RECONSTRUCTION OF THE ACTIVE SITE OF A BACTERIAL PHOSPHOTRIESTERASE FOR THE CATALYTIC HYDROLYSIS AND DETOXIFICATION OF ORGANOPHOSPHATE NERVE AGENTS

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The bacterial phosphotriesterase (PTE), originally purified from the bacterium *Pseudomonas diminuta*, catalyzes the hydrolysis of the organophosphate insecticide paraoxon at a rate near the diffusion-controlled limit. The protein has been crystallized and the three-dimensional structure determined to high resolution. The protein adopts a $(\beta/\alpha)_8$-barrel structural fold and the active site is dominated by a binuclear metal center with a bridging hydroxide that is used for direct nucleophilic attack on the phosphorus center of the substrate. The wild-type enzyme is stereoselective for the hydrolysis of chiral organophosphate substrates. For example, the wild-type enzyme preferentially hydrolyzes the S_P-enantiomer of methyl phenyl *p*-nitrophenyl phosphate by a factor of 93:1. The mutation of Gly-60 to alanine (G60A) enhances the preference for hydrolysis of the S_P-enantiomer to a factor of 13,000:1 by reducing the rate of hydrolysis of the R_P-enantiomer. The stereoselectivity of PTE can be reversed by mutation of three residues within the active site (I106G/F132G/H257Y) and now the R_P-enantiomer is preferentially hydrolyzed by a factor of 118:1. Therefore, the stereoselectivity can be changed by more than four orders-of-magnitude by mutation of only four amino acids changes in the active site and thus PTE variants can be used to resolve racemic mixtures of chiral organophosphate esters. PTE was demonstrated to catalyze the hydrolysis of the organophosphate nerve agents tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), VX, and VR. Screening of a small library of active site mutants identified one mutant (H257Y/L303T) that was particularly efficient toward the hydrolysis of the G-agents (in collaboration with Dr. Steve Harvey). The YT mutant of PTE hydrolyzed GB with a k_{cat} of 3.1×10^4 min^{-1}. This mutant, at a dose of 1 mg/Kg, was shown to significantly protect guinea pigs from exposure to sarin. The LD_{50} increased by more than a factor of 65 (in collaboration with Dr. Douglas Cerasoli). Coating of the surface of the YT variant with a thin ultrahydrophilic semipermeable poly(carboxybetaine) polymer enhanced protein stability, reduced immunological complications and protected rodents from repeated exposures to sarin over a period of 1-week (in collaboration with Dr. Shaoyi Jiang). From a 30,000 member mutant library, multiple variants were identified for the catalytic hydrolysis of VX and VR. The best mutants identified to date for the hydrolysis of (S_P)-VX and (S_P)-VR were enhanced more than 10,000-fold, relative to the wild-type PTE (in collaboration with Dr. S. Harvey). These results demonstrate that the active site of PTE can be readily manipulated to engineer protein variants with enhanced catalytic properties for hydrolysis of highly toxic organophosphate nerve agents.