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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

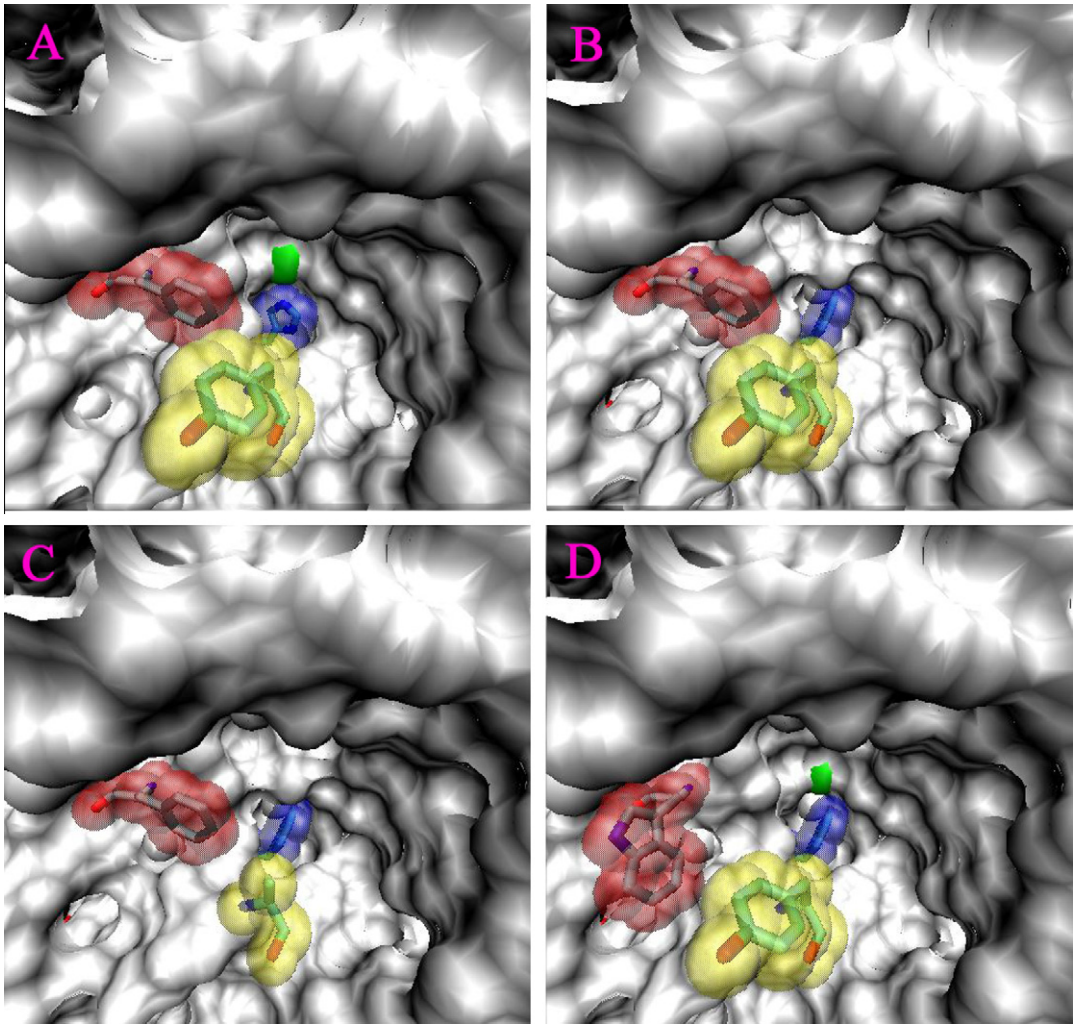

Fig. 1. Molecular models of PON1 putative active site. Transparent highlighted residues include 115 (blue), 71 (yellow), and 347 (red). Panel A shows wild type PON1 (H115, Y71 and F347). Panel B shows mutant H115W. Panel C shows mutant H115W/Y71A. Panel D shows mutant H115W/F347W. The green sphere is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

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