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FIRST REACTIONS

Nature's Enzymes Tricked with Xenobiotic Oxidants

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The relationship between enzyme engineers and synthetic organic chemists is simultaneously symbiotic and competitive. Synthetic chemists often find their inspiration in the active sites of enzymes, but celebrate any ability to outmaneuver nature's catalysts. Scientists who create new enzymes, for their part, are quick to point out how elegantly and rapidly catalytic proteins stitch together molecules under ambient conditions and without need for protecting groups.

What happens, then, when scientists introduce enzymes to chemicals out of their element?

Arnold and co-workers report a new enzymatic method to put together aziridines (strained three-membered nitrogen-centered rings) relying on *in vitro* evolution of cytochrome P450.[1] Aziridines are useful tools for installation of carbon−nitrogen bonds, the glue that holds together biologically active amines and amides in chemical synthesis. These three-membered rings have garnered a lot of attention in the area of amine synthesis. However, development of synthetic transformations of aziridines has lagged behind that of epoxides, the analogous oxygen-containing molecules. This comparative lack of application is why some in the synthesis community regard aziridines as epoxides' "ugly cousins".[2]

Looking back into the natural world, the question arises as to why there are so few aziridine-containing natural products (less than 20) compared to epoxides (in the thousands). The answer, without a doubt, lies in the accessibility of a direct biosynthetic path to three-membered nitrogenous rings. For oxygen, P450 enzymes have evolved into competent systems for epoxidation, using dioxygen as the terminal oxidant. Within the active sites of these enzymes, transiently generated Fe═O intermediates transfer oxygen atoms to alkenes (Figure 1). There is no comparable nitrogen variant in nature because the energy required for the generation of the corresponding Fe═NR intermediate is substantially higher. Arguably, there has never been any evolutionary pressure to produce the high-energy nitrogen analogue of dioxygen. Perhaps the existence of only a few aziridine-containing natural products is a testament to that. In fact, all

The relationship between enzyme design and synthetic methodology is evaluated with regard to Arnold and co-workers' report of a novel aziridination enzyme.

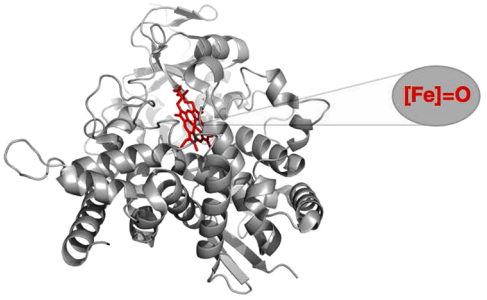

Figure 1. Cytochrome P450s are powerful oxidation enzymes.

of the aziridine natural products are believed to come from intramolecular S_N2 reactions, a distinctly different, and longer path.[4]

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Synthetic chemists have created several imaginative approaches to transition metal-based aziridination of alkenes by using metal catalysts including those with salen ligand-based systems.[5] Interestingly, some methods rely on heme-inspired metal porphyrins, much like the center of the P450. The rarity of aziridines in nature provides an impetus to investigate these rings, particularly their nucleophilic ring-opening transformations.[3] Because of the poor leaving group ability of the "RNH^-" functionality, the parent NH aziridines are of limited utility compared to their epoxide congeners

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that enjoy a healthier relationship with the more stable "RO^-" leaving group. Thankfully, in the lab, aziridines' electrophilic properties can be modulated by the attachment of an electron-withdrawing group to nitrogen.

Arnold and co-workers were able to teach P450 a new trick, coaxing this powerful enzyme to accept nitrenes as opposed to the more familiar oxene ligands (Figure 2).[1]

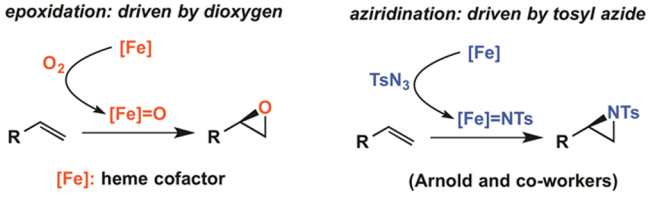

Figure 2. Arnold and co-workers were able to change cytochrome P450 from an epoxidation enzyme (left) to an aziridination enzyme (right) through the power of *in vitro* evolution coupled with a xenobiotic oxidant (tosyl azide).

Toluenesulfonyl azide acts as the nitrogen-based oxidant in this chemistry. The dinitrogen evolution that accompanies aziridination is the driving force of this new synthesis. From a chemist's standpoint, high enantioselectivity of the reaction is a particularly useful feature, although the reliance on toluenesulfonyl chemistry is somewhat limiting, given the relatively harsh conditions needed to remove this protecting group at a later stage. Screening of additional generations of these enzymes could select for those able to use the nosyl group and would result in a more user-friendly version of the reaction. In addition, the substrate scope is heavily skewed toward aromatic group-containing alkenes. It will be interesting to see how aliphatic molecules, which are often more synthetically useful than their aromatic counterparts, fare in this transformation.

Arnold's report is an important milestone in this storied field of inquiry as it shows that biological systems can be quite proficient in transferring nitrene ligands to alkenes, provided that P450s are tricked into this new role by unnatural oxidants such as azides.

In closing, synthetic chemists have long had an upper hand over nature in the area of nitrene transfer to alkenes. The absence of nature's variant of this chemistry has provided a compelling rationale for the development of many synthetic catalysts. Arnold's report is an important milestone in this storied field of inquiry as it shows that biological systems can be quite proficient in transferring nitrene ligands to alkenes, provided that P450s are tricked into this new role by unnatural oxidants such as azides. Arnold astutely points out that this aziridination represents only one of myriad reactions that might be possible by manipulating the natural promiscuity of enzymes through evolution; this opportunity should continue to inspire scientists' creativity.

Professor Yudin blogs about chemistry at the amphoteros blog.

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