Rational Analysis of Structural and Functional Changes of Oxidoreductases in Non-Conventional Reaction Media

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Establishing biotransformation in non-aqueous media is recognized as a hot-topic in the bio-catalysis community owing to several advantages: (1) high-solubility of synthetically interesting hydrophobic substrates, (2) reduced risk for water-induced protein denaturation, and (3) elimination of undesired side reactions. Whereas a high number of research studies on the application of hydrolases (EC3) in low-water media are published, very few publications are available on the use of oxidoreductases (EC1) in this non-conventional environment. This is unfortunate as oxidoreductases catalyses synthesis of valuable chemical compounds which are of high interest for pharma and agro, but as well as for bulk chemical industries. Among the oxidoreductase enzymes (e.g., dehydrogenases/reductases, oxygenases, oxidases, and peroxidases), alcohol dehydrogenases (ADHs) are predominantly applied in organic synthesis not only at laboratory but also at industrial scale. However, these applications are mainly based on the use of aqueous media.

Due to the lack of an understanding for the effects of organic solvents, which would replace the water as the reaction environment, in this project we focus on a rigorous evaluation of these effects on the ADH-catalysis. For this purpose, two model ADHs will be characterized in commonly used organic solvents (e.g., ethyl acetate and methyl tert-butyl ether), in ‘greener’ ones (e.g., 2-methyltetrahydrofuran) as well as in deep-eutectic-solvents, which are a new generation of solvents with more sustainable characteristics compared to some ionic liquids. Thereby, the focus will be on relating solvent properties (e.g., hydrophobicity, molecular structure, and water content) to ADH catalysis characteristics (e.g., activity, stability, and selectivity). Molecular dynamics simulations will be performed for a deep understanding of the interactions between the protein, solvents, and water. Having elucidated and understood the effects of the organic solvent properties on the enzyme catalysis, our final goal is to develop general solvent selection rules taking into account the solvent properties. Overall, the here presented project topic has not been investigated yet and it represents a clear strategy to elucidate the protein-solvent-water interactions, which possess a considerable potential for understanding the ADH-catalysis in non-aqueous media. Especially the close collaboration between experimental methods and molecular simulations will lead to new insights on different scales. Moreover, this research project will open new possibilities to evaluate other enzyme classes for the effects of organic solvents on enzyme characteristics.