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Title and subtitle: Amine transaminase: Process development aspects for chiral amine synthesis			
<p>Many modern drugs contain chiral amine moieties and the need for more efficient processes to synthesize amines in high optical purity and concentration are greatly demanded by the fine chemical and pharma industry. Amine transaminases (ATA) allow for direct asymmetric synthesis of optically pure amines from ketone substrates. Although academia and industry have been researching ATAs extensively since more than 20 years, the development of an efficient ATA-based process for chiral amine synthesis remains challenging.</p> <p>In this doctoral work, process development aspects for the ATA-catalyzed synthesis of chiral amines was investigated. The work can be divided into four parts: i) method development in order to obtain quickly detailed kinetic and stability data, ii) identify the inactivation mechanism that renders ATAs inefficient under process conditions, iii) utilize these biochemical information to stabilize ATAs through enzyme and process engineering, and iv) development of process concepts that employs <i>in situ</i> product removal (ISPR) methods to overcome the thermodynamic and inhibition barrier of ATA-catalyzed reactions.</p> <p>The characterization of ATAs was accelerated by developing an automated high performance liquid Chromatography (HPLC) platform. Equipped with a size exclusion column, this HPLC platform constitutes a substrate-independent assay that allows quantification of enzyme-bound and free cofactor species. The combination of kinetic and stability analysis (e.g. via melting point determination) revealed that ATAs suffer from amine donor-induced inactivation mechanism. Besides cofactor concentration and substrate ratio, the quaternary structure of ATAs was found to affect the operational stability and the tendency to lose the aminated cofactor intermediate (pyridoxamine 5'-phosphate, PMP). The dimeric ATAs from <i>Chromobacterium violaceum</i> and <i>Vibrio fluvialis</i> exhibited lower stability than a novel tetrameric ATA. The tetramer structure stabilizes a key element for cofactor binding and reduces dissociation of PMP during catalysis.</p> <p>Targeted enzyme engineering yielded a mutant with improved stability towards high substrate concentrations, solvent and reaction temperature that contained two mutations in the cofactor-ring binding motif, which revealed it as a 'hot spot' for ATA engineering. Moreover, medium engineering strategies are demonstrated that improve ATA stability and productivity, e.g. employing optimal substrate ratios.</p> <p>A novel process concept was developed to address the unfavorable reaction equilibrium, product and coproduct inhibition as well as amine product purity. This integrated process concept uses alanine as amine donor in combination with two ISPR methods, i.e. a three-phase extraction system to recover the inhibiting amine product (1-methyl-3-phenylpropylamine) and an enzymatic cascade reaction to remove the inhibiting coproduct (pyruvate). The double ISPR concept yields the amine product in high purity ($\geq 98\%$) without any additional purification step. Advantageously, even when poorly water-soluble ketone substrates (here: benzylacetone) are used, the amine product can be enriched <i>in situ</i> to high concentrations, which will ease the downstream process.</p>			
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