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Review

Enantiopreference of *Candida antarctica* lipase B toward carboxylic acids: Substrate models and enantioselectivity thereof

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ABSTRACT

Free or immobilized *Candida antarctica* lipase B (CALB) is well recognized for tolerating polar organic solvents, low water activity, alkaline pH, and elevated temperature. It can exhibit a very high degree of activity and enantioselectivity for preparing optically active hydroxy and amino but not carboxylic acid compounds. Taking the current interests of preparing chiral carboxylic acids via CALB into account, this review is aimed to summarize the published articles related to the topic, and proposes comprehensive substrate models for predicting the enzyme enantiopreference for the stereoisomers and prochiral compounds, yet with high reliability only for the former. The kinetic and thermodynamic analysis is then addressed with emphasis on extracting the quantitative information for rationalizing the enzyme enantioselectivity. Moreover, interesting and recent advances on improving the enzyme enantioselectivity via medium, substrate, or enzyme engineering approach are highlighted.

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1. Introduction

Lipases (E.C. 3.1.1.3) have been widely applied as robust biocatalysts in industry for the synthesis of a variety of lipids, food and flavors, pharmaceuticals, fine chemicals, cosmetics, biodiesels, polymers, among others [1–6]. A very useful feature of the enzyme is the enantiodiscrimination, with which the preparation of single-isomer chiral drugs in the pharmaceutical industry is fulfilled, either by kinetic resolution of racemic alcohols, acids, and amines or by desymmetrization of prochiral and meso compounds [6–13]. This may be attributed to the stereodifferentiating pocket of enzyme active site for the affinity and transformation of acyl or leaving group of the substrate containing stereocenters.

The catalytic machinery of lipases, consisting of a Ser-His-Asp/Glu triad and several oxyanion-stabilizing residues, follows an acylation–deacylation displacement mechanism as demonstrated in Fig. 1 [2,14]. In the acylation step, the imidazole moiety of histidine of the catalytic triad abstracts a proton from the serine to increase the nucleophilic attack at the carbonyl carbon of the acyl donor, while the resultant imidazolium donates the proton to the leaving group oxygen or nitrogen from the tetrahedral intermediate to form the acyl-enzyme intermediate. In the deacylation step, hydrolysis proceeds via a second tetrahedral intermediate with water as the acyl acceptor to give the acid product.

Depending on the stereocenter allocated in the acyl or leaving group, a variety of pre-steady-state or steady-state techniques as well as molecular modeling methods have been employed for determining whether the acylation or deacylation step is rate-limiting in the overall reaction [15–18]. Therefore the complicate rate equations based on Ping–Pong Bi Bi mechanism for both enantiomers can be simplified to those via Michaelis–Menten mechanism [19]. Moreover as the importance of catalytic histidine as a general acid–base catalyst is highlighted in the whole acylation step, solvent isotope effects and structure–reactivity correlations have been interpreted in terms of rate-limiting tetrahedral adduct formation or breakdown [20–22]. If the three dimensional structural properties of active site are further released by multidimensional NMR spectroscopy or X-ray crystallography, all kinetic constants for each enantiomer in the rate-limiting acylation or deacylation step can be estimated via molecular modeling and determine the enzyme activity, enantiopreference, and enantioselectivity [16,23–25]. Unfortunately, the estimated kinetic constants and hence the enantioselectivity are usually in qualitative agreements with the experimental data.

The transition-state model based on Michaelis–Menten mechanism for the rate-limiting acylation or deacylation step has been proposed to simplify the calculating steps and time for estimating the enantiopreference or even enantioselectivity via molecular modeling methods [17,18,26–31]. Yet the calculation procedures are still very tedious and time-consuming in comparison with a substrate model based on substituent size, electronic attractions, and/or charge effects at the stereocenter. Indeed a reliable substrate model represents a simple and fast way for accelerating the enzyme screening process for the fast-reacting enantiomer. For primary alcohols and amines as well as secondary alcohols, several empirical rules, known as Kazlauskas rule, have been proposed for successfully predicting the enantiopreference for CALB and lipases from *Alcaligenes sp.*, *Aspergillus niger*, *Burkholderia cepacia* (or *Pseudomonas cepacia*), *Pseudomonas fluorescens*, *Candida rugosa* (or *Candida cylindracea*), porcine pancreatic [7,11,13]. This rule was moreover extended to carboxylic esters containing one stereocenter in the acyl part only for *C. rugosa* and *A. niger* lipases, yet with low reliability for the later [32–34].

CALB is a globular α/β type protein with approximate dimensions of 30Å × 40Å × 50 Å with formula weight of 33,273 Da. Unlike most lipases, the enzyme active site of approximately 10Å × 4 Å wide and 12 Å deep is readily accessible, implying that CALB has no lid to exhibit interfacial activation and control substrate entry to the active site [35]. The enzyme especially after immobilization is well recognized for tolerating a great variation in experimental conditions such as polar organic solvents, lower water activities, alkaline pH, and elevated temperature. Therefore, a wide range of academic researches and industrial applications for synthesizing low molar mass and polymer is realized, as illustrated in Fig. 2 for the increasing numbers of articles [4,36–39]. Nowadays CALB in free or immobilized formula is commercially available and sold under the trade names of Novozym 435 (or SP435, a recombinant enzyme, immobilized product) and SP 525 (a powder product) from Novozymes, Chirazyme L-2, c.-f., C2, lyo and Chirazyme L-2, c.-f., C3, lyo from Roche Diagnostics, CALB-AH-42 from Almac, CV-CALBY (free), IMMCALB and IMMCALBY (immobilized) from ChiralVision, CALB lyo and CALB immo from c-LEcta, ELCAB from Eucodis, and IMMCALB from Syncore Laboratories, among others.

CALB exhibits a very high degree of activity and enantioselectivity for the resolution of alcohols and amines in industrial preparation of optically active hydroxy and amino compounds [7,9,13,40–42]. However, it also shows low to modest enantioselectivity toward carboxylic acids, and has been attributed to fewer and/or weaker contacts between the substrate and spacious acyl binding site, in contrast to the excellent enantiopreference for secondary alcohols and primary amines in the restricted active pocket. In order to realize CALB as a versatile biocatalyst, various approaches via substrate, medium, and/or enzyme engineering can be employed for overcoming the drawback [43,44]. Taking the low enzyme enantioselectivity and current interests for preparing chiral carboxylic acids into account, it is aimed at first to summarize the articles related to the topic from a literature survey, and propose comprehensive substrate models for the prediction of CALB enantiopreference toward various substrates. The kinetic and thermodynamic analysis is then addressed with the emphasis on elucidating the quantitative parameters and enantioselectivity that may shed insights on how to manipulate the enzyme stereodiscrimination. Moreover, interesting and recent advances on improving CALB enantioselectivity are highlighted.

2. Acyl donor spectra

The enzyme-catalyzed kinetic resolution and desymmetrization of acyl donors are classified according to the substrate structure (i.e. racemates with the carbonyl carbon in or not in a