EXPENANTIO

Towards green synthesis of chiral molecules: innovative experimental and theoretical approaches for understanding molecular basis of lipase and esterase enantioselectivity

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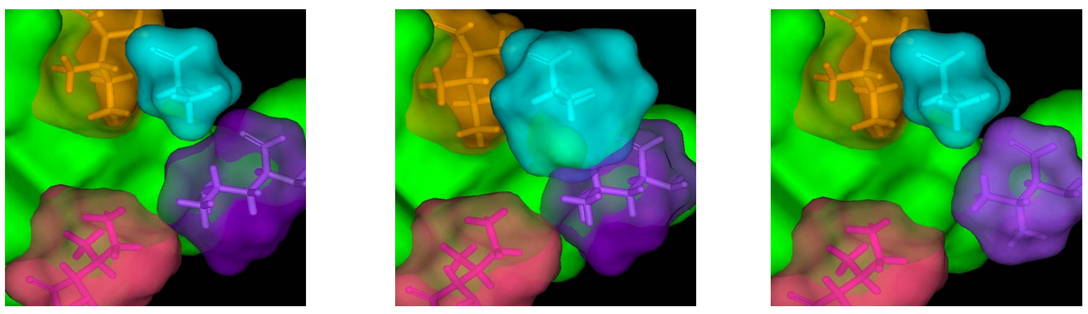
Lipase engineering for increasing enantioselectivity

Candida antarctica lipase B (CALB) presents high enantioselectivity towards many chiral substrates, such as secondary alcohols. To investigate the structural basis of this selectivity and to increase it, modified enzymes were produced using expression systems in *Pichia pastoris* . For example, amino acids located in the short tunnel to the enzyme active site (I189, L278, A282 and I285) were modified into amino acids with more or less bulky side chains, in order to affect substrate trajectory to the active site, and increase enantioselectivity. With the A282L and L278V mutants, an increase in enantioselectivity towards pentan-2-ol and hexan-3-ol compared to the wild type was observed. In addition, these modified enzymes presented a higher activity.

Marton Z, Léonard-Nevers V, Syren P-O, Bauer C, Hult K, Tran V, Graber M (2010) Mutations in the stereospecificity pocket and at the entrance of the active site of Candida antarctica lipase B enhancing enzyme enantioselectivity, J. Mol. Catal. B: Enzym. 65: 11–17.



WT A282L L278V

Shape of the entrance tunnel for wild type and modified CALB (I189 (pink), L278 (purple), A282 (blue) and I285 (orange)).

Substrate engineering for increasing lipase selectivity

CALB catalyzed resolution of secondary alcohols by transesterification with esters as acyl donors, occurs through a Ping Pong Bi Bi mechanism, which includes two steps. The first is the acylation of the enzyme by the ester substrate, to yield the acyl–enzyme intermediate. In the second step, the chiral alcohol interacts with the acyl-enzyme to form a product ester. CALB enantiopreference for the R-form of the chiral alcohol can be modified through a molecular imprinting effect or “ligand-inducedenzyme memory” caused by the shape of the ester substrate :The more the structure of the first substrate resembles the preferred enantiomer, the higher the enantioselectivity.

In case of CALB-catalyzed acylation of amino-alcohols the chemoselectivity strongly depends on the substrate structure : a nucleophilic group in β of an amino group enhances rates of N-acylation. Rates of N-acylation is improved for the short chain amino-alcohol whereas O-acylation is favored for the long chains amino-alcohols. Our kinetic results allows to propose a previously unacknowledged hydrogen shuttling mechanism in the transition state for lipase catalyzed N-acylation of amino alcohols.

Chaput L, Marton Z, Pineau P, Domon L, Tran V, Graber M, Enhancing the Enantioselectivity of CALB by substrate imprinting: a combined experimental and molecular dynamics simulation model study. J Mol Catal B: Enzym (accepted).

Le Joubioux F, Ben Henda Y, Bridiau N, Achour O, Graber M, Maugard T The effect of substrate structure on the chemoselectivity of Candida antarctica lipase B-catalyzed acylation of amino-alcohols. J Mol Catal B: Enzym (in revision).

Lipase enantioselectivity prediction by FEP studies

Enantioselectivity of enzyme is related to the difference in free energy ΔΔG* between transition states for both enantiomers. For the first time, we used the free energy perturbation (FEP) method to evaluate the free energy difference between tetrahedral intermediates formed with R and S alcohol enantiomers for a series of secondary alcohols, in case of CALB-catalyzed resolution of these chiral alcohols. This is a valid model for ΔΔG*. Computational results were found to be in qualitative agreement with experimental data, and enable the determination of substrate orientation in the active site with fair confidence.
Notwithstanding, FEP calculations can provide results for novel substrates, without the need for a significant number of experimental data to adjust the model, as is the case, for instance, with 3D-QSAR methods. In the future FEP method could become a very interesting tool for the pharmaceutical industry.

Chaput L, Sanejouand Y-H, Balloumi A, Tran V, Graber M (2012) Contribution of both catalytic constant and Michaelis constant to CALB enantioselectivity; use of FEP calculations for prediction studies. J Mol Catal B: Enzym 76: 29– 36.

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