

Engineering of Phosphotriesterase to Detoxify Organophosphates in Extreme Environments

Peter James Baker¹ Yogesh T. Ganesan¹ and Jin Kim Montclare^{1,2}

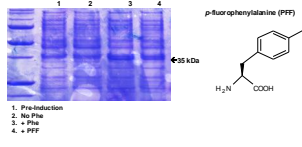
¹Department of Chemical and Biological Sciences, Polytechnic Institute of New York University
Brooklyn, New York 11201

²Department of Biochemistry, SUNY-Downstate Medical Center,
Brooklyn, New York, 11203

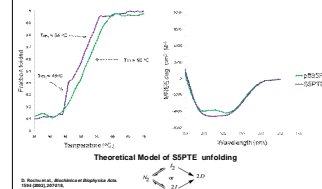
Abstract

Phosphotriesterase (PTE) is a well-studied naturally occurring enzymes capable of degrading and detoxifying organophosphates. Detailed kinetic analysis of the wild-type enzyme has shown a decreased activity in extreme environments such as high temperatures, low pH and in the presence of organic solvents. An enzyme which is functional in these environments has a great potential for bioremediation and anti-terrorism applications. Here we describe the residue specific incorporation of *p*-fluorophenylalanine into PTE and the effects on its thermostability and thermoactivity.

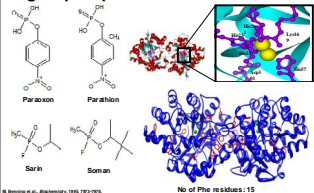
SDS-PAGE analysis of PTE expression in the presence of PFF



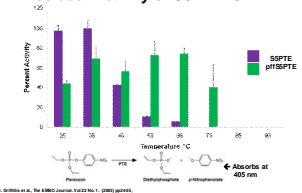
CD Analysis of S5PTE and pffS5PTE



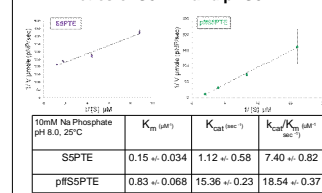
Organophosphates and S5PTE Structure



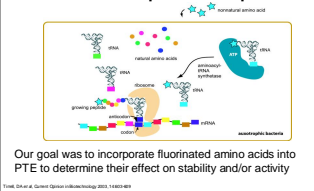
Residual Activity of S5PTE +/- PFF



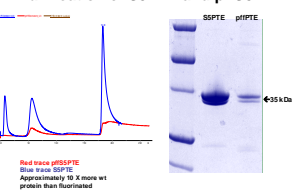
Kinetics of S5PTE and pffS5PTE



In vivo Residue-Specific Incorporation



Purification of S5PTE and pffS5PTE



Conclusion

- We were able to successfully express and purify S5PTE and pffS5PTE
- pffS5PTE appears to have enhanced thermoactivity over the non-fluorinated enzyme
- There is an effect on secondary structure as well as kinetics. Although there is a loss in affinity (K_M) for the substrate, a higher K_{cat} and higher catalytic activity is observed when S5PTE is fluorinated.

Acknowledgements

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