the potential of encapsulated OPAA in an *in vivo* system, Pei et al. used OPAA capable of catalyzing paraoxon, encapsulated in resealed erythrocytes of mouse formed by hypotonic dialysis method. OPAA produces *p*-nitrophenol and diethylphosphate after hydrolysis of paraoxon. After an HCl mediated stoppage of reaction, *p*-nitrophenol was extracted in dichloromethane in sequential steps. Extracted *p*-nitrophenols were quantified with a spectrometer at 400 nm. This approach has determined the OPAA activity encapsulated within mouse erythrocytes, but because of UV-absorbing chemicals in the blood, the activity determination suffered interferences [40]. The detection limit of the method was found to be 0.01 mM of *p*-nitrophenol and the half-life of enzyme activity for OPAA in RBCs is about 1.05 h. This method involved several steps of encapsulation and extraction of product formed during the catalytic reaction and, in this process, the chances of hemolysis of RBC are also a probability that may or may not affect the enzyme activity. Another example of comparing the OPAA biosensing in an encapsulated form is the use of alginate polymer microspheres tested on paraoxon by Jain et al. [36] (Figure 3(f)**)**. *Escherichia coli* cells were used to obtain the OPAA-FL variant for testing the biosensing activity. The *p*-nitrophenol synthesis and association of protons synthesized in the catalyzed process with fluorescein isothiocyanate-dextran were predicted using colorimetric and fluorimetric detection, respectively. Ethyl paraoxon was measured colorimetrically with a sensing range of 0.01–1 mM. Fluorometric ethyl paraoxon was measured between 0.1 and 0.5 mM after a short 3-min incubation period. The research has proven that the use of the OPAA-FL variant can detect OP insecticides and can be used as both colorimetric or fluorimetric biosensors in both encapsulated and unencapsulated forms. The fluorescence intensity of FITC decreased after 3 min, resulting in a linear detection range of 0.1–0.5 mM and a detection limit of 0.056 mM [36].

Electrochemical biosensors

Electrochemical sensors are based on the amperometric response of an enzyme biosensor. The enzyme, OPH/OPAA, hydrolyzes OPs to an electrically active species. This technique is also built onto a single-channel microprocessor for the quick disguising of nerve agents [52]. The main mechanism is the determination of enzyme activity along with other physical properties to identify the amount that is associated with this rate. Since the enzyme could be readily attached to the pH-sensitive surface, the pH-sensitive field-effect transistor (pHFET)

is another appropriate technology for detecting these pH changes. This improves the detection of enzyme hydrolysis products. Because numerous pH sensors can be placed on a single sensor substrate where different enzymes are attached, these also provide a platform that lends itself to the construction of a multi-enzyme sensor array [53].

In research by Simonian *et al.*, the ability of OPAA to hydrolyze the P–F bond of fluorine-containing OPs was used to develop a biosensor for the detection of DFP (having P–F bond), paraoxon (P–O bond), and demeton-S (P–S bond) [23]. The amperometric method of transduction has been utilized by developing and testing the activity of OPAA in the form of immobilization in silica gel particles and immobilization on pH-sensitive field-effect transistor (pH-FET). The electrical measurements in the former were performed using a pH glass electrode in a batch analysis method and the latter using a pH-FET in a stop-flow analysis method [23]. When the stability of such a system was evaluated the silica gel particle-based system showed a 5-month long maintenance of activity to an extent of 60%.

Goud et al., in 2020, described a manufacturing chip for the rapid detection of G-type chemical warfare weapons employing a potentiometric all-solid-state fluoride transducer with OPAA [48]. Micro-fabricated, solid fluoride potentiometric biosensors with optimized parameters that affect the sensor efficiency were studied. The resulting fluoride sensing chip had appealing analytical properties, such as Nernstian slope, excellent selectivity, and reliability. The fluoride sensor was subsequently used in conjunction with the immobilized OPAA enzyme to detect DFP, a model G-type OP nerve agent surrogate, quite sensitively and selectively. Here, the OPAA hydrolyzes the P–F bond, releasing fluoride ions promptly recorded on the adjacent fluoride detector. The limit of detection was 100 μM [48]. It shows sensitivity for DFP detection and linearity over the entire concentration range of 250–3000 μM with an estimated detection limit of 100 μM. Three different sets of SPEs were used to test the reproducibility of biosensing chip in detecting DFP throughout a concentration range was calculated to be 3% with a standard deviation of 4.58 mV with a stability of 8–12 days.

The first wearable solid-contact fluoride sensor was based on textiles for the selective recognition and biodetection of fluorine-containing G-type nerve agents [49]. The achievement of selective potentiometric biosensing of G-type nerve agents in conjunction with immobilized OPH or OPAA enzymes has been made possible by overcoming the challenge of developing an effective on-body fluoride sensor. The resulting textile-based nerve-agent biosensor is easily interchangeable and has been combined with a tiny potentiostatic to enable wireless detection of the G-type neurotoxic DFP surrogate chemical.

The spandex fluoride potentiometric sensor's performance allowed it to be converted into enzymatic textile biosensors for detecting DFP, a frequent simulant of F-containing G-type nerve poisons. The detection of DFP in connection with the new flexible fluoride transducer, miniaturization of the entire textile-based sensor system toward a user-friendly field operation in connection with a compact potentiostatic interface, and wireless signal transmission to a smartphone were all made possible using OPAA and OPH enzymes, which both offer facile biocatalytic hydrolysis. The corresponding calibration curve showing a sensitive (25.11 μV/μM DFP) and nearly linear ($R^2 = 0.987$) response to DFP was obtained with the OPAA-F textile sensor and a sensitive (37.87 μV/μM DFP) and linear ($R^2 = 0.998$) response to DFP with the OPH-F sensors, with lower concentrations of 50–600 μM and 50–250 μM, respectively, over the concentration 250–2500 μM DFP for further and 250–2750 μM DFP solutions for the later sensor with RSD being 1.52% and 1.93%.

Over a decade, spectroscopic and electrochemical sensors are acquiring recognition as an emerging technique for qualitative analysis. As previously stated, the fluorescence approach is a highly specific, selective, and cost-effective method for detecting chemical compounds. Due to its sensitivity and selectivity, the enzymatic fluorescence sensor plays a critical role in identifying targets. Although their results suggest changes in the secondary structure of OPAA all through catalytic reaction with DFP in order to have access to liberated fluoride ions, which may alter the polarity of tryptophan moieties, it is more specific than CD fluorescence intensity tempering and can be used as a biosensor of effectiveness with a significantly lower detectability threshold. Pei et al. used a procedure that involves many steps of encapsulation and separation of the product created during the catalytic reaction, hemolysis of RBC is also a possibility that might or might not impair enzyme function and a complex process [40].

Electrochemical DNA biosensors, in comparison to fluorescent and colorimetric biosensors, have received much attention in the field of biochemical research. Thermal and long-term drifts, caused by the pHFET approach, limits the accuracy of monitoring systems for continuous readings. The recovery and reusability of immobilized enzymes lower the overall cost of the enzyme. Goud et al. reported a chip-based detection

that improves coverage and dynamic range while being accessible and costly [48].

These techniques need to be more specific and sensitive enough to detect even the minor outputs received and should work on ways to have less instrumentation and exhibit more portability. It can be more efficient to detect and to further less detection limits with a quick response time. It should be catalytically efficient to have good binding and catalytic events between the enzyme and the substrate.

Having good binding efficiency of enzyme and substrate also depends on the carrier which provides a nest for the OPAA enzyme. The selection of carriers depends on the nature, size, and charge of the polymer. Biopolymer is a widely accepted and efficient carrier for encapsulating OPAA due to its biocompatibility. Biopolymers also serve as part of the green revolution, as they are biodegradable, biocompatible, and have the tendency to inculcate in the environment without causing any damage [54]. Many biopolymers, such as alginate, chitosan, and RBCs are extensively used to fabricate nano and microparticles to encapsulate OPAA.

Other sensing approaches of OPs

Various other analytical methods are also being used for the determination of Ops, such as chromatographic techniques, surface acoustic wave sensor, capillary electrophoresis, enzyme-linked immunosorbent assay, and electrospray ionization–mass spectrometry [55]. Gas chromatography is an accurate analytical separation method for both high- and low-volatility analytes. It is an effective, but also a very complex method to operate regularly. Liquid chromatography can be used for nonvolatile compounds, but high pressure and tiny particles limit the column length. It is also a slow analytical method because of low diffusion rates in liquids [55].

The surface acoustic wave sensor (SAW sensor) is very popular for the observation of chemical warfare agents, which can be made small and portable [56]. It is based upon the mechanical vibration that propagates on a cross-digit transducer or an inter-digited electrode pattern across the solid surface of piezoelectric material. Methods like the ion mobility spectrophotometer are a segregation method in which ions of gas-phase, made by the vapor-phase ionization of neutral molecules are separated according to the differences in their velocities through a gas in an electric field, used for the detection of chemical warfare agents like soman but suffers from the problem of very low resolving power.

The enzyme-linked immunosorbent assay (ELISA), an immunochemical sensor, is a useful method for the sensing of several nerve agents. The stability of the antibody, cost, time, etc. are some of its limiting factors, that may take as long as 2 h for detection. However, when combined with other selective detectors like pulsed-flame photometric detectors, the time can be decreased markedly [57].

Capillary electrophoresis is a separation method for ionizable and ionic compounds. It can be coupled with selective sensors like the flame photometric detector. In several degrading goods, the electrokinetic injection can be employed for improved detection limits. For example, methylphosphonic acid, isopropylmethylphosphonic acid ethylmethylphosphonic acid, and pinacolylmethylphosphonic acid were detected at the ppb level in 3 min from water samples.

The use of an oxidized porous silicon interferometer (PSi) screen with a hydrolysis catalytic and surfactant (II) may also be used to identify chemical warfare chemicals. HF gas, which destroys the silicon oxide and creates a blue shift, also a reduction in the Fabry-Perot fringes from the Psi interferometer is created, when nerve drugs are hydrolyzed. Its detection time is 5 min [58]. Hollow fiber micro-extension liquid-phase (HF-LPME) depends upon the exhaust of objective analyses in the pores of the wall of the permeable hollow fibers, and then the acceptor phase (which may be watery or natural), of the hollow fiber, from watery instances into the supported liquid membrane. The acceptor solution is subjected to a synthetic examination immediately after extraction. HF-LPME is a successful way to employ complicated samples of liquid and gas phases.

Subsequently, numerous endeavors are being undertaken to develop new techniques for detecting pesticides in a quick, sensitive, specific, and easy-to-use manner. Currently, several researchers are working on developing new sensing systems for detecting pesticides with optimization, substrate specificity, and varying complexity, such as GC/MS, which is quite complex for the assessment of OP compounds. Although this technique is not best suited for thermally labile samples, it is highly efficient and allows the isolation of the components of complex mixes in a reasonable amount of time. Liquid chromatography, on the other hand, is a sluggish analytical procedure due to poor diffusion rates in liquids' peak or band-broadening, resulting in lower resolution than GC.

SAW sensors are compact, portable, and widely used for the detection of chemical warfare weapons, however, they are unstable owing to moisture affinity [56]. The ion mobility technique has its limitations such as low resolving power and poor reproducibility. ELISA procedures, on the other hand, are simple, quick, and

highly specific but are very expensive when using sophisticated techniques. Capillary electrophoresis also has inconsistencies in retention times. Sohn et al.'s PSi interferometer is a single-device calibration that is chemically unstable, yet it is inexpensive and simple to use [59].

Efforts should be focused on cost-effectiveness, easy-to-detect, and quick reaction methods. Instead of solid films, any metal may be utilized in SAW sensors to produce interdigital transducers. Electrophoresis resolution must be improved, and gel-to-gel repeatability must be improved. There is a need for less sophisticated procedures and fewer instruments, which can reduce reaction time by up to 4–5 times and improvisation must be required for some parameters, such as detection limit, sensitivity, and specificity.

Future prospects and challenges

Biosensors based on OPAA/OPH can directly detect OP compounds without requiring multiple steps, but more investigation is required for their use in real samples. Considerable efforts are made toward this for reliable OPAA biosensors that can be used for a wide range of purposes in agriculture and other fields, as for the detoxification of toxic agents. It is crucial to design novel microbial enzymes that detoxify OP chemicals to mitigate their hazardous impact on the environment.

Studies have been conducted to completely alter enzymes applying molecular biology and protein designing methods, boosting their substrate-binding affinity and catalytic properties. Although identification of other enzymes is imperative for improving the efficiency by analyzing the structure, functionality, and catalysis of OP degrading enzymes like that of OPH and OPAA [10], nonetheless, using the available engineered enzymes and coupling theme to nanomaterial chemistry to develop biosensors is also equally essential [14]. OPAA has some inherent disadvantages like temperature-sensitivity and being destroyed by organic solvents which can be overcome by immobilization of OPAA, long-term durability and catalytic efficiency can be increased by mesoporous silica particles, MOFs, nanocapsules, etc., which significantly increase OPAA's performance for *in situ* study applications [44].

Encapsulation of OPAA can enhance the sensitivity, stability, and performance of detection/degradation of OP compounds like OPAA encapsulation in a liposome, or porous MOFs. Nanomaterial-enabled (bio)scavengers have been recognized as new therapeutic treatments, owing to their nm size and charge, providing OP-targeted reaction without any unwanted percutaneous penetration [50]. Using diverse nanoparticles such as quantum dots (QDs), gold nanoparticles (AuNPs), and others, an enzymatic biosensor based on electrochemicals was developed, which sustains the bioactivity. Researchers are concentrating on OPAA-based electrochemical biosensors to increase sensor efficiency [60].

Reports are still meager for enhancement of these by nanomaterials-based OPAA encapsulation coupled with a fluorophore or colorimetric agents. The most extensively used enzyme sensors benefit from the large range of pesticides that may be detected with high sensitivity, often at ranges of nM–pM. Methods that have been able to detect the OP compounds in the concentration ranges of μM–mM, LOD needs further improvement considering the hazardous nature of these molecules. Signal-to-noise ratios of up to 3:1 or 2:1 should be the goal of detecting techniques. Additional efforts should be made to improve cost-effectiveness, technique, portability, and detection ease. More precise, reliable, and reproducible equipment is needed, with rapid reaction time. The efficiency and the long-term stability of OPAA are critical issues that are not addressed in most of the studies evaluated. Furthermore, for real-world applications in OP screening, multi-detection abilities should be regarded as essential. At the current state of the art, efforts in this direction should be prioritized.

Several challenges still require to be addressed, such as the considerable distance to be covered in the development of enzymes as potential biomaterials. Genetic modification of OPAA is another way of improving the properties and kinds of substrates the enzyme can hydrolyze. More specific point mutations studies need to be carried out for widening the substrate types. pH detection needs further exploration for OP detection using OPAA. More precise, reliable, and reproducible equipment are needed, with rapid reaction time. The efficiency and long-term stability of OPAA are critical issues that are not addressed in most of the studies evaluated and yet require further investigation. The direction of approach toward the point of care devices and multiplexed analysis of pesticides must be focused on near future.

Various POC approaches were investigated for biosensing through different elements, such as: fluorophore dye [36], quantum dots [61], and nanorods [62] using transducers such as chips, microfluidics, paper-strip, which were analyzed using sensing devices like smartphones, arduino, microprocessor on the phenomenon of calorimetric [36], fluorometric [61], and bioluminescence [62]. These approaches can be used further for the detection of OP compounds through

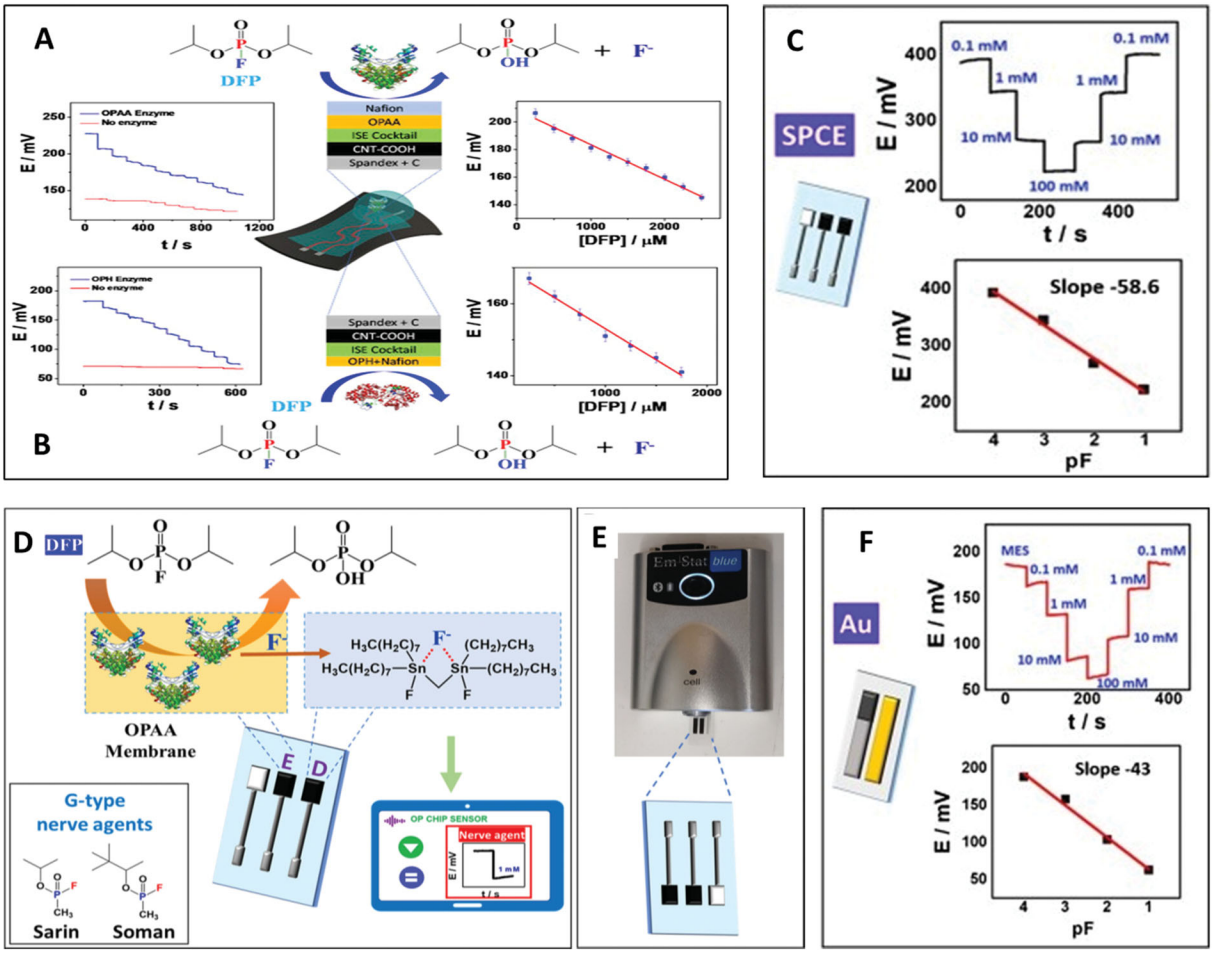

Figure 5. Textile-based POC potentiometric biosensor having (A) immobilized OPAA enzyme on spandex solid contact sensor for potentiometric detection of DFP in the concentration range of 25-2500 µm, with calibration graph with respect to the concentration of DFP as substrate, (B) Immobilized OPH enzyme on spandex solid contact sensor for potentiometric detection with a detection range of 250–2750 µm for DFP as substrate along with calibration curve with respect to concentration. (C) and (F) Evaluation of electrode's substrate SPCEs & Au, along with their calibration plot for optimization of solid-state potentiometric-based fluoride sensor. (D) OPAA-based enzymatic and transduction reaction resulting in hydrolysis of DFP with fluoride ion sensing using potentiometric ISE, which have capability of interfacing to smartphone for rapid & onspot detection of nerve agents. (E) Picture of PalmSens electrochemical device for interfacing with printed electrochemical chips. Reprinted from References [48,47], Copyright (2022), with permission from Elsevier.

OPAA as POC systems. At present, many of the studies are investigated like Arduini et al. proposed device which is a 3D paper origami-based biosensor for the detection of various pesticides by combining different enzyme-based biosensors [63]. Another study in which they immobilized OPAA/OPH enzyme on a textile-based solid contact flexible based sensor, which are fluoride ion-sensitive for the detection of G-nerving agent (sarin and soman) [49] (Figure 5). Other than this, wearable devices for plants, strip-based direct colorimetric detection, electrochemical sensing with the fabrication of nanomaterials should be approached in the future with smart biomaterials which can be 2D structures, such as borophene flatland [64], transitional metal carbides layered structures like MXenes [65] for better stability of the enzyme, as part of biosensing mechanism.

Conclusions

Over the last few decades, enzyme-based biosensors have been greatly explored and established to show qualitative/quantitative measurement of biomarkers. An enzyme-based system to catalyze toxic compounds provides a swift way for their removal and are also environmentally safe ways suitable for detection. Immobilization of OPAA has largely been studied for rapid and high-efficiency hydrolysis of nerve agents. The susceptibility of OPAA to organophosphorus-

containing chemicals, particularly fluoride-containing G-type nerve agents, such as sarin, tabun, cyclosarin, and soman, has sparked attention. MOFs have drawn attention with applications including bio storage, cascade reaction, biosensing, etc. OPAA-FL variant recombinant production and incorporation into a biosensing assay can aid in the quantification of OPs. The use of the OPAA-FL version for the creation of a biosensing assay for ethyl paraoxon, employing a colorimetric and fluorometric estimate, is described in this research. The OPH and OPAA enzymes can hydrolyze OP compounds present in pesticides, and chemical nerve agents. The OPAA is more restricted as it can only catalyze P-F bonds, whereas the OPH can also catalyze: P–O, P–S, and P–CN bonds in OP compounds. Biosensors based on them can directly detect OP compounds without requiring multiple steps but their use in real samples needs improvements. Thus, engineered enzymes and nanomaterial-based biosensors are essential to improve their efficiency. In this field, significant efforts are being made to develop a reliable OPAA biosensor that may be utilized for a variety of purposes in agriculture as well as other fields such as toxic agent detoxification.

Disclosure statement

The authors report no declarations of interest.

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