Improved Whole Cell Biocatalysts for P450 catalyzed API Metabolite Synthesis

Martin Held (ETH, Basel)

Project Summary

Cytochrome P450s monooxygenases (P450s) catalyze the regio- and stereoselective hydroxylation of non-activated carbon skeletons. No homologous reaction can be found in the world of organic chemistry, which makes P450 monooxygenases an indispensable tool for many applications. In particular, the pharmaceutical industry frequently employs these enzymes to reconstitute the metabolism of xenobiotics and pharmaceuticals and their elimination from the body, which is mainly caused enabled by human P450s, particularly those found in the liver[1]. This re-enactment is important as due to the high structural homology of the P450's hydroxylation products to the original drugs, these substances can either contribute to the pharmacodynamics in a similar or even larger extent than the API itself or alternatively may affect off-targets, with the risk of triggering undesired side effects.

The detailed characterization of the effects of such so-called drug metabolites in the course of pharmacokinetic and pharmacology studies require gram or (multi-)gram amounts[2] of these substances. Unfortunately, preparative scale synthesis is not trivial with any of the currently, typically recombinant, systems or liver tissue preparations. Regarding the former, one major thrust is the recombinant expression of P450s in microbial hosts, which has been impeded by enzyme instability in these systems[3]. At least part of the poor operational stability is due to oxidative damage caused by the so-called "uncoupling reaction"[4]. "Uncoupling" refers to the unspecific breakdown of the P450's heme-Fe(II)-oxygen complex, i.e. at the enzyme's active site, or to the escape of electrons during transport from the cytochrome P450 reductase (CPR) to the P450. In both cases, highly reactive oxygen species (ROS, mainly hydrogen peroxide (H_2O_2), but also superoxide ions ($O2^-$)) are formed instead of the desired hydroxylated products. These ROS can then react with other oxidizable substance available close by, including parts of the enzyme itself, and thus severely damage the (whole cell) biocatalyst. In this way, consumption of an electron donor (NAD(P)H) is uncoupled from the formation of the desired product while the cell's survival is jeopardized. The degree of the uncoupling reaction and therefore the efficiency of catalysis hence depends on the tightness of all partners involved in the electron transport chain – from NAD(PH)H up to the point at which electrons are discharged onto the substrate. Unfortunately, it is also one of the most difficult parts of the system to be experimentally evaluated and optimized.

We will develop a high-throughput screening assay that will allow measuring the degree of uncoupling directly in a recombinant E. coli cell by quantifying the concentration of hydrogen peroxide in vivo. The assay system will find broad application for assessing and modulating the coupling efficiencies of oxygenases in general. Within this project, we will apply the system for reducing unproductive reductase-P450 electron shunts for the most prominent P450 enzyme involved in xenobiotic degradation, CYP3A4 from human liver[5], and concomitantly for reducing the degree of uncoupling. To this end, we will (I) screen the metagenome for new heterologous reductase partners, (II) further improve the performance of the enzyme system by means of protein engineering of the reductase component, and, (III) generate artificial self-sufficient reductase-CYP fusions with already known and novel CPRs isolated from the metagenome. This way, we aim to provide a collection of optimized CYP catalysts for preparative scale synthesis of drug metabolite at reduced costs.

References:

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