Catalytic methods for the destruction of chemical warfare agents under ambient conditions

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This *tutorial review* – which should particularly appeal to chemists, biochemists, and molecular biologists interested in catalysis, redox processes, and enzymology – summarizes the recent progress toward developing catalysts capable of destroying one or more of the classical chemical warfare agents under ambient conditions. Specifically, we explore the reactions of sulfur mustard, the G-series of organophosphorus nerve agents including sarin and soman, and the organophosphorus nerve agent, VX. Catalysts range from metal-centered oxidation catalysts to engineered catalytic antibodies.

Introduction

Even before the deadly 1995 release of sarin in the Tokyo subway, researchers were working to develop catalytic methods to destroy sarin and other well known and easily prepared chemical warfare agents. Since 2001, as terrorist activity and regional instability have increased, efforts to mitigate the threat from chemical weapons have also increased in both breadth and depth. This tutorial review will discuss the recent research into and development of catalysts capable of converting chemical warfare agents into much less toxic products under ambient conditions. In some cases mere destruction of a chemical agent is inadequate, since the resulting products can also be highly toxic, and special attention must therefore be paid to the product distribution to ensure that the agent has been effectively detoxified. In the sections below, we review the chemistries of detoxifying reactions for the various classical agents and the recent advances in developing catalytic technologies to facilitate these reactions.

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Catalysts capable of detoxifying chemical warfare agents would find many applications. Generally, they would allow military planners and civilian first responders to prepare for known threats with a minimum of equipment and logistical support. It goes without saying that chemical attacks are expected to be exceptionally rare events, and planners therefore desire countermeasures that are exceptionally small, light, and shelf stable so that they may be widely available when and where they are needed. Detoxifying chemical agents by catalytic reactions under ambient conditions offers the possibility of dramatically improved decontaminants that would be smaller, lighter, more efficient and less expensive than current options. The possibilities are especially appealing in cases where the consumed reactant is either molecular oxygen or water. Detoxifying catalysts could also be used for the destruction of chemical agent stockpiles (known as demilitarization), they could be applied to coatings and fabrics to minimize the need for traditional decontamination of vital equipment, and they could be added to protective garments and masks to increase the protection of emergency responders.

Because the particular chemical agents are rather easy to prepare and are continuing threats, most of the published research has focused on three groups of classical agents: the 'mustard gas' blister agent, the G-series of organophosphorus nerve agents, and the organophosphorus nerve agent, VX. Detoxification and decontamination of these agents has been an ongoing priority for decades.[1] While these classical chemical agents are still a primary research focus, there is also an increased interest in hazardous industrial chemicals and related materials that might be more readily available. For this reason, chemistries with general oxidative or hydrolytic power for detoxifying broad ranges of chemicals are being investigated with increasing intensity.

1. HD

Sulfur mustard, bis-2-chlorethyl sulfide, is a potent vesicant and can be detoxified by dehydrohalogenation to form the chloroethyl vinyl sulfide, by nucleophilic attack to displace an activated aliphatic halogen, or by oxidation (see Fig. 1). Sulfur

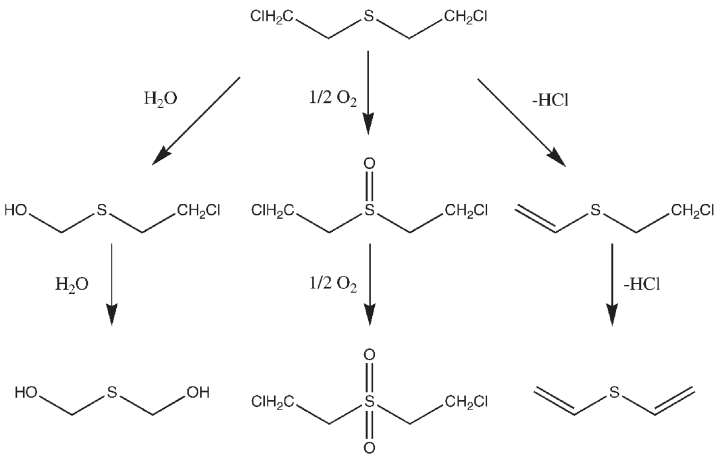

Fig. 1 Some possible detoxifying reactions of sulfur mustard. Note that the reaction pathways are not mutually exclusive, that the hydrolysis product may be oxidized and the sulfone may undergo dehydrohalogenation or hydrolysis. The hydrolysis reactions are also complicated by polymerization resulting from nucleophilic substitution of glycol groups onto unreacted 2-chloroethyl fragments.

mustard is known as agent H and is commonly considered in its distilled form and is often referred to as agent HD; except in very technical discussions, the designation HD is used routinely. HD may be detoxified by partial oxidation to the sulfoxide (HD-O), but further oxidation to the sulfone (HD-O_2) is not welcome since the sulfone (but not the sulfoxide) of HD is also a vesicant (*i.e.* causes blisters). While hydrolysis of HD to produce thiodiglycol does occur in water with a half life estimated to be on the order of 5 minutes, HD has very low solubility in water and drops of the agent dispersed in water tend to form a polymerized crust, limiting the effectiveness of hydrolytic catalysis.[2] The closest approach to promoting hydrolysis of sulfur mustard has probably been the formation of microemulsions that bring the agent and the water nucleophile into close proximity. Most of the work on ambient catalytic detoxification of agent HD has focused on the partial oxidation of the agent and its surrogates.

As with all chemical warfare agents, testing with the actual agent is often very expensive and not universally available, so much of the developmental work on a catalyst or process is often done on a surrogate chemical that possesses many of the important features of the agent. Surrogates, also called simulants or analogs, must be carefully chosen to reasonably substitute for the target agent in the specific reaction pathway or reaction conditions under investigation. In the case of the partial oxidation of sulfur mustard, for example, an appropriate surrogate should clearly have a sulfide bond. Ideally, the surrogate would have similar structural features allowing for the same reaction mechanisms that would detoxify the agent, and would also have similar physical properties like solubility and vapor pressure. Also, one should consider secondary reactivities when possible, allowing for foreseeable side reactions. For example, tetrahydrothiophene may have the required sulfide linkage (although it may be more active toward oxidation), but it does not offer the 2-chloroethyl fragment that can undergo dehydrohalogenation or nucleophilic substitution, which might be important side reactions in an oxidative decontamination. Because one surrogate rarely offers all the important features of the agent, multiple surrogates are often used before, and occasionally instead of, testing with the actual chemical agent. Despite the expense and inconvenience, there is ultimately no substitute for testing with actual chemical agents.

1.1. Aerobic oxidation

An appealing possibility is to develop a catalyst to activate ambient molecular oxygen to selectively convert sulfur mustard, agent HD, to its sulfoxide HD-O. Such a catalyst would allow a rapid and simple response to HD contamination, and might be applied in a prophylactic surface treatment for critical items to render them effectively immune from the threat of HD contamination.

In recent work, Dr Hill's group at Emory University reported a highly active iron-substituted polyoxometalate (POM) catalyst for the selective conversion of 2-chloroethyl ethyl sulfide (CEES) to CEES-O in an acetonitrile solution under an air atmosphere.[3] The exact chemical identity of the catalytic species could not be definitively determined, but it appears to involve an iron-substituted tungsten-based Keggin structure with an associated $H(ONO_2)_2$ fragment. In one experiment using a pure oxygen atmosphere, oxygen consumption was monitored by a digital manometer and was found to track nicely with the observed CEES conversion. The details of the catalytic reactions are uncertain and may involve gaseous NOx species, since the activity of the catalyst declines upon storage (activity reduced by half in about 50 days) and reactions in vials of differing headspace volumes suggest that a gas phase reaction is significant. Furthermore, this work explored neither the effects of the acetonitrile solvent nor the particular oxidation of agent HD, so the extent to which the results would translate to the neat chemical agent is unknown. Nonetheless, this effort suggests the intriguing possibility of developing sufficiently active and selective catalysts to passively detoxify HD by converting it to HD-O using only ambient molecular oxygen.

Also using ambient oxygen, Noradoun and colleagues developed an iron-based oxidation catalyst that used zero-valent iron in combination with ethylenediaminetetraacetic

acid (EDTA) and air to initiate a radical-based Fe^{3+}/Fe^{2+} redox cycle. They demonstrated the ability to degrade malathion into low molecular weight acids.[4] This aqueous oxidation chemistry could conceivably be used to detoxify agent HD as well as the nerve agents by effectively mineralizing them. Unfortunately, the catalytic system was also found to rapidly degrade the EDTA chelating agent and would almost certainly attack other organic materials it might contact without significant selectivity, making it less desirable as a decontaminant.[5]

1.2. Oxidation using peroxides

Although less appealing than aerobic oxidation using ambient molecular oxygen as the terminal oxidant, oxidation using peroxides is a widely known approach to chemical decontamination. Hydrogen peroxide is an ingredient in a decontaminant formulation known as Decon Green that uses molybdate and carbonate to activate the peroxide,[6] and hydrogen peroxide is also featured in several commercial decontaminants. A group at the University of Florida reported that carbonate catalysts of hydrogen peroxide, as counterions to cationic surfactants, showed dramatic acceleration for the oxidation of aryl sulfides in aqueous systems.[7] Borates are also known activators of hydrogen peroxide, and a recent paper from Northumbria University describes the specific peroxoborate chemistry of sulfide oxidations.[8] Both of these reports also compare the reactivity of their particular peroxide activator with that of other hydrogen peroxide derivatives, including peroxymonosulfate.

In addition to hydrogen peroxide, other peroxide species have also been used to detoxify surrogates for chemical agents. For example, a group at the University of Vermont identified a series of vanadium oxide catalysts, supported on mesoporous silica, that catalyze the oxidation of CEES to CEES-O by *tert*-butyl hydroperoxide, with only a small fraction of the product being further oxidized to the undesired sulfone, CEES-O_2.[9] They found that increasing the vanadium loading in the catalyst led to larger vanadium oxide clusters which were less potent, but more selective catalysts for the desired partial oxidation. Unfortunately, all of the tested catalysts produced a significant fraction of the undesired sulfone, suggesting that under the conditions of excess oxidant that would be used in any sulfur mustard decontamination scenario, the resulting products would ultimately retain much of the blistering potential of the original agent HD.

Barker and Ren, then at the University of Miami, reported the oxidation of sulfides with *tert*-butyl hydroperoxide catalyzed by mononuclear manganese-based oxidation catalysts.[10] Here also, sulfone products were commonly produced. Additionally, several side reactions were noted, including the formation of disulfides and the dehydrohalogenation of 2-chloroethyl fragments to form vinyl sulfoxides or vinyl sulfones. The production of a disulfide product suggested a single-electron transfer reaction mechanism with a sulfenium radical intermediate as indicated in Fig. 2.

An Israeli group successfully used hydrogen peroxide in the enzyme-mediated haloperoxidation of chemical agents, including agent HD. Chloroperoxidase enzyme obtained from the filamentous fungus *Caldariomyces fumago*, in combination with urea hydrogen peroxide (50 mM) and sodium chloride (0.5 M), was found to rapidly oxidize sulfur mustard in solution, producing both the HD sulfoxide and the HD sulfone, in addition to the 2-chloroethyl vinyl sulfoxide and other products.[11] However, as with other potent non-specific oxidants, further treatment would be needed to degrade the HD sulfone due to its vesicant properties. The active oxidant was not identified in this work, but was very likely an oxidized halogen species because the efficacy of the enzyme system was strongly and non-linearly dependent on the concentration of chloride salt and because hydrogen peroxide had only a small effect on the HD in the absence of the chloroperoxidase enzyme. This same enzyme system was also found to rapidly degrade agent VX in solution as well, suggesting that it may lead to a generic decontaminant if the enzymes can be stabilized for long active life in storage and in use.

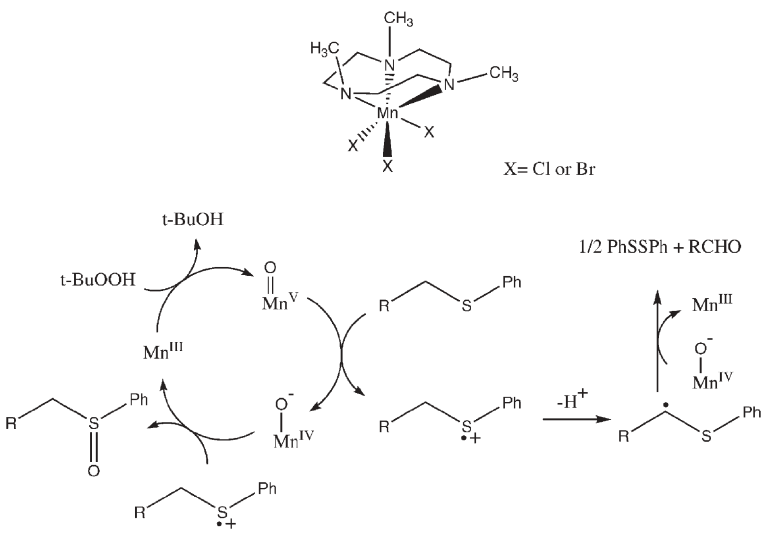

Fig. 2 Mononuclear Mn catalyst (top) and proposed mechanism of sulfide oxidation to produce both sulfoxide and disulfide products through a sulfenium radical cation intermediate.

1.3. Photooxidation

There has been considerable effort spent developing a variety of photocatalysts that generate oxidizing species in the presence of light; titanium dioxide is a common active ingredient. Generally, photocatalysts have limited utility and significance from a military perspective since a premium is put on nocturnal operations, and even civilian first responders must be protected both day and night. Recent efforts by Lombardi at Ventana Research appear to have succeeded in developing long-lived reactive intermediates, effectively storing some of the reactive capacity of the photocatalysts and slowly releasing the activated singlet oxygen produced by the photocatalysts.[12] While this ability to store the reactive intermediates may expand the possibilities for detoxifying chemical agents by photochemistry, applications will undoubtedly remain in which the lack of light is a limiting factor.

1.4. Dehydrohalogenation

Nanoparticulate calcium oxide appears to have some catalytic activity for the dehydrohalogenation of HD.[13]Partially hydrated nanoparticulate CaO exposed to agent HD produced a large fraction of divinyl sulfide with an agent half life on the order of 4 hours. It is not clear to what extent the reaction with CaO was truly catalytic, since an excess of CaO was used and islands of $CaCl_2$ (which is a known catalyst for such dehydrohalogenation reactions) were likely formed during the reaction. It is also interesting to note that surface hydration must play an important role in the reaction, since dried nanoparticulate CaO reacted more slowly than partially hydrated nanoparticulate CaO and produced both a much smaller fraction of vinyl elimination products and a much greater fraction of glycol products (adsorbed alkoxides). Further, the common mustard simulant CEES only reacted slowly, even on the partially hydrated nanoparticulate calcium oxide surface, and produced mostly alkoxides.

2. G-agents

The G-class nerve agents are organophosphorus compounds that exert their neurotoxic effects by inhibiting acetylcholinesterase enzymes. As a class, G-agents have relatively high vapor pressures, are moderately soluble in water and hydrolyze in water with half lives on the order of a day.[2] For these reasons, decontamination of G-agents is often considered to be less of a technical challenge than decontamination of the less volatile, less soluble, and less labile agents. Nonetheless, considerable work has gone into developing catalytic methods to detoxify these highly neurotoxic agents under ambient conditions, and this work has been largely successful. Fig. 3 shows the structures of the most common G-agents and a typical hydrolysis reaction.

2.1. Enzymatic hydrolysis

Several enzymes have been shown to accelerate the hydrolysis of the G-agents and related compounds, including organophosphorus pesticides and, in some cases, agent VX. Two recent reviews have been published, "Bacterial detoxification of organophosphate nerve agents"[14] and the exceptionally thorough "Microbial degradation of organophosphorus compounds",[15] covering much of the effort in detoxifying nerve agents by hydrolysis since the vast majority of such hydrolysis catalysts are bacterially derived. The reader is referred to these reviews for detailed discussions of the topic.

One limitation to enzymatic hydrolysis of the G-agents is that, as shown in Fig. 3, the hydrolysis products are acidic and the enzyme activity typically drops dramatically at pH values below about 6. Acid by-products also occur during the hydrolysis of agents VX and HD. Using hydrolysis catalysts to decontaminate or detoxify relatively large quantities of agent therefore requires a buffer to maintain the pH of the reaction in the neutral to slightly alkaline range. This buffering capacity is commonly provided by stoichiometric acid–base pairs, but could also be induced by a competing enzymatic reaction such as the formation of ammonia from urea by urease to neutralize the acidic organophosphorus hydrolase (OPH) hydrolysis products on demand.[16] Urease conversion of urea to ammonia had a maximum activity around pH 6.5, while the OPH enzyme used had a maximum activity around pH 8.5, so the competing reactions stabilized the pH values of the test solutions until one of the reagents was largely consumed or until significant product inhibition slowed one of the reactions. By changing the relative concentrations of the two enzymes, the authors demonstrated the ability to achieve and maintain a predicted pH set point by this dynamic buffering mechanism, and without the need for classical acid–base buffers.

Another practical limitation to enzymatic hydrolysis relates to the mass transport of the agent to the active enzyme. Whether the hydrolysis enzymes are native to the bacteria cultured to express them or are engineered into the cultured

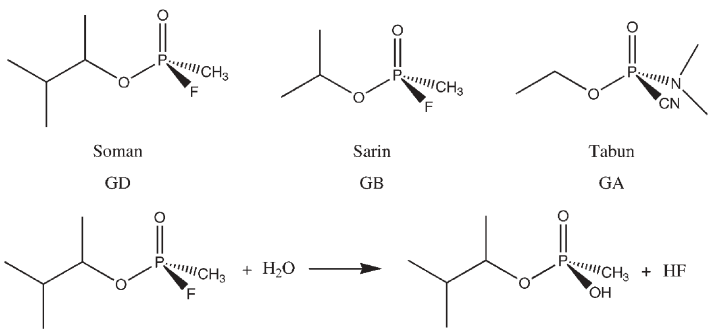

Fig. 3 G-agent structures and the soman hydrolysis reaction. Note the acidic reaction products.

organism, the active enzymes are typically confined to the intracellular matrix. To reduce mass transport limitations inherent in having the enzyme and the target substrate on opposite sides of cell walls, studies on enzymatic detoxification have usually used purified enzymes or cell lysate. An alternative approach to improving mass transfer, attaching the catalytic enzyme to the surface of the bacteria and exposing the whole cell to the substrate, has been explored for the past decade by Chen and Mulchandani and their colleagues at the University of California Riverside. By coupling the DNA coding for OPH enzymes to DNA coding for particular proteins expressed on the cell surface, they have been able to produce cell lines that express the fusion proteins on their surfaces and retain their catalytic activity. They have produced a variety of bacterial lines with surface-expressed OPH enzymes, and have recently collaborated with experts in yeast surfaces to develop a strain of bakers' yeast with surface-displayed OPH using a similar genetic engineering approach.[17]

Chen and Mulchandani have also engineered *E. coli* bacteria that express both the OPH and a cellulose-binding domain on the cellular surface, then used the cellulose-binding domain to attach the whole cells to a hollow fiber bioreactor.[18] An incomplete monolayer of cells adhered strongly to the cellulose surface of the bioreactor and maintained 90% of its activity for paraoxon hydrolysis over the course of seven weeks and at least 15 repeated uses. The bioreactor was regenerated to about 90% of its initial functionality by stripping the cells from the membrane using sodium dodecyl sulfate, which denatures the cellulose-binding proteins, followed by rinsing and loading a new set of cells onto the surface. This work combined their previous genetic engineering of the bacterial surface proteins with more traditional chemical engineering considerations for the large scale detoxification of nerve agents.

2.2. Non-enzymatic hydrolysis

In addition to enzymatic catalysts, iodosylcarboxylates also appear to promote catalytic hydrolysis of the G-agents and other selected organophosphates. The mechanism appears to be nucleophilic substitution at phosphorus, followed by hydrolytic attack at the central iodine to displace the phosphorus center from the iodosylcarboxylate (Fig. 4). A thorough review of the chemistry for this and related nucleophiles (including other alpha-effect nucleophiles and metal complexes) was published in 2002 and is recommended for further reading.[19]

3. VX

The catalytic detoxification of chemical agent VX under ambient conditions presents a particular challenge to chemists because the compound is much less labile than the G-agents, and uncatalyzed hydrolysis of VX does not occur at useful rates in pH-neutral solutions. Additionally, one of the possible hydrolysis products (EA-2192) is both much less reactive toward further nucleophilic attack and nearly as toxic as VX itself.

As Fig. 5 suggests, agent VX is susceptible to nucleophilic attack at several places, possibly cleaving C–O or C–S bonds in addition to the more commonly considered P–O and P–S cleavage. Different pathways may be more or less important depending on the nucleophile, catalyst, and reaction conditions. For example, P–S bond cleavage dominates in the autocatalytic hydrolysis of VX with stoichiometric amounts of water[20] but P–O or C–O cleavage is significant in highly alkaline aqueous solutions and produces about 15% EA-2192. In their very thorough review of chemical warfare agent degradation products, Munro *et al.* note the appearance of 2-diisopropylaminoethanol and bis(2-diisopropylaminoethyl)-sulfide, two degradation products that can best be explained by nucleophilic attack at carbon (by water and by diisopropylaminoethylthiol, respectively), cleaving the S–C bond.[2] Furthermore, the presence of 2-diisopropylaminoethyl ethyl sulfide in ton containers of VX suggests that C–O cleavage can also occur through nucleophilic attack by diisopropylaminoethylthiol (produced by previous hydrolysis of another

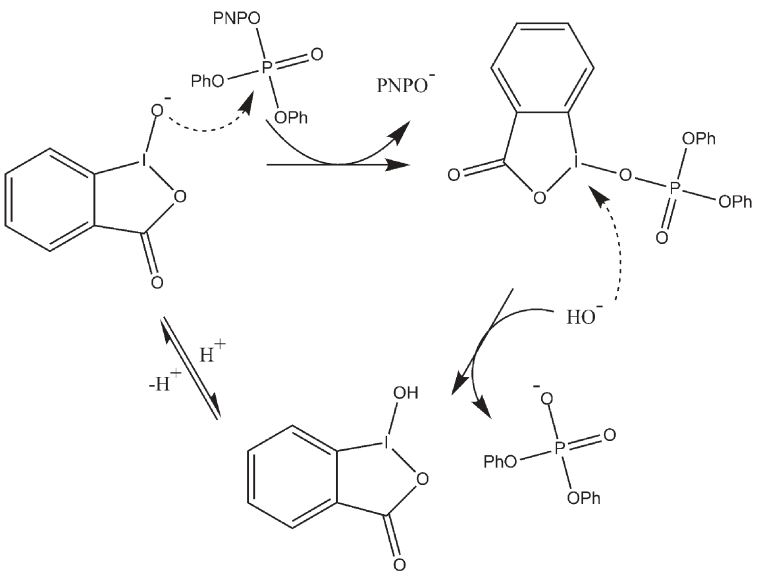

Fig. 4 Mechanism of iodosylcarboxylate hydrolysis of a *p*-nitrophenyl phosphate.

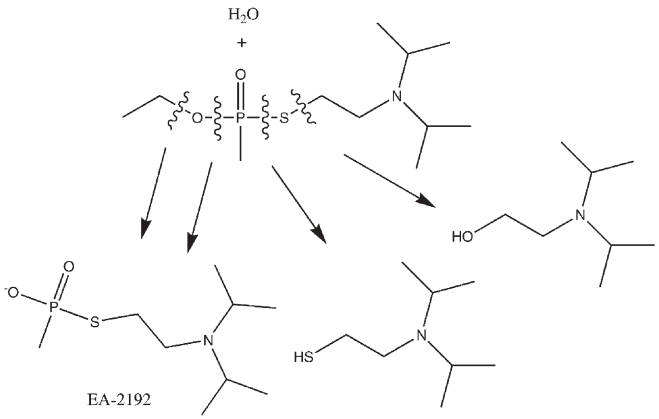

Fig. 5 Possible initial hydrolysis products of agent VX.

molecule) at the ethoxy carbon. These mechanisms of nucleophilic attack at carbon are suggested by the available data and may be of minor importance in some systems, but with the exception of the study on VX with stoichiometric amounts of water mentioned above (which included experiments with $H_2{}^{18}O$) conclusive data have not been reported for most reaction systems.

3.1. Oxidation

In the mid 1990s Yang noted that hydrogen peroxide can be a useful decontaminant for agent VX, particularly at slightly alkaline pH values where a fraction of the peroxide is deprotonated into the nucleophilic hydroperoxide anion.[21] This oxidizing α-effect nucleophile selectively cleaved the targeted P–S bond, but required about a 20-fold molar excess of peroxide to react with all the VX, since the initial reaction products were also susceptible to further oxidation. The peroxide activators discussed in the section on agent HD (bicarbonate, borate, molybdate, sulfate) are also expected to facilitate peroxide reactions with agent VX.

Additionally, a new type of peroxide activator developed by Collins's group at Carnegie Mellon has recently been demonstrated against a series of organophosphorus pesticide analogs of VX.[22] These catalysts, iron-centered tetraamido macrocyclic ligands (Fe-TAML), are water soluble and serve to activate hydrogen peroxide toward both hydrolysis and oxidation, resulting in deep oxidation (near mineralization) of the pesticides in a matter of hours. Raising the pH value of the reaction solution from 8.0 to 10.0 allowed for an increased concentration of hydroperoxide, HO_2^- (pK_a of $H_2O_2 \approx 11.5$), which was credited with accelerating initial hydrolysis over oxidation and leading to a more desirable product distribution. However, the TAML catalysts also appear to catalyze their own degradation in the presence of excess peroxide, so long term storage may require a combination of separating the two components and gradually releasing the Fe-TAML catalysts into the peroxide matrix.

3.2. Haloperoxidation

The enzymatic haloperoxidation noted above for agent HD was also tested against agent VX. The aqueous reaction was buffered at pH 2.75, so the peroxide was certainly not the hydrolytically active hydroperoxide anion described by Yang. The activity was strongly dependant on the concentration of chloride ion, indicating an oxidized chlorine intermediate, such as hypochlorous acid or elemental chlorine.[11] Using either the urea hydrogen peroxide complex or a combination of glucose and glucose oxidase as a source for peroxide, the authors were able to demonstrate rapid breakdown of agent VX (*e.g.* $t_{1/2}$ of 8 s and 30 s). Unlike in their work with agent HD, the VX decomposition products were not reported.

3.3. Methanolysis

A group at Queen's University in Ontario has developed a series of catalysts to detoxify agent VX by methanolysis instead of hydrolysis. They have studied and confirmed the mechanism of the reaction for both Zn^{2+} and La^{3+} based methanolysis catalysts, which involves coordination of the substrate to the metal ion and subsequent intramolecular nucleophilic attack by a coordinated methoxide ligand,[23] very similar to the understood mechanism of hydrolysis by the OPH enzymes mentioned above (Fig. 6). The methanolysis reaction has an advantage over hydrolysis for the decontamination of agent VX in that P–O bond cleavage – effectively substituting methoxy for ethoxy – does not result in a significantly less labile product and the P–S bond will also ultimately be cleaved. Although encouraging kinetics have been observed for analogous compounds and the trend indicates that VX would

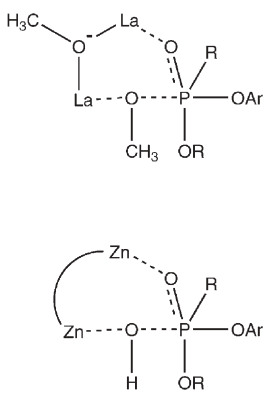

Fig. 6 Intermediates of binuclear La^{3+} catalyzed methanolysis reaction (top) and binuclear Zn^{2+} OPH-mediated hydrolysis reaction.

also be decontaminated in a reasonable time,[24] the technology has the obvious limitation of requiring methanol as solvent and reactant. Also, one must question the stability of these catalysts in the presence of water, and, consequently, their utility in field applications.

3.4. Hydrolysis

Where it is technically feasible, detoxification by hydrolysis tends to be preferred since the water reactant is second only to atmospheric oxygen in its availability. The published research into catalyzing the hydrolysis of agent VX has taken three main approaches: tailoring the existing OPH enzymes to better match the VX substrate, positioning nucleophilic moieties near the VX binding site in cholinesterase enzymes to induce catalytic activity, and developing catalytic activity in antibodies by inoculating the immune system with fragments resembling the desired VX hydrolysis transition state. Reported successes in degrading agent VX have been modest, and even though the production of EA-2192 is both possible and undesired, hydrolysis product distributions have rarely been reported.

3.4.1. Mutagenesis of OPH enzymes. Several of the organophosphorus hydrolase enzymes that are such good catalysts for G-agent hydrolysis have also been tested for their ability to hydrolyze VX. Generally, their activity has been low. To improve the activity of OPH enzymes toward VX hydrolysis, Gopal *et al.* conducted site directed mutagenesis of the wild type enzyme to induce four types of mutations to specifically tailor the enzyme active site to accommodate and hydrolyze agent VX.[25] These efforts were only able to improve the relative VX hydrolysis rate by about 33% over the native OPH enzyme. Importantly, the authors also observed no correlation between the activity of these enzymes on VX and any of five other less toxic chemicals commonly used as analogs for hydrolysis reactions, again suggesting that the use of analogs for the development of catalysts to detoxify agent VX is probably best left to the early proof-of-principle stage of research.

In addition to this sort of targeted site-directed mutagenesis described above, an excellent paper by a group at the Australian National University describes the directed evolution of an OPH enzyme by repeated mutation and selection for their activity hydrolyzing three organophosphate pesticides which were rapidly degraded by an organophosphate degrading enzyme (OPDA) but only slowly by the wild type OPH.[26] They found that the evolved OPH mutants, which had similar reactivity to the wild OPDA, differed from the wild type OPH enzyme in many of the same amino acid substitutions by which that the wild type OPDA differed from the wild OPH, and that these similarities between the evolved OPH and the wild type OPDA were especially pronounced around the active sites. They also found some evolved amino acid alterations in parts of the OPH enzyme that were more remote from the active site, suggesting more subtle effects that would be difficult to predict and engineer by site-directed mutagenesis. Similar directed evolution of existing OPH or OPDA enzymes to directly improve the kinetics of VX hydrolysis remains a promising area for future development.

3.4.2. Mutagenesis of butyrylcholinesterase. Both acetylcholinesterase (AChE) and butyrylcholinesterases (BuChE) are strongly inhibited by nerve agents binding to the active sites of the enzymes. In the mid 1990s, an effort was made to transform the binding site to a site of catalytic hydrolysis for nerve agents by introducing a nucleophilic histidine residue into the active site of human BuChE by site-directed mutagenesis.[27] One of the mutants, G117H, was found to hydrolyze agents GB and VX at modest rates. These agents would rapidly bind to the mutant enzyme, but the histidine residue in the oxyanion hole of the active site would allow the complex to slowly dephosphorylate instead of locking the phosphorylated fragment into the active site as happens with the wild type BuChE enzyme. Subsequent work using a variety of organophosphorus compounds demonstrated the breadth of hydrolytic activity from this single mutant.[28]

This same human G117H BuChE mutant was later induced in transgenic mice, where it provided a stable genetic line with a significant resistance to the toxic effects of the organophosphorus (OP) compound and VX analog echothiophate.[29] The mechanism of the induced resistance to OP poisoning was not clear, however, since inhibition of the native AChE and BuChE enzymes by the challenge echothiophate was apparently not affected by the G117H BuChE and the known kinetics of echothiophate hydrolysis suggested that the hydrolysis of echothiophate by the G117H mutant was too slow to significantly lower the effective concentration of the OP during the course of the experiment. Further, although the G117H mutant did retain some esterase activity and was more resistant to inhibition by OP compounds, the available esterase activity of the G117H BuChE was only about 20% of the inhibited native AChE activity, leading to doubts that the significant protective effect of the G117H mutant was due to its residual esterase activity.

3.4.3. Catalytic antibodies. Several research groups have worked to enlist the variety and precision of the immune response to develop antibody proteins containing catalytic sites for the desired reactions. In the case of nerve agent hydrolysis, the produced catalytic antibodies could conceivably be used to provide persons at risk of exposure to nerve agents with significant resistance to their toxic effects. Removed from the biological system, such catalytic antibodies may also provide a chemically gentle method for decontaminating certain equipment that might be sensitive to the oxidative and/or nucleophilic environment typical of modern decontaminating solutions. Furthermore, the catalytic antibodies developed might be isolated and characterized in sufficient detail to elucidate their specific mechanisms of catalytic activity, potentially adding significantly to our fundamental understanding of agent chemistries.

In its simplest form, the development of catalytic antibodies involves exposing the immune system to a protein-bound hapten, a small molecular fragment that in these cases resembles the transition state of the reaction to be catalyzed and may also include polar or charged groups to elicit complimentary structures in the catalytic pocket. In responding to the protein–hapten conjugate, the immune system develops a variety of antibodies, some of which may contain

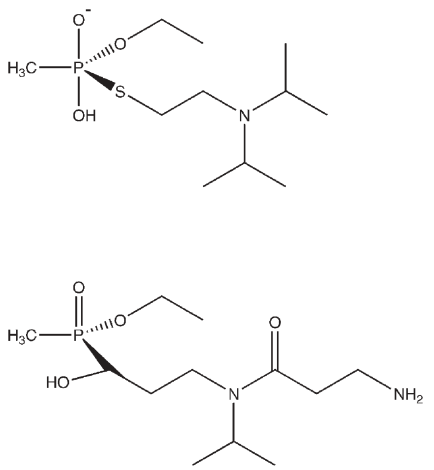

Fig. 7 Imagined intermediate in VX hydrolysis (top) and the corresponding hapten synthesized for immunological engineering of catalytic hydrolysis. The primary amino group is used to bind the hapten to a carrier protein prior to inoculation.

enzymatic pockets fitting the hapten fragment, and some of these may be catalytic for the desired reaction. Several variations on this theme have been explored, with varying success, as described below.

In their recent CSR tutorial review, Eubanks *et al.* gave a good summary of their work in this field, using the hydrolysis of paraoxon as a model reaction.[30] In their efforts to induce catalytic activity in antibodies by exposure to haptens resembling the targeted transition state for paraoxon hydrolysis, they were able to accelerate the desired hydrolysis reaction by factors of about 50 to 500. By adding functionality to their hapten design to make it capable of binding an additional nucleophile once in the antibody pocket, they were able to increase the rate of antibody reactivity with their target substrate, but the observed reactivity was not catalytic hydrolysis in that the reactive site appeared to bind stoichiometrically and covalently to the paraoxon substrate.[31]

A research team in France has worked since the late 1990s to develop catalytic antibodies specifically for the hydrolytic detoxification of agent VX. Their first targets were antibodies designed to stabilize the targeted transition state wherein a water molecule approaches the central phosphorus atom to create a pentacoordinate phosphorus-centered intermediate for the $S_N2(P)$ reaction.[32] These efforts, using the hapten shown in Fig. 7, led to catalytic antibodies with specific and significant catalytic activity. Although a well developed set of control experiments demonstrated that the catalytic activity was due to the elicited antibodies, was inhibited by the haptens that helped shape the catalytic cavity, and was specific to agent VX over other analogous compounds, the observed catalytic activity was too low to provide useful products.

Recently, this French group has focused on more elaborate chemical engineering of catalytic antibodies using rather complicated haptens in multi-step preparations of the active antibodies. Specifically targeting P–S bond cleavage in agent VX, they sought to develop a catalytic site that both included a specific reactive nucleophile and oriented the nucleophile toward back-side nucleophilic attack of the substrate to facilitate the desired $S_N2(P)$ displacement reaction between the built-in oxime nucleophile and the bound VX to selectively displace the thiol leaving group. The nucleophile–substrate adduct itself would then be cleaved by hydrolysis, regenerating the catalytic site. Fig. 8 shows their overall strategy, including initial immunization with a hapten containing not only a transition state analog but also a fragment resembling a nucleophilic residue which will later be covalently bonded adjacent to the substrate binding site to provide a catalytic moiety in the proper orientation and proximity to facilitate the catalysis of the bound substrate.[33] In practice, this strategy has been difficult to implement, since the hapten initially designed was both moderately challenging to synthesize and was ultimately not sufficiently stable under the biological conditions required for immunology.[34] Their efforts in this area continue.

Although it does not specifically address chemical warfare agents, a review by Ostler *et al.* provides a good introduction to the development and applications of polyclonal catalytic antibodies.[35] They note two important trends: despite the inherent variability of the polyclonal antibodies, mixtures of antibodies consistently display the kinetic behavior of just a single type of catalytic site; and the ability of the antibodies to bind the target molecules develops relatively early in the immune response but does not correlate strongly with the catalytic activity that tends to develop later in the immune response.

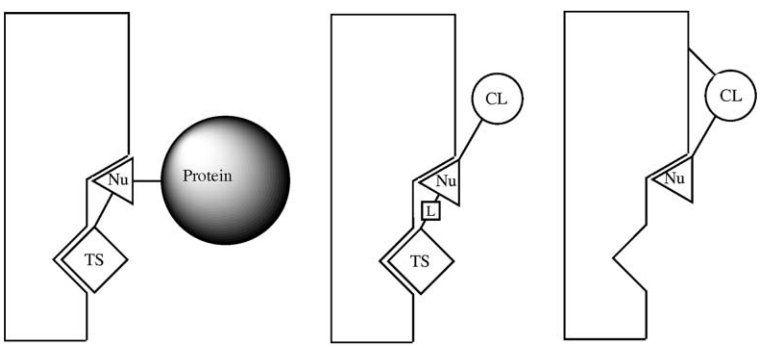

Fig. 8 Engineering a catalytic antibody. The catalytic cavity is initially formed by a hapten containing both a nucleophilic fragment and a hydrolysis transition state analog. Then this cavity is filled by a smaller molecule containing both a cross linking fragment (CL) to bind the nucleophile in place in the catalytic cavity and a cleavable linkage (L) between the transition state analog and the nucleophile.

Conclusions

Progress in both oxidation and hydrolysis catalysts offer the promise of improved protection from and response to releases of chemical warfare agents. In all cases, especially with sulfur mustard and the nerve agent VX, testing against chemical analogs and simulants can provide only limited developmental guidance and cannot replace tests with the actual chemical agents. For sulfur mustard, agent HD, oxidation is a promising detoxifying method but the desired selective partial oxidation has been difficult to achieve. For the G-series nerve agents, enzymatic hydrolysis offers high rates of detoxification, and biomolecular and chemical engineering have been applied to improve access of the substrates to the enzymes. For all enzymatic hydrolysis reactions, the pH of the reaction matrix must be maintained in the useful range for the enzymes. Iodosobenzoate, an alpha-effect nucleophile, is also reported to serve as a hydrolysis catalyst for G-agents. Agent VX can be detoxified by catalytic hydrolysis, methanolysis, or oxidation most likely coupled with nucleophilic substitution. Hydrolytic catalysis is particularly attractive in that the water reagent is practically ubiquitous, but efforts to modify OPH enzymes and to generate new catalysts from cholinesterases or from engineered antibodies have so far failed to produce sufficiently active catalysts.

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References

1 Y.-C. Yang, J. A. Baker and J. R. Ward, *Chem. Rev.*, 1992, **92**, 1729–1743.
2 N. B. Munro, S. S. Talmage, G. D. Griffin, L. C. Waters, A. P. Watson, J. F. King and V. Hauschild, *Environ. Health Perspect.*, 1999, **107**, 933–974.
3 N. M. Okun, J. C. Tarr, D. A. Hilleshiem, L. Zhang, K. I. Hardcastle and C. L. Hill, *J. Mol. Catal. A: Chem.*, 2006, **246**, 11–17.
4 C. E. Noradoun, C. S. Mekmaysy, R. M. Hutcheson and I. F. Cheng, *Green Chem.*, 2005, **7**, 426–430.
5 C. E. Noradoun and I. F. Cheng, *Environ. Sci. Technol.*, 2005, **39**, 7158–7163.
6 G. W. Wagner and Y.-C. Yang, *Ind. Eng. Chem. Res.*, 2002, **41**, 1925–1928.
7 H. R. Yao and D. E. Richardson, *J. Am. Chem. Soc.*, 2003, **125**, 6211–6221.
8 D. M. Davies, M. E. Deary, K. Quill and R. A. Smith, *Chem.–Eur. J.*, 2005, **11**, 3552–3558.
9 C. R. Ringenbach, S. R. Livingston, D. Kumar and C. C. Landry, *Chem. Mater.*, 2005, **17**, 5580–5586.
10 J. E. Barker and T. Ren, *Tetrahedron Lett.*, 2005, **46**, 6805–6808.
11 G. Amitai, R. Adani, M. Hershkovitz, P. Bel, I. Rabinovitz and H. Meshulam, *J. Appl. Toxicol.*, 2003, **23**, 225–233.
12 US Pat., 7 259 122, 2007.
13 G. W. Wagner, O. B. Koper, E. Lucas, S. Decker and K. J. Klabunde, *J. Phys. Chem. B*, 2000, **104**, 5118–5123.
14 F. M. Raushel, *Curr. Opin. Microbiol.*, 2002, **5**, 288–295.
15 B. K. Singh and A. Walker, *FEMS Microbiol. Rev.*, 2006, **30**, 428–471.
16 A. J. Russell, M. Erbeldinger, J. J. DeFrank, J. Kaar and G. Drevon, *Biotechnol. Bioeng.*, 2002, **77**, 352–357.
17 K. Takayama, S.-I. Suye, K. Kuroda, M. Ueda, T. Kitaguchi, K. Tsuchiyama, T. Fukuda, W. Chen and A. Mulchandani, *Biotechnol. Prog.*, 2006, **22**, 939–943.
18 A. A. Wang, W. Chen and A. Mulchandani, *Biotechnol. Bioeng.*, 2005, **91**, 379–386.
19 H. Morales-Rojas and R. A. Moss, *Chem. Rev.*, 2002, **102**, 2497–2521.
20 Y.-C. Yang, L. L. Szafraniec, W. T. Beaudry, D. K. Rohrbaugh, L. R. Procell and J. B. Samuel, *J. Org. Chem.*, 1996, **61**, 8407–8413.
21 Y.-C. Yang, *Acc. Chem. Res.*, 1999, **32**, 109–115.
22 A. Chanda, S. K. Khetan, D. Banerjee, A. Ghosh and T. J. Collins, *J. Am. Chem. Soc.*, 2006, **128**, 12058–12059.
23 R. E. Lewis, A. A. Neverov and R. S. Brown, *Org. Biomol. Chem.*, 2005, **3**, 4082–4088.
24 S. A. Melnychuk, A. A. Neverov and R. S. Brown, *Angew. Chem., Int. Ed.*, 2006, **45**, 1767–1770.
25 S. Gopal, V. Rastogi, W. Ashman and W. Mulbry, *Biochem. Biophys. Res. Commun.*, 2000, **279**, 516–519.
26 H. Yang, P. D. Carr, S. Y. McLoughlin, J. W. Liu, I. Horne, X. Qiu, C. M. J. Jeffries, R. J. Russell, J. G. Oakeshott and D. L. Ollis, *Protein Eng.*, 2003, **16**, 135–145.
27 C. B. Millard, O. Lockridge and C. A. Broomfield, *Biochemistry*, 1995, **34**, 15925–15933.
28 O. Lockridge, R. M. Blong, P. Masson, M.-T. Froment, C. B. Millard and C. A. Broomfield, *Biochemistry*, 1997, **36**, 786–795.
29 Y. Wang, A. T. Boeck, E. G. Duysen, M. Van Keuren, T. L. Saunders and O. Lockridge, *Toxicol. Appl. Pharmacol.*, 2004, **196**, 356–366.
30 L. M. Eubanks, T. J. Dickerson and K. D. Janda, *Chem. Soc. Rev.*, 2007, **36**, 458–470.
31 Y. Xu, N. Yamamoto, D. I. Ruiz, D. S. Kubitz and K. D. Janda, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4304–4307.
32 P. Vayron, P.-Y. Renard, F. Taran, C. Créminon, Y. Frobert, J. Grassi and C. Mioskowski, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 7058–7063.
33 S. Dias, F. Jovic, P.-Y. Renard, F. Taran, C. Créminon, C. Mioskowski and J. Grassi, *J. Immunol. Methods*, 2002, **269**, 81–98.
34 F. Jovic, L. Louise, C. Mioskowski and P.-Y. Renard, *Tetrahedron Lett.*, 2005, **46**, 6809–6814.
35 E. L. Ostler, M. Resmini, K. Brocklehurst and G. Gallacher, *J. Immunol. Methods*, 2002, **269**, 111–124.

