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In situ NMR and STD NMR to study mechanisms of enzyme catalyzed reactions to optimize use of enzymes in organic synthesis

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Online monitoring of enzyme catalyzed reactions by *in situ* ¹H NMR is a valuable approach to gain data about kinetics and mechanisms of biotransformations. In addition Saturation Transfer Difference NMR spectroscopy (STD NMR) can be used as time efficient method to study comprehensive mapping of enzyme/substrate interactions in global as well as in site-specific fashion. The resulting binding patterns represent a molecular foundation of specific substrate, co-substrate, and co-enzyme binding to an enzyme. These interactions are determined during catalysis as well as under binding-only conditions. The STD NMR technique is hence applied to delineate non-covalent enzyme/ligand interactions of several wild type enzymes as well as corresponding mutant enzyme variants. Combination of both STD and *in situ* ¹H NMR techniques allows comprehensive studies of mechanistic details, which can be supported and visualized by *in silico* molecular docking. The gained results can be correlated with macroscopic features like substrate specificity and catalytic activity (k_{cat}). Transformations catalyzed by varying enzymes like phosphorylases [E.C.2.4.1.X], UDP-xylose synthase [E.C.4.1.1.35], and xylose reductase [E.C.1.1.1.21] are exemplary investigated. The NADH dependent *Candida tenuis* xylose reductase (CtXR) catalyzed reductions are of particular interest due to formation of ternary enzyme/co-enzyme/substrate complexes. Studying transformations of natural substrates, artificial substrate analogues, and not accepted compounds allows conclusions how this enzyme uses non covalent interactions to bind and transform its substrates. An application of CtXR for the stereoselective formation of pharmaceutical active compounds indicates the meaning of the presented NMR concept for synthetic approaches in the field of green chemistry.

Biography

Lothar Brecker received his diploma and PhD in Chemistry from the University of Dortmund in 1993 and 1996, respectively. After working at Graz University of Technology and Research Center Borstel, he became an Associate Professor at the University of Vienna. There he actually serves as Vice Head of the Institute of Organic Chemistry and Deputy Director of the Chemistry Studies Program. His main research activities are in the fields of using NMR to study enzyme ligand binding, interactions between small molecules, and structure determination of natural products. He has published more than 80 papers in reputed journals.

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