Evolution of Structure and function in the b-lactamase/DD-peptidase fold

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The β-lactamase (Bla) hydrolytical activity has arisen several times from DD-transpeptidases (PBPs), suggesting that these activities are close in catalytical space. We have been able to replicate the evolutionary process of Bla activity emergence on a PBP2X using a semi directed mutagenesis scheme. Some of the most interesting changes, like modifying an enzyme's catalytic properties, require several mutations in concert; it is therefore essential to explore efficiently sequence space by generating the right diversity. We designed a biased combinatorial library using an evolutionary engineering approach in which biochemical and structural information were incorporated by site directed mutagenesis on relevant residues near or in direct contact with the substrate and then subjected to random mutagenesis to allow for mutations in unanticipated positions. We isolated mutants conferring 10-fold higher cefotaxime resistance levels than the WT through mutations exclusively in the coding sequence. We show that only three substitutions in the PBP active site, two produced by the directed and one by the random mutagenesis, are sufficient to acquire this activity. One mutant had a 10^5–fold increase in cefotaxime deacylation rate allowing it to hydrolyze efficiently ß-lactams yet conserving PBP activity.

We have analyzed the effect of the starting amino acid or nucleotide sequences on the functional adaptation of enzymes in directed evolution experiments.

For this purpose we have selected variants of the Bla TEM-1 and PC-1 which now exhibit a cephalosporinase activity. These enzymes are homologous and have a very similar structure are only 27% identical at the amino acid level. We subjected both genes to gene-shuffling and mutagenic PCR cycles, selecting in increasing cefotaxime concentrations. We have generated a variant of the TEM-1 encoding gene which has synonymous mutations in codons 164 and 238. The codons were changed so that two nucleotide changes were necessary in each to obtain the usual E164K and G238S changes. This gene has allowed us to obtain new mutations, different from those usually found, confering a specificity change.

In the case of PC-1 we have found an altogether different set of unique mutations when compared to natural and *in vitro* generated wide-spectrum β-lactamases. Both sets of mutations are located around the active site but seem to induce the change in specificity through different mechanisms.