GENERATION OF NEW IMINE REDUCING ENZYMES – EXPANSION OF THE IMINE REDUCTASE SEQUENCE SPACE

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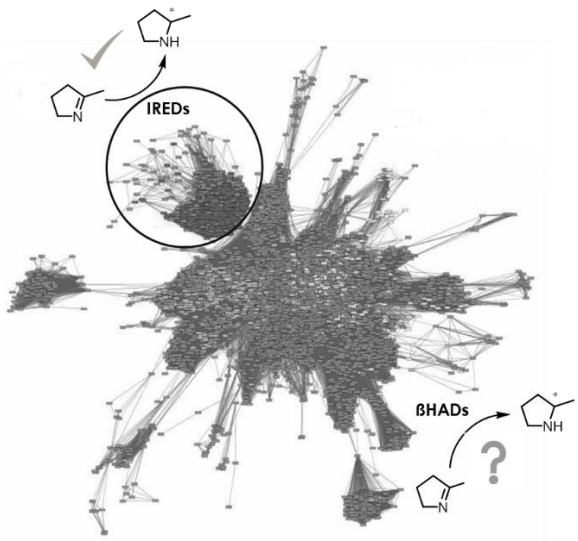
The synthesis of chiral molecules by asymmetric hydrogenation applying rhodium and ruthenium-based metal catalysts and molecular H_2 as hydride donor is an important and established technology in the pharmaceutical and fine-chemical industries.[1] The tremendous progress in enzyme discovery, enzyme engineering and process development made in recent years enable to extend these technologies by biocatalytic alternatives. Many reductase enzymes catalyzing the stereocontrolled addition of hydrogen from NAD(P)H to α,ß-unsaturated carbonyl compounds, cyclic/acyclic imines and aldehydes/ketones are described.[2] The latest discovered enzymes in this field are imine reductases (IREDs), catalyzing the reduction of various C=N bonds.[3] Recently the structural similarity of IREDs to ß-hydroxyacid dehydrogenases (ßHADs) was investigated[4], suggesting a common ancestry to C=O reducing enzymes.
In previous work we demonstrated that two enantiocomplementary imine reductases from Streptosporangium roseum and Paenibacillus elgii display promiscuous activities for highly reactive carbonyl compounds.[5] The results of analysis on the asymmetric reduction of carbonyls by IREDs and the high structural similarity of IREDs and ßHADs prompted us to investigate the imine reducing capability of ßHAD family members. We will present the interconversion of ßHADs into imine reducing enzymes by introducing single amino acid substitutions. Applying a rational protein design approach by comparing the active sites of one IRED family member and three selected ßHADs, we were able to identify crucial amino acid positions for the desired switch in chemoselectivity.



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