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A rationally designed mutant of plasma platelet-activating factor acetylhydrolase hydrolyzes the organophosphorus nerve agent soman

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Organophosphorus compounds (OPs) such as sarin and soman are some of the most toxic chemicals synthesized by man. They exert toxic effects by inactivating acetylcholinesterase (AChE) and bind secondary target protein. Enzymes can be engineered by amino acid substitution into OP-hydrolyzing variants (bioscavengers) and used as therapeutics. Some enzymes associated with lipoproteins, such as human plasma platelet-activating factor acetylhydrolase (pPAF-AH), are also inhibited by OPs; these proteins have largely been ignored for engineering purposes, because of complex interfacial kinetics and a lack of structural data. We have expressed active human pPAF-AH in bacteria and previously solved the crystal structure of this enzyme with OP adducts. Using these structures as a guide, we created histidine mutations near the active site serine of pPAF-AH (F322H, W298H, L153H) to generate novel OP-hydrolase activity. Wild-type pPAF-AH, L153H, and F322H have essentially no hydrolytic activity against the nerve agents tested. In contrast, the W298H mutant displayed novel somanase activity with a kcat of 5 min-1 and a KM of 590 μM at pH 7.5. There was no selective preference for hydrolysis of any of the four soman stereoisomers. The kcat/KM for W298H is 8x103 M-1 min-1, a significant enhancement over the wild-type enzyme.

Biography

Stephen D Kirby has been working in the field of chemical threats for the US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Grounds for 30 years, to include areas of research such as cyanide detoxification, sulfur mustard metabolism, and edemagenic pulmonary threats. The focus of his current research area has been developing and evaluating catalytic and stoichiometric protein bioscavengers for organophosphorus compounds. Current research has investigated enzyme platforms that include human carboxylesterase, human paraoxonase-1, platelet activating factor acetylhydrolase and the bacterial enzyme phosphotriesterase.

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