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Human paraoxonase double mutants hydrolyze V and G class organophosphorus nerve agents

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ABSTRACT

Variants of human paraoxonase 1 (PON1) are being developed as catalytic bioscavengers for the organophosphorus chemical warfare agents (OP). It is preferable that the new PON1 variants have broad spectrum hydrolase activities to hydrolyze both G- and V-class OPs. H115W PON1 has shown improvements over wild type PON1 in its capacity to hydrolyze some OP compounds. We improved upon these activities either by substituting a tryptophan (F347W) near the putative active site residues for enhanced substrate binding or by reducing a bulky group (Y71A) at the periphery of the putative enzyme active site. When compared to H115W alone, we found that H115W/Y71A and H115W/F347W maintained VX catalytic efficiency but showed mixed results for the capacity to hydrolyze paraoxon. Testing our double mutants against racemic sarin, we observed reduced values of K_M for H115W/F347W that modestly improved catalytic efficiency over wild type and H115W. Contrary to previous reports, we show that H115W can hydrolyze soman, and the double mutant H115W/Y71A is nearly 4-fold more efficient than H115W for paraoxon hydrolysis. We also observed modest stereoselectivity for hydrolysis of the P(−) stereoisomer of tabun by H115W/F347W. These data demonstrate enhancements made in PON1 for the purpose of developing an improved catalytic bioscavenger to protect cholinesterase against chemical warfare agents.
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1. Introduction

The main goal in combating the effects of organophosphorus compounds (OP) in the body is to protect acetylcholinesterase (AChE) from inhibition in both the peripheral (PNS) and central nervous system (CNS). This paradigm has been the cornerstone for therapeutic intervention against OP intoxication since the inception of OP countermeasures. Blood vessels act as the collection and distribution conduit for the nerve agent and can be considered as the best compartment for therapeutic intervention upon intoxication. Current chemotherapeutic treatments for OP intoxication promote survivability but do not afford complete protection from CNS effects such as cognitive deficits and, particularly in exposures involving soman, neuronal pathology induced by seizures. Traditional pharmacological approaches to alleviate the effects of OP poisoning have reached a point where only modest incremental therapeutic improvements are being realized. An alternative approach to protect against neurological impairments is needed.

The use of stoichiometric bioscavengers, such as human butyrylcholinesterase (BuChE), protects against the toxic effects of nerve agents alone with no cognitive impairment, as assessed by behavioral responses [1]. Importantly, stoichiometric bioscavengers, which includes BuChE, demonstrate no unwanted side effects when administered as a pre-treatment to rhesus monkeys [1]. Specifically, sophisticated operant performances requiring visual recognition memory are unaffected by a large bolus dose of human BuChE, which was large enough to protect against several LD_{50}s of nerve agent [2,3]. While these results are encouraging, a second generation of bioscavengers that have catalytic activity against some OPs, such as human paraoxonase 1 (PON1), provide a unique platform on which to engineer desired activities for improved rates of OP binding and hydrolysis over those rates achieved by wild type BuChE alone.

Human serum paraoxonase 1 (EC 3.1.8.1) is a calcium-dependent enzyme secreted by the liver into the bloodstream. It is associated with high-density lipoproteins and may function to reduce the oxidation level of low-density lipoproteins [4]. Human PON1, like squid DFPase, is a six-bladed β-propeller enzyme [5]. It has been identified as a candidate enzyme for developing

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improved activities against OP nerve agents because of its residence in blood and its nascent ability to hydrolyze OPs like paraoxon [6]. The aim of this study was to explore the active site of human wild type (WT) PON1 and to develop variants (Fig. 1) with an enhanced catalytic efficiency to hydrolyze the nerve agents tabun (GA), sarin (GB), soman (GD) and VX. Improving the ability of human PON1 to react with OP nerve agents at lower concentrations is a goal we aim to achieve by mutating F347 to the larger, non-polar residue tryptophan. The F347 position was chosen because it helps to frame one side of the putative active site, and by mutating this site to a tryptophan we can determine the effect an incrementally larger non-polar residue has within the active site. Tyrosine 71 was chosen because it appeared to be a bulky amino acid side chain that partially blocked access to the putative active site residues. By reducing the bulky side chain at position 71, we hoped to improve the flow of substrates and by-products around this site. The H115W mutant of PON1, which hydrolyzes VX better than WT does, serves as a benchmark of catalytic efficiency, together with the WT enzyme as a baseline activity level [7]. The results presented will guide future efforts to create an improved catalytic PON1 that can provide protection for our soldiers and citizens from the effects of nerve agent intoxication.

2. Material and methods

2.1. Molecular modeling of human PON1

Amino acid residues to be mutated were selected through the use of computational molecular modeling. We chose the structure of a gene shuffled variant of PON1 [5] (pdb code: 1V04) as the backbone model for WT PON1 due to its similarity in primary amino acid sequence to that of human PON1, and viewed the file in visual molecular dynamics (VMD) version 1.8.6. Residues were assessed, based on their position, charge and hydrophobicity, for their possible involvement in substrate binding and a putative active site mechanism [7]. Changes in residues surrounding the PON1 putative active site have been shown to change the k_{cat} and K_M of PON1, as others have shown with H115W for the substrate paraoxon [7]. In our approach, if changing a residue led to a more hydrophobic active site or a more open active site pocket *in silico*, the amino acid was marked as a possible mutation site to be produced as a novel variant of human PON1. Seven sites in the human PON1 gene were chosen to be modified by site directed mutagenesis: Y71, Y190, N168, N224, H285, F347, and F222. The residue H115 was also modified to form double mutants of some of these

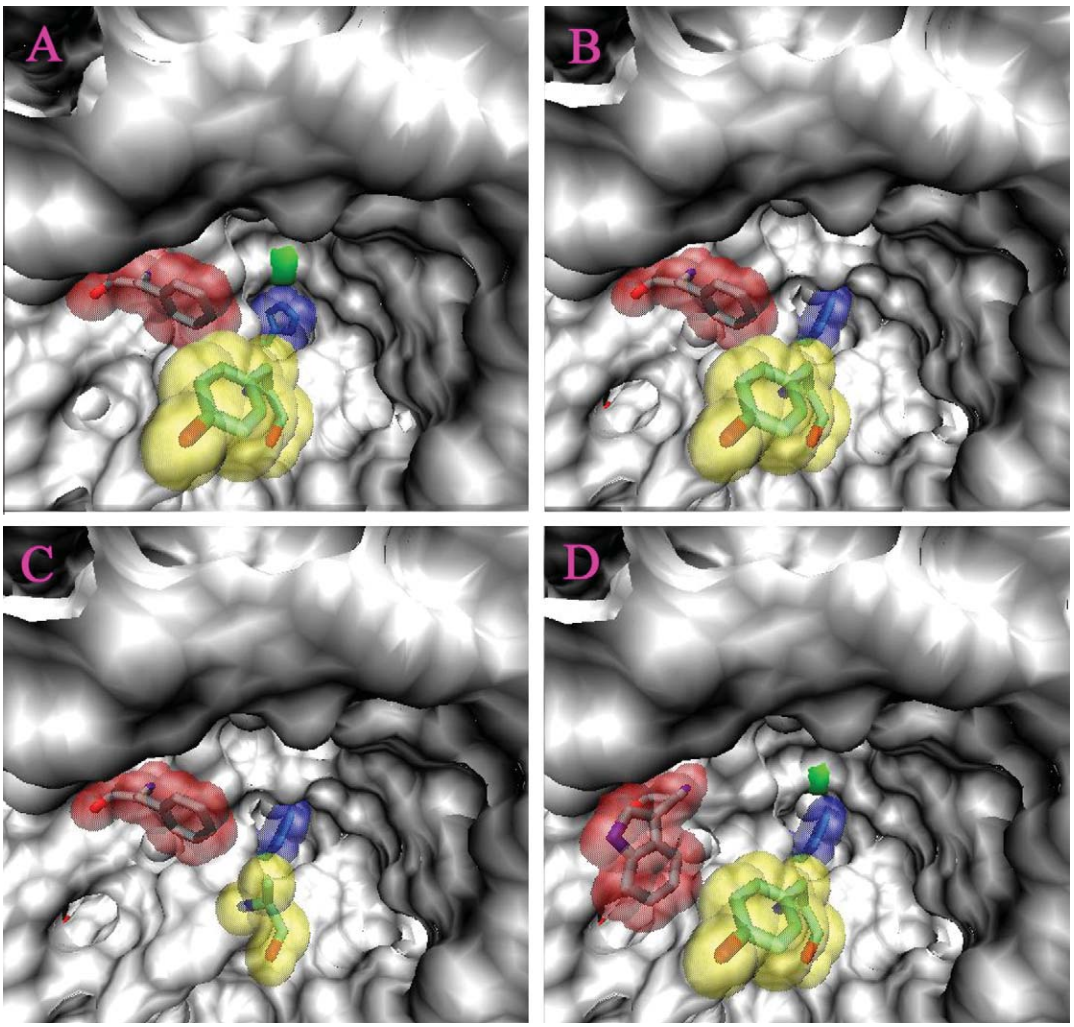

Fig. 1. Molecular models of PON1 putative active site. Transparent highlighted residues include 115 (blue), 71 (yellow), and 347 (red). Panel A shows wild type PON1 (H115, Y71 and F347). Panel B shows mutant H115W. Panel C shows mutant H115W/Y71A. Panel D shows mutant H115W/F347W. The green sphere is one of two coordinated calcium ions.

positions. The mutations Y71A/H115W, Y190A, N168L, N168L/H115W, N224L, H285W, H285F/H115W, F347 W, F347 W/H115W, and F222A were modeled using the program Deep View, choosing the lowest energy rotamer possible. The new protein database (pdb) files were then imported into VMD to be examined to ensure reasonable geometry and contacts in 3-dimensional space.

2.2. Mutagenesis of human PON1

The DNA of ten site-directed mutants of human PON1 or a WT human PON1 (1092 basepairs; with alleles M55 and R192) was synthesized *de novo* as constructs in the pcDNA3.1 vector (5428 basepairs) at HindIII/ApaI cloning sites by GeneArt (Regensburg, Germany). Plasmids were provided as 10 μg lyophilized samples, which were resuspended in de-ionized water prior to use. The plasmid containing the H115W PON1 mutant in pcDNA3.1 was supplied by Dr. Tamara Otto (United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Grounds, MD).

2.3. Preparation of human PON1 plasmid DNA

Competent DH5-α bacteria were transformed with DNA of each mutant PON1 plasmid construct. The transformation mixture was plated onto luria broth (LB) agar plates containing ampicillin (100 μg/mL) and grown overnight at 37 °C. A single colony was picked from the selective plate and grown in 5 mL of liquid selective starter culture for 8 h at 37 °C. The starter cultures were used to inoculate 500 mL of LB/ampicillin broth for 16 h. Cultures were then processed by QIAGEN Plasmid Maxi Kit. Yield of plasmid DNA was determined by optical density at 260 nm using a NanoDrop spectrophotometer (Thermo Scientific).

2.4. Transfection of 293T cells with human PON1 plasmid constructs

NIH 293T cells were grown to about 80% confluency on complete media consisting of Dulbecco's Minimal Essential Medium (25 mM HEPES buffer and 4.5 g/L glucose; DMEM; Lonza Inc.,) enhanced with 5% fetal bovine serum and 4 mM L-glutamine. Transfection of pcDNA3.1/PON1 constructs was performed as follows: 10 μL of the DNA from the maxi-preparation was added to 1.5 mL DMEM and in a separate tube, lipofectamine (60 μL) was added to 1.5 mL DMEM and allowed to incubate for 5 min at 27 °C. The DNA/DMEM mixture was then added to the lipofectamine/DMEM tube and allowed to incubate for 20 min at 27 °C. This final mixture was added to a 10 cm^2 petri dish containing 293T cells in complete media and allowed to incubate for 4 h at 37 °C, 5% CO_2. After 4 h, the transfection mixture was aspirated off and 10 mL of fresh complete media was added. The cells were incubated at 37 °C, 5% CO_2 for 48 h, after which time the supernatant was collected and sterile filtered. "Empty vector" pcDNA3.1 was also transfected in a separate culture as a negative control.

2.5. Polyacrylamide gel electrophoresis, western blot and protein quantitation

Wild type and mutant PON1 supernatants were electrophoresed on 12% tris–glycine precast polyacrylamide gels (Invitrogen) in the presence of sodium dodecyl sulfate. Recombinant human PON1 expressed in *E. coli* (Randox Laboratories Ltd., Antrim, UK) was run at a known concentration in parallel with the experimental supernatants as a positive control. Protein gels were then transferred to polyvinylidene fluoride (PVDF) membrane using an iBlot dry transfer system (Invitrogen). Membranes were probed with a monoclonal mouse anti-human PON1 antibody (gift provided by Dr. Richard James, University Hospital, Geneva, Switzerland) diluted 1:5000 in SuperBlocker T20 (Thermo Scientific) overnight at 4 °C. Membranes were washed three times with tris-buffered saline tween-20 (TBST-20) for 5 min each. Goat anti-mouse alkaline phosphatase (AP) conjugated antibody at a 1:10,000 dilution was used as the secondary antibody, incubating for 1 h at 25 °C. Membranes were washed four times with TBST-20 for 5 min each. The western blots were then developed with 5-bromo-4-chloro-3′-indolylphosphate *p*-toluidine salt (BCIP) and nitro-blue tetrazolium chloride (NBT) reagent (Sigma), identifying AP activity. Western blots were scanned with an EPSON scanner and the images were quantitated using the densitometry program Un-Scan-It V6.0 (Silk Scientific Corp., Orem, UT, USA). A standard curve was generated from the Randox positive controls and protein quantities were determined for the PON1 supernatants.

2.6. Paraoxon hydrolysis assay

The activity of WT and variant PON1 against the OP insecticide paraoxon was tested in a 96-well plate format, essentially as described [8]. The assay contained 20 μL of 500 mM Glycine/10 mM $CaCl_2$ (pH 10.5; 25 °C), 100 μL of supernatant, and 80 μL of paraoxon dilutions to give final concentrations of 920, 460, 180, and 90 μM. Initial velocity of the formation of para-nitrophenol (ε = 17,000 M^{-1} cm^{-1}) was tracked for 20 min at OD_{412}. Data were recorded as mOD/minute from the SpectraMax Plus plate reader and converted to concentration/min using Beer's Law. Values shown are the average and standard deviation of three independent biological samples (n = 3) unless noted otherwise.

2.7. VX hydrolysis assay

VX hydrolysis was measured following the formation of the S-2-(diisopropylaminoethyl) leaving group as a mixed disulfide with the Ellman reagent DTNB (5,5′-dithiobis-2-nitrobenzoate) at 412 nm (ε = 13,600 M^{-1} cm^{-1}) [9]. The assay was measured for 4 h in the presence of 50 mM Tris/10 mM $CaCl_2$ at pH 7.4, 25 °C. Buffer only and "empty vector" samples were used as controls for spontaneous and non-specific hydrolysis.

2.8. Hydrolysis reaction with racemic sarin, soman, or tabun

We studied hydrolysis of sarin, soman, or tabun using gas chromatography/mass spectrometry methods modified from Yeung et al. [7] and, Smith and Schlager [10]. A similar approach was used to assess the stereospecific hydrolysis of GA using chiral gas chromatography. A 150 μl sample of wild type or variant human PON1 expression media or buffer (ranging in pH from 7.5 to 8.5) was incubated up to 60 min at room temperature with racemic sarin, soman, or tabun (final concentrations: 2000–31.2 μM). At various time points, the samples were extracted with an equal volume of dry ethyl acetate with 50 μM DFP as an internal standard. The majority of the organic layer containing the unbound nerve agent was removed and passed over type 4A (grade 514) alumina-silicate molecular sieve to remove excess water. Samples were transferred to 11 mm crimp top vials for gas chromatography/mass spectrometry (GC/MS) analysis. One microliter of the sample was injected into an Agilent 6890 gas chromatograph (GC) fitted with a chiral GC column. For soman analysis, the GC column was a 20 m × 0.25 mm internal diameter Astec G-TA Chiraldex γ-cyclodextrin trifluoroacetyl column, 0.12 μm film thickness (Supelco, Bellefont, PA). For sarin and tabun analysis, a γ-cyclodextrin butyryl stationary phase column of the same dimension and manufacturer was used. The GC was interfaced to an Agilent 5973 mass spectrometer (MS) with an electron impact ion source. The MS was operated using selected ion monitoring. For OP quantitation, characteristic ions for the nerve agents were monitored (m/z 99,

125 for GB; *m/z* 99, 126 for GD; and *m/z* 133, 162 for GA). Two ions (*m/z* 101,127) were monitored for the internal standard DFP. Total area under the curve was used to develop ratios of each isomer between samples of GA. For GB and GD, where no stereoselectivity was detected, the peak areas of the individual isomers were combined and reported as racemic reactivity. Peak area was normalized against the DFP peak within each GC/MS run. The chromatographic identification of the P(+) and P(−) stereoisomers of tabun was determined using previously identified stereospecific reactions with rat plasma for tabun [11]. Kinetic parameters (K_M and k_{cat}) were determined by Michaelis–Menten steady-state kinetics and experimental data were fit to this model using Prism 4.0 (GraphPad, La Jolla, CA).

3. Results and discussion

Organophosphate insecticides like paraoxon are helpful in quickly determining OP hydrolase activity in PON1 variants. However, there can be drawbacks to using them as substitutes for the *bona fide* nerve agents. Each OP compound, whether insecticide or chemical warfare agent, has its own unique stereochemical structure and therefore may react and be hydrolyzed at different rates. In this study, paraoxon was initially used to screen mutants for turn-over rates greater than wild type and to eliminate those variants that were non-active. Paraoxon hydrolysis was enhanced nearly 9-fold with H115W/Y71A (Table 1). This was due to both an enhanced k_{cat} and a decrease in observed K_M. When this variant was tested against sarin, however, there was no difference compared to wild type and it had reduced activity relative to H115W alone (Table 2). Those mutants that displayed either reduced or undetectable hydrolysis of paraoxon relative to WT PON1 were not analyzed with other OP substrates. This category included: F347W, F222A, Y190A, N168L, N224L, H285W, N168L/H115W, and H285F/H115W. While it remains formally possible that some of these variants may have altered activity against nerve agents or other OP compounds, their reduced capacity or inability to catalyze hydrolysis of paraoxon suggests that they are likely to be ineffective bioscavengers.

The addition of F347W to H115W decreased the catalytic efficiency of this double-mutant against paraoxon while the H115W/Y71A was nearly 9-fold higher than wild type. This indicates that paraoxon is a poor surrogate for OP nerve agent hydrolysis because the improved catalytic efficiencies switched when we examined Y71A/H115W and F347W/H115W for GB hydrolysis. This trend has been noted in other mutant sets.

Contrary to a prior report [7], H115W does hydrolyze GD (Table 3). This may have been overlooked in the previous report due to saturation of the GC/MS detector. Only at low substrate concentrations were we able to determine the kinetic constants reported here. The same can be said for GA hydrolysis (Table 4). Here we report the first PON1 mutants that can hydrolyze GA. Again, both double mutants are improved over H115W alone by increased values of k_{cat}, but offset by increased values for K_M (data not shown). As presented in Table 4, H115W/F347W shows a very modest stereoselective preference for the P(−) isomer of GA.

We determined that substituting a tryptophan (F347W) near the putative active site of PON1, in combination with H115W, lowered the value of K_M for sarin hydrolysis by 10-fold over wild type (Table 2). This improvement in K_M is promising for developing human PON1 with an improved binding affinity. One of our main objectives engineering human PON1 was keeping K_M values low so that nerve agent hydrolysis can be effective even at sub-lethal doses. The introduction of tryptophan at position 347 not only improved GB binding but also did not disrupt the improved VX rates of hydrolysis afforded by H115W alone.

Saturation kinetics with VX could not be achieved due to restrictions on the concentrations to be used in the laboratory as established by USAMRICD's SOPs for surety material. For this reason, an assumption is made that only catalytic efficiencies could be estimated from the rate plots since we could not use concentrations of VX greater than the apparent K_Ms that developed (Table 5). VX hydrolysis was achieved in both double mutants. We were pleased to find that VX catalytic efficiency was not significantly diminished by the combination of these mutations with H115W.

The idea of developing an improved catalytic bioscavenger for nerve agent hydrolysis based on a platform with existing capabilities like PON1 seems practical, but without an established catalytic mechanism, this goal is much more difficult. A mechanism can be dissected, albeit with difficulty, through meticulous mutagenesis and defined kinetic parameters for expressed variants. It is important to note that we are only reporting apparent kinetic parameters that are internally comparable within the presented data sets. When we are able to routinely use isolated OP stereoisomers in hydrolysis assays, we will be able to determine single substrate kinetic parameters for each variant.

Alternatively, structure determination through crystallography would give a definitive, global view of the critical residues involved in catalysis if an intermediate could be trapped. At this point in time, no crystal structure of wild type human PON1 or a PON1 with trapped intermediates exist. PON1 variants developed for this study were based on a structural homology to a crystal structure developed from a chimeric PON1 derived from multiple mammalian sources [5]. Interestingly, recent structural analyses of chimeric PON1 variants have identified many of the same amino acid residues altered in this study as important for PON1 activity and substrate specificity [12,13]. Despite the currently available information regarding PON1 structure, designing a human PON1 variant with significantly improved catalytic efficiencies using rational mutagenesis remains a daunting task.

It is also paramount to characterize the stereoselective preference of a catalytic bioscavenger for the OP "substrate" and how it can be engineered to preferentially hydrolyze the more toxic isomer. The vast majority of known bioscavenger variants show a preferential hydrolysis of the less toxic stereoisomer (P+). While these activities are encouraging and advance the progress in bioscavenger development, they only support OP degradation, and not OP detoxification, which is less robust for a protein drug that will have its site of action within the bloodstream. Detoxification through hydrolysis of the more toxic stereo isomer must be the goal in bioscavenger development and should always be considered throughout the engineering phase. It is the distinct difference between detoxification and degradation of an OP that will make for an improved bioscavenger. In our study, we showed a slightly

Table 1
Paraoxon hydrolysis kinetic constants for wild type and variant PON1s ($n = 3$).

	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/K_M ($min^{-1}\,mM^{-1}$)	k_{cat}/K_M ratio to WT
WT	62.0 ± 14	0.77 ± 0.2	82.8 ± 3.7	1
H115W	76.1 ± 3.7	0.41 ± 0.05	187 ± 12.6	2.3
Y71A/H115W	213 ± 35	0.30 ± 0.1	715 ± 121	8.6
F347 W/H115W	27.7 ± 5.1	0.38 ± 0.01	72.7 ± 16.0	0.88

Table 2
Apparent sarin (GB) hydrolysis kinetic constants for wild type and variants of human PON1 ($n = 2$).

	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/K_M (min^{-1} mM^{-1})	k_{cat}/K_M ratio to WT
WT	483 ± 25.5	0.430 ± 0.073	1124 ± 103.1	1
H115W	638 ± 202	0.076 ± 0.023	8373 ± 332.5	7.5
Y71A/H115W	470 ± 241	0.449 ± 0.214	1038 ± 57.5	0.9
F347 W/H115W	414 ± 146	0.041 ± 0.011	10001 ± 1247	8.9

Table 3
Apparent soman (GD) hydrolysis kinetic constants for wild type and variants of human PON1 ($n = 3$).

	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/K_M (min^{-1} mM^{-1})	k_{cat}/K_M ratio to WT
WT	317 ± 126	0.23 ± 0.12	1458 ± 160	1
H115W	216 ± 51.7	0.21 ± 0.14	1453 ± 962	0.99
Y71A/H115W	713 ± 196	0.32 ± 0.05	2235 ± 461	1.5

Table 4
Apparent k_{cat} tabun of variants of human PON1 with (GA) ($n = 1$). P = chirality around the phosphorus atom of tabun.

	k_{cat} (min^{-1})		Ratio of P(−)/P(+)
	P(+)	P(−)	
H115W	1886	1996	1.06
Y71A/H115W	3009	3365	1.12
F347 W/H115W	4499	6017	1.34

Table 5
Apparent catalytic efficiency for VX hydrolysis for the wild type and PON1 variants ($n = 3$).

	k_{cat}/K_M (min^{-1} mM^{-1})	k_{cat}/K_M ratio to WT
WT	70.3 ± 51.5	1
H115W	153 ± 142	2.2
Y71A/H115W	142 ± 49.4	2.0
F347 W/H115W	121 ± 109	1.7

enhanced stereoselective preference for the more toxic P(−) isomer of tabun in the H115W/F347W double mutant (Table 4).

4. Conclusions

We attempted to improve upon the catalytic efficiency of the H115W mutant, which has been the best PON1 mutant developed to date for the hydrolysis of select nerve agents. Our findings show that the PON1 variants tested maintained their catalytic efficiency for VX compared to H115W. Improvements in sarin hydrolysis by H115W/F347W was achieved by a lower K_M, which was nearly half the K_M value for H115W. Hydrolysis of tabun by wild type PON1 was not detected and only modest improvements in catalytic efficiency over H115W were achieved in the double mutants by increases in k_{cat}. Future engineering of human PON1 should attempt to combine the mutations presented here into a triple mutant (H115W/F347W/Y71A) and assess its activity. The mutants presented in this paper move the science forward in honing the human PON1 platform as a catalytic bioscavenger for OP nerve agents.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

[1] A. Saxena, W. Sun, C. Luo, T.M. Myers, I. Koplovitz, D.E. Lenz, B.P. Doctor, Bioscavenger for protection from toxicity of organophosphorous compounds, Journal of Molecular Neuroscience 30 (2006) 145–148.
[2] T.M. Myers, W. Sun, R.S. Naik, M.G. Clarck, A. Saxena, Characterization of human serum butyrylcholinesterase in rhesus monkeys: behavioral and physiological effects, Neurotoxicology and Teratology 34 (2012) 323–330.
[3] S.M. Matzke, J.L. Oubre, G.R. Caranto, M.K. Gentry, G. Galbicka, Behavioral and immunological effects of exogenous butyrylcholinesterase in rhesus monkeys, Pharmacology Biochemistry and Behavior 62 (1999) 523–530.
[4] D. Rochu, E. Chabriere, P. Masson, Human paraoxonase: a promising approach for pretreatment and therapy of organophosphorus poisoning, Toxicology 233 (2007) 47–49.
[5] M. Harel, A. Aharoni, L. Gaidukov, B. Brumshtein, O. Khersonsky, R. Meged, H. Dvir, R. Ravelli, A. McCarthy, L. Toker, I. Silman, J. Sussman, D. Tawfik, Structure and evolution of the serum paraoxanase family of detoxifying and anti-atherosclerotic enzymes, Nature Structure and Molecular Biology 11 (2004) 412–419.
[6] T.C. Otto, C.K. Harsch, D.T. Yeung, T.J. Magliery, D.M. Cerasoli, D.E. Lenz, Dramatic differences in organophosphorus hydrolase activity between human and chimeric recombinant mammalian paraoxonase-1 enzymes, Biochemistry 48 (2009) 10416–10422.
[7] D.T. Yeung, J.R. Smith, R.E. Sweeney, D.E. Lenz, D.M. Cerasoli, Direct detection of stereospecific soman hydrolysis by wild type human serum paraoxonase, FEBS Journal 274 (2007) 1183–1191.
[8] D.T. Yeung, D. Josse, J.D. Nicholson, A. Khanal, C.W. McAndrew, B.J. Bahnson, D.E. Lenz, D.M. Cerasoli, Structure/function analyses of human serum paraoxonase (HuPON1) mutants designed from a DFPase-like homology model, Biochimica et Biophysica Acta 1702 (2004) 67–77.
[9] G.L. Ellman, K.D. Courtney, V. Andres, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase, Biochemical Pharmacology 7 (1961) 88–95.
[10] J.R. Smith, J.J. Schlager, Gas chromatographic separation of the stereoisomers of organophosphorus chemical warfare agents using cyclodextrin capillary columns, Journal of High Resolution Chromatography 19 (1996) (1996) 151–154.
[11] H.P. Benschop, L.P.A. De Jong, Nerve agent stereoisomers: analysis, isolation, and toxicology, Accounts of Chemical Research 21 (1988) 368–374.
[12] M. Goldsmith, Y. Ashani, Y. Simo, M. Ben-David, H. Leader, I. Silman, J.L. Sussman, D.S. Tawfik, Evolved stereoselective hydrolases for broad-spectrum G-type nerve agent detoxification, Chemico Biological Interactions 19 (2012) 456–466.
[13] M. Ben-David, M. Elias, J.J. Filippi, E. Dunach, I. Silman, J.L. Sussman, D.S. Tawfik, Catalytic versatility and backups in enzyme active sites: the case of serum paraoxonase 1, Journal of Molecular Biology 418 (2012) 181–196.