A Molecular Mechanism for Decreased Activity in Mutant pnb CE Enzymes

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The human carboxylesterase 1 (hCE1) enzyme is catalyzes the hydrolysis of xenobiotic esters. Hence, it is one of the body's primary defenses against ester-containing toxicants. In addition, hCE1 has been implicated in the hydrolysis of cholesteryl oleate, and thus in the transport and removal of cholesterol from cells. An eight-fold difference in rates for the hydrolysis of the pyrethroid insecticide transpermetherin were found between eleven human liver samples.[1]

The *p*-nitrobenzyl esterase enzyme from *Bacillus subtilis* (pnb CE) is an analogue to hCE1. To better understand the source of the variability in carboxylesterase activity, we compared a series of mutant pnb CE enzymes to the wild type. The mutant enzymes were formed by replacing the leucine residue 362 at the "side door" of the wild type pnb CE with either a neutral alanine (L362A), a negatively charged glutamate (L362D), or a positively charged arginine (L362R). We showed the L362R mutant had the largest decrease in activity.[2]

This poster presents our molecular dynamics studies designed to reveal the mechanism for these observed differences. We believe that the primary cause of the loss in activity in the L362R mutant is because of a change in the hydrogen bonding pattern relative to the wild type. Specifically, the Arg362 side chain in L362R reaches across the side door opening and hydrogen bonds to Gln276, thereby reducing conformational flexibility in the enzyme. None of the other mutants show this behavior. Another possibility is that the Arg362's positive charge leads to an electrostatic effect at the active site. However, based on the experimental data we consider this to be less likely.

References:

[1] M. K. Ross, A. Borazjani, C. C. Edwards, and P. M. Potter, Biochemical Pharmacology **71** (5), 657 (2006).

[2] T. M. Streit, A. Borazjani, S. E. Lentz, M. Wierdl, P. M. Potter, S. R. Gwaltney, and M. K. Ross, Biol. Chem. (in press).

The project described was supported by Grant Number P20RR0177661 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources of the National Institutes of Health.