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Developing flavoprotein alkene reductases into synthetically useful catalysts for asymmetric reductions

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Alkene reductases have recently emerged as promising catalysts for reducing electron-deficient *C*=*C* bonds with very high stereoselectivities. Two key problems remain, however. First, the substrate range of these enzymes is somewhat limited, generally favouring smaller alkenes. In addition, their high stereoselectivities make it difficult to access the "other" product enantiomers. Directed evolution studies of *Pichia stipitis* Old Yellow Enzyme 2.6 (OYE 2.6) as remedy to both issues has been carried out. These studies focused on three representative substrates whose reduction products are important chiral building blocks in organic synthesis. Residues located near the substrate binding region of the active site by site-saturation mutagenesis were surveyed and was found that four appeared to play important roles for two of the substrates. On the other hand, no improvement in stereoselectivity for the third alkene substrate was found. This problem was solved by carrying out further, parallel directed evolution studies on the other two substrates and was found that a key intermediate mutant identified during the course of these efforts regained activity toward the third substrate and also showed a reversed stereoselectivity. This process allowed us to cross a "desert" during directed evolution: Starting from an active protein and arriving at a catalytically active mutant via inactive intermediates. Subsequent rounds of directed evolution allowed further improvements and yielded synthetically useful catalysts. Crystallographic studies of the wild-type and key mutant OYE 2.6 variants were also carried out to understand how the mutations impacted both substrate binding and stereoselectivity.

Biography

Jon Stewart earned a PhD from Cornell University in 1991, then carried out Postdoctoral work at Penn State University as a Helen Hay Whitney Fellow from 1991-1994. He joined the Department of Chemistry at the University of Florida as an Assistant Professor in 1994, where he is now Professor of Chemistry. Developing new synthetic methodologies based on enzymes has been a major focus of his research. In addition to publishing extensively in this area, he has also served as an Editor for the Journal of Molecular Catalysis B: Enzymatic since 2002.

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