

Evolution of a new catalytic mechanism: from esterases to hydroxynitrile lyases

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Enzymes within a superfamily often catalyze different, but related, reactions. For example, the α/β -hydrolase-fold superfamily contains both esterases and amidases, which both catalyze hydrolysis of carboxylic acid derivatives. The reactions have common intermediates and similar catalytic steps. One can imagine gradual steps that evolve an esterase into an amidase.

In other cases, enzymes within a superfamily catalyze unrelated reactions. For example, the α/β -hydrolase-fold superfamily also contains hydroxynitrile lyases, which catalyze the addition of cyanide to aldehydes to form a cyanohydrin. The mechanism for this addition differs significantly from hydrolysis. It is difficult to imagine gradual steps that could create hydroxynitrile lyase activity. Understanding this natural path would aid the protein engineering of enzymes that catalyze new reactions.

We reconstructed and characterized ancestral enzymes in the evolution of hydroxynitrile lyases from esterases. Several ancestral enzymes were esterases with esterase-like catalytic residues, one ancestral enzyme was a hydroxynitrile lyase with HNL-like catalytic residues, but one (EST3) showed an intermediate active site. Mutagenesis and modeling suggests that EST3 follows a transitional mechanism that bridges the two reaction types.

Attempted protein engineering of an esterase into an HNL has not yet yielded an efficient HNL. Exchange of catalytic residues switched activity, but the efficiency was low. Exchange of all residues within the active site also did not yield an efficient enzyme. Engineering an efficient HNL will require changes both inside and outside the active site.