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Obstacles in the Development of Stereo Selective Enzymes for Organic Chemistry

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Abstract

Thermo stability, substrate range, and enantioselectivity are just a few of the catalytic parameters of enzymes that can be improved through directed evolution for use in synthetic organic chemistry and biotechnology. When using synthetic organic chemistry with biocatalysts, stereo selectivity is very crucial. This article focuses on recent advances in approach for stereo selective enzyme evolution in the laboratory using hydrolases and monooxygenases as the enzymes. ISM or iterative saturation mutagenesis, has been found to be an incredibly effective way to evolve enzymes with improved or reversed enantioselectivity, a wider range of substrates, and/or increased thermo stability.

Keywords: Biocatalysis; Monooxygenase; Acinetobactor; Thermo stability; Laboratory

Description

Modern synthetic organic chemistry relies heavily on asymmetric catalysis. Enzymes and chiral synthesized transition metal complexes are the two options available to organic chemists in practice. For a certain type of transformation, no trigger can be really universal. Therefore, it is wise to build up a sizable toolbox with all three types of catalysts represented, frequently acting in a complementary manner. For more than a century, enzymes have been used in organic chemistry, with a number of important industrial applications. Biocatalysis has historically been plagued by severe restrictions related to a limited range of substrates, inadequate enantioselectivity and insufficient robustness.

In 1997, guided evolution of enantioselective enzymes was developed, which made it possible to overcome some of the drawbacks and restrictions of biocatalysis. In fact, 12 years after the proof of principle study was published, practically all classes of enzymes are being used in more and more academic and industry laboratories, using this novel technique to asymmetric catalysis.

The basic idea is based on repeatedly cycling through gene mutagenesis, expression and screening (or selecting) for enantioselectivity.

Prior to the introduction of this idea, directed evolution of protein stability has already been proposed. The error prone Polymerase Chain Reaction (epPCR), saturation mutagenesis and DNA shuffling are the gene mutagenesis techniques most frequently employed in all studies of this form of protein engineering. These techniques were used in the initial proof of concept experiment on the hydrolytic kinetic resolution of rac 1, which was facilitated by *Pseudomonas Aeruginosa* Lipase (PAL) With only a minor enantioselectivity in favor of (S)-2 (E=1.1), the Wild Type (WT) PAL exhibits weak enantioselectivity. The Darwinian principle is well demonstrated in this early study as a way to adjust a biocatalyst; however an E value of 11 is hardly realistic. E=13 was obtained after the fifth cycle of epPCR, which indicated that a more effective plan needed to be developed. As a result, many theories were investigated over a period of years, including saturation mutagenesis at hot sites that had previously been detected by epPCR. Finally, saturation mutagenesis at a four residue position near to the binding pocket with the creation of

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what is now known as a targeted library and DNA shuffling produced the best results. After the best mutant with six point mutations was developed from the 50,000 trans formants produced by these mutagenesis techniques, it displayed a strong selectivity factor of E=51. It was unexpected to find that five of the six mutations were on the surface and just one was right adjacent to the binding pocket. Only two of the point mutations are required and a relay mechanism is in operation, according to a thorough QM/MM investigation. It was discovered that the double mutant (E=63) was significantly more enantioselective. This was a victory for theory, but it also showed that the practical strategies including the inversion of enantioselectivity were not as effective as one may have liked. Picking up unneeded mutations requires extra screening work and hinders "fast directed evolution.

Using the Cyclo Hexanone Mono Oxygenase (CHMO) from acinetobacter, the first such endeavor focused on the oxidative desymmetrization of 4-hydroxycyclohexanone. This intriguing synthetic reaction is catalyzed by WT CHMO, although with low enantioselectivity (ee=9%). The evolution of (R) and (S) selective mutants with 90% ee enantioselectivity was accomplished using the traditional epPCR approach as in the initial proof of principle lipase study. One of the mutants, Phe432Ser, was subsequently tested as a catalyst in the desymmetrization of a collection of additional structurally diverse substrate in each library. Subsequent rounds of saturation mutagenesis were then conducted at the corresponding other sites using this template, and so on. You can also use the second or third best hit in a library.