Promiscuous Biocatalysis

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Enzyme Promiscuity: Using a P450 Enzyme as a Carbene Transfer Catalyst

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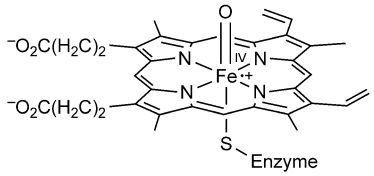
cyclopropanation · cytochromes · enzyme promiscuity · enzyme catalysis · stereoselectivity

Enzymes can be used as biocatalysts in many synthetic organic transformations, but they are incapable of catalyzing most of the synthetically important reactions mediated by (chiral) transition-metal complexes. This is one of the reasons why researchers active in the area of enzyme promiscuity have attempted to induce enzymes to catalyze unnatural reaction types using a variety of approaches[1] including the design of artificial metalloenzymes.[2] The latter include Fe^{III} corrole complexes bound in serum albumins[2a] and Cr^{III} salophen complexes in apo-myoglobin[2b] as catalysts for H_2O_2-mediated asymmetric sulfoxidation as well as Cu^{II} phthalocyanine complexes bound in serum bovine albumin as catalysts for enantioselective Diels–Alder reactions;[2c] the respective proteins do not catalyze these transformations. The de novo design of enzymes lacking or containing metal centers is yet another approach.[1e,f]

The latest contribution to the reactions of promiscuous enzymes based on a different approach was recently reported by Arnold et al., who demonstrated that the iron–heme moiety in a cytochrome P450 enzyme can function as a catalyst in the cyclopropanation of olefins under anaerobic conditions.[3] P450 monooxygenases normally catalyze O_2-mediated oxidative C–H activation (R–H→R–OH), a radical process initiated by the reactive intermediate "Compound I".[4] Epoxidation of olefins is also possible, a process in which Compound I transfers its O atom directly onto the olefinic function.



Compound I

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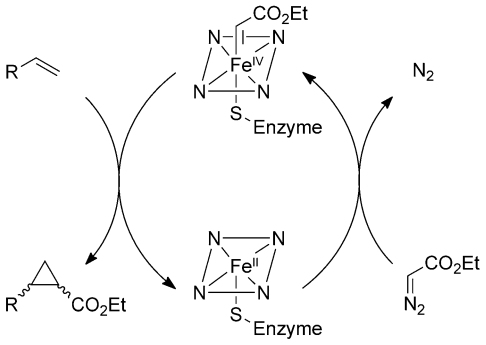
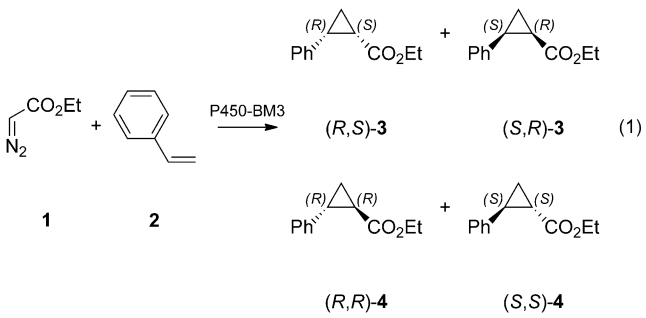
In the new study highlighted here,[3] the authors tested the idea of using P450-BM3 as a catalyst in carbene transfer reactions with the formation of cyclopropanes, in analogy to olefin epoxidation induced by Compound I. They expected the iron(II)–heme catalytic center to react with diazo compounds leading to the formation of a reactive high-valent iron–carbenoid complex which then transfers the respective carbene to an olefin (Scheme 1). In a model reaction the



Scheme 1. Envisioned P450-catalyzed cyclopropanation.[3]

authors employed ethyl diazoacetate (**1**) as the carbene source and styrene (**2**) as the olefin; the reaction products were the two *cis* enantiomers, (*R*,*S*)-**3** and (*S*,*R*)-**3**, and in the diastereomeric regime the two *trans* enantiomers, (*R*,*R*)-**4** and (*S*,*S*)-**4** [Eq. (1)]. It is interesting to note that the identical reaction and the same compounds were employed by Nozaki, Noyori, and co-workers in the first asymmetric transition-metal-catalyzed reaction in 1966,[5a] which marked the beginning of a new era in research, namely transition-metal-catalyzed asymmetric transformations.[5b] Since then, cyclopropanations of this kind have been optimized employing more efficient ligands in Cu, Rh, Ru, and Au complexes.[6] Extremely high enantio- and diastereoselectivity and pronounced activity have been achieved, which means that the synthetic problem is essentially solved.

Prior to the report of Arnold et al.,[3] a number of studies described the formation and reactivity of porphyrin-type transition-metal carbenoids, including those based on synthetic iron–porphyrin complexes.[7] For example, Kodadek, Woo, and co-workers utilized various synthetic achiral



iron(II)–porphyrin complexes to catalyze the model reaction **1** + **2** → **3** + **4** (*trans*/*cis* up to 13:1).[7b] They reported that the iron(II)–porphyrin complexes required for carbene transfer are extremely air sensitive, undergoing rapid oxidation to the inactive Fe^{III} form. This means that for catalytic purposes an inert gas atmosphere or a stoichiometric amount of a reducing agent such as cobaltocene has to be used.[7b,c] Interestingly, iron–heme bound carbenoids have been reported for P450 enzymes themselves. In the P450-catalyzed metabolism of 1,3-benzodioxole, the intermediacy of a 1,3-benzodioxole-2-carbenoid at the iron–heme center appears to be involved.[8] In a more recent study, Carreira et al. devised a user-friendly olefin-cyclopropanation method employing in situ generated diazomethane under basic conditions (KOH); several transition-metal complexes served as carbene-transfer catalysts, including an iron(III)–porphyrin complex under aerobic conditions.[7e] If prior reduction to Fe^{II} is in fact necessary for catalysis,[7b,c] it is not clear how electron transfer occurs in this system.[7e]

Independent of these mechanistic questions, the Arnold strategy proved to be successful.[3] All reactions were performed under an inert gas atmosphere in aqueous medium containing methanol as the cosolvent and sodium dithionite as the reductant. Wild-type P450-BM3 was used as well as a number of mutants produced earlier for the oxidative hydroxylation of structurally completely different substrates, and acceptable activity and in some cases reasonable stereoselectivity were observed. Moreover, a few mutagenesis experiments based on saturation mutagenesis were performed, leading to improved selectivities.[3] The best mutant delivers the *cis* product (*S*,*R*)-**3** with high diastereoselectivity (92:8) and enantioselectivity (97% *ee*). The yields are generally in the range of 30–60%. Several other styrene-type substrates were also tested, but in these cases diastereo- and enantioselectivity proved to be moderate to poor.[3]

This is a fascinating proof-of-principle study. Its purpose at this stage is not to provide a method that is more efficient than traditional transition-metal catalysis.[5b,6] Rather, it demonstrates that unusual enzyme promiscuity can in fact be designed. The next step is to confirm the proposed working hypothesis in mechanistic and structural studies. Testing different reaction types and other heme-bound metals and is yet another perspective.

A fundamental problem that persists to this day in essentially all registered examples of enzyme promiscuity including those cases involving artificial enzymes, is the low activity of the respective biocatalysts.[1–3] For example, the already mentioned Diels–Alder reactions catalyzed by the artificial metalloenzyme comprising Cu^{II} phthalocyanine complexed to bovine serum albumin proceed with more than 95% *ee*, but the reaction rate is very low.[2c] Evans-type Cu^{II} catalysts are much more active in similar asymmetric Diels–Alder reactions.[9] Developing general methods for enhancing the activity and selectivity of promiscuous enzymes, including those generated by de novo design, remains a challenge.[1e,f]

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