

Structural Studies of a Novel Phosphotriesterase Capable of Degrading Soman

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Phosphotriesterase (OPH) is an enzyme that is capable of hydrolyzing organophosphorus neurotoxins such as those commonly found in a variety of insecticides and chemical warfare agents. This enzyme is naturally found in a variety of organisms including bacteria, squid, insects and humans. We have cloned, expressed, purified and determined the x-ray structure of an OPH enzyme isolated from an extremophile that has increase thermostability and solubility compared to the most commonly studied enzyme from *Pseudomonas diminuta*. More over, our enzyme has increase activity toward soman gas. Unfortunately, none of the enzymes studied to date have activity toward the most lethal and abundant chemical warfare agent on earth, Russian VX-gas. For this reason, we are looking to re-engineer our enzyme to broaden its substrate specificity range by means of site-directed and saturation mutagenesis, as well as other directed evolution approaches.

In order to be successful, we are using x-ray crystallography to map the reaction coordinate of the enzyme and to identify residues that play important roles in catalysis. We have determined the high resolution structure of OPH in complex with an intermediate analog by using monochromatic x-rays. We have also used polychromatic x-ray methods to determine the structures of 3 separate time points (T = 0, 30, 60 minutes) on a single crystal that was subjected to a slow-reacting substrate in a flow cell. All data sets were taken at BioCARS at the Advanced Photon Source (Argonne National Laboratory). The final structures and progress in analysis of the data will be presented. This research is supported by Office of Naval Research award N000140210956.

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