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Computational study of the enantioselectivity of the *O*-acetylation of (*R*,*S*)-propranolol catalyzed by *Candida antarctica* lipase B

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ABSTRACT

Candida antarctica lipase B (CalB) displays moderate enantioselectivity when it catalyzes the acetylation of (*R*,*S*)-propranolol, favoring the faster transformation of the *R*-propranolol. With the aim to better understand the enantioselectivity of this reaction, we have performed a molecular dynamics (MD) study of the enzyme substrate complexes. Reactive enzyme substrate complexes were identified for both enantiomers of propranolol, which differ in their temporal stability and in their ability to reach the corresponding transition states (TS). Reactive complexes of *R*-propranolol present a better ability to be transformed by CalB than those of *S*-propranolol. This allows us to explain the enantioselectivity. Analysis of the enzyme–substrate interactions suggests that the CH-π interactions between the naphthyl rings of propranolol and the residues of the CalB binding pocket may play an important role in stabilizing the transition states involved in the transformation of the *R*-propranolol. The residues Ile189, Ala282 and Leu278 were identified as key residues for the enantioselectivity of CalB.

1. Introduction

Propranolol ((*R*,*S*)-1-iso-propylamino-3-(1-naphthoxy)-2-propanol), a beta-adrenergic blocking agent used for treatment of arterial hypertension and other cardiovascular disorders [1–3], is commercially available as a racemic mixture. However, only the *S*-enantiomer has the desired therapeutic effect, and administration of the racemic propranolol mixture may cause side effects such as bronchoconstriction or diabetes [4–6].

Several strategies to obtain *S*-propranolol in enantiomerically pure form have been proposed, including chemical, enzymatic and chemoenzymatic synthesis routes [7–16]. Recently immobilized *Candida antarctica* lipase B (CalB) was used as a biocatalyst to carried out the acetylation of (*R*,*S*)-propranolol in toluene. The enantioselectivity was moderate ($E = 57$), but higher than or comparable to the enantioselectivity observed in the kinetic resolution of propranolol either via esterification or hydrolysis using other enzymes [13]. Using immobilized CalB allows to reuse the biocatalyst and to simplify its separation from the reaction products. This makes the reaction attractive for industrial applications, but the enantioselectivity has to be improved for this purpose.

Lipases are characterized by a catalytic triad consisting of Serine, Histidine and Aspartate (Ser105, His224 and Asp187 in CalB). The accepted general mechanism of lipase-catalyzed reactions involves two steps. The first step of the reaction is the addition of an acyl-group to the catalytic serine of the enzyme, yielding the acyl-enzyme (acylation step). In the second step, the acyl-group can react with several nucleophiles, such as water, alcohols, amines or peroxides (deacylation step) [17]. Acylation as well as deacylation proceed via an initial noncovalent enzyme–substrate complex (Michaelis complex; MCC) and a tetrahedral intermediate (TI). The latter is stabilized by NH and OH functions in the so-called oxyanion hole of the enzyme, constituted by the residues Thr40 and Gln106 in CalB. According to this mechanism, the deacylation step in the CalB-catalyzed acetylation of (*R*,*S*)-propranolol is expected to be chemo- (*N*- or *O*-acetylation) and stereoselective (acetylation of *R*- or *S*-propranolol) (see Scheme 1).

We previously studied the chemoselectivity of this reaction and found experimentally that the *O*-acylated product is formed exclusively [18]. To rationalize this result we applied an enzyme–substrate docking protocol to model the MCCs of the deacylation reaction, which showed that both *R*- and *S*-propranolol accommodate within the binding pocket of CalB in two binding

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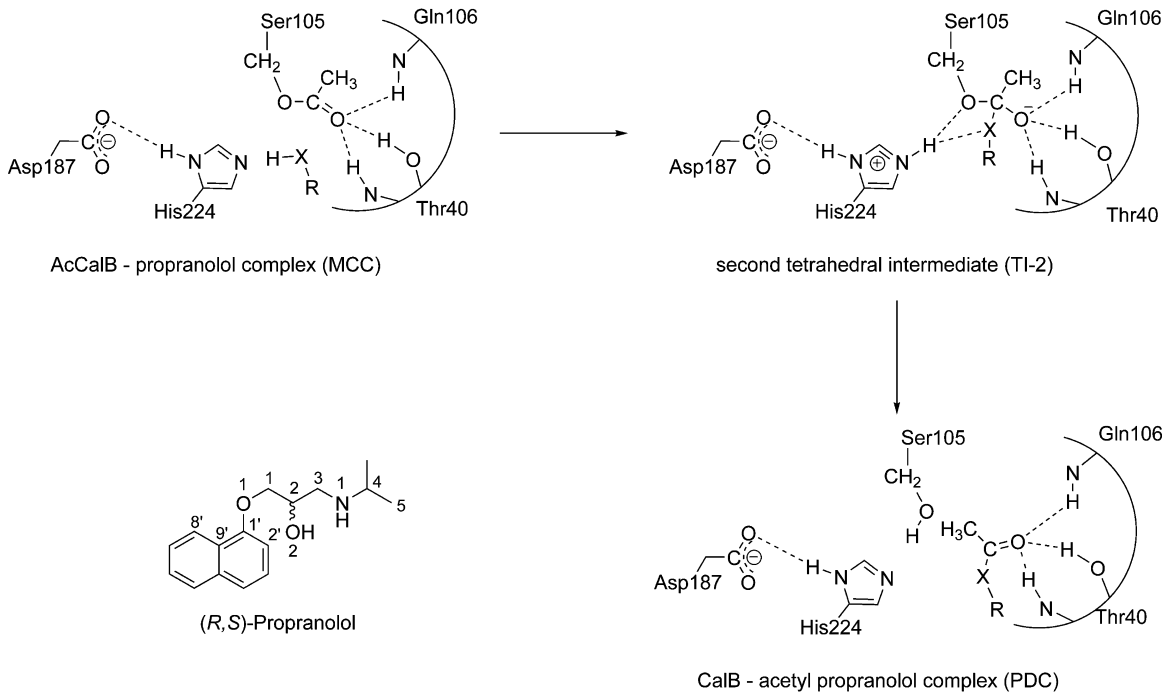

Scheme 1. Deacylation step in the CalB-catalyzed acetylation of (*R,S*)-propranolol (X = N or O, according to the nucleophile groups of propranolol). This reaction step occurs sequentially via an initial noncovalent acetyl-CalB (AcCalB) substrate complex (Michaelis complex; MCC) and the second tetrahedral intermediate (TI-2), to give a noncovalent CalB-product complex (PDC). The structure of propranolol with the corresponding atom numbering is shown down at the left.

modes (namely, binding modes I and II). Viewed with the catalytic triad Asp-His-Ser oriented from left to right, the binding pocket of CalB is constituted by a large hydrophobic pocket above the catalytic triad and a medium size pocket below it. In binding mode I the naphthoxy side chain of propranolol is located in the large hydrophobic pocket. The isopropylamine side chain is in the medium pocket and part of it may extend toward the entrance of the pocket (i.e. toward the solvent). In binding mode II the orientation of propranolol is reversed (see Figs. 1 and S1 of the Supplementary data). Only conformations of the substrate were identified in which its hydroxyl group is close to the catalytic His224 and the acetylated serine. This explained the experimentally observed chemoselectivity of CalB. In addition, to check the reliability of the complexes identified by docking, they were subjected to short

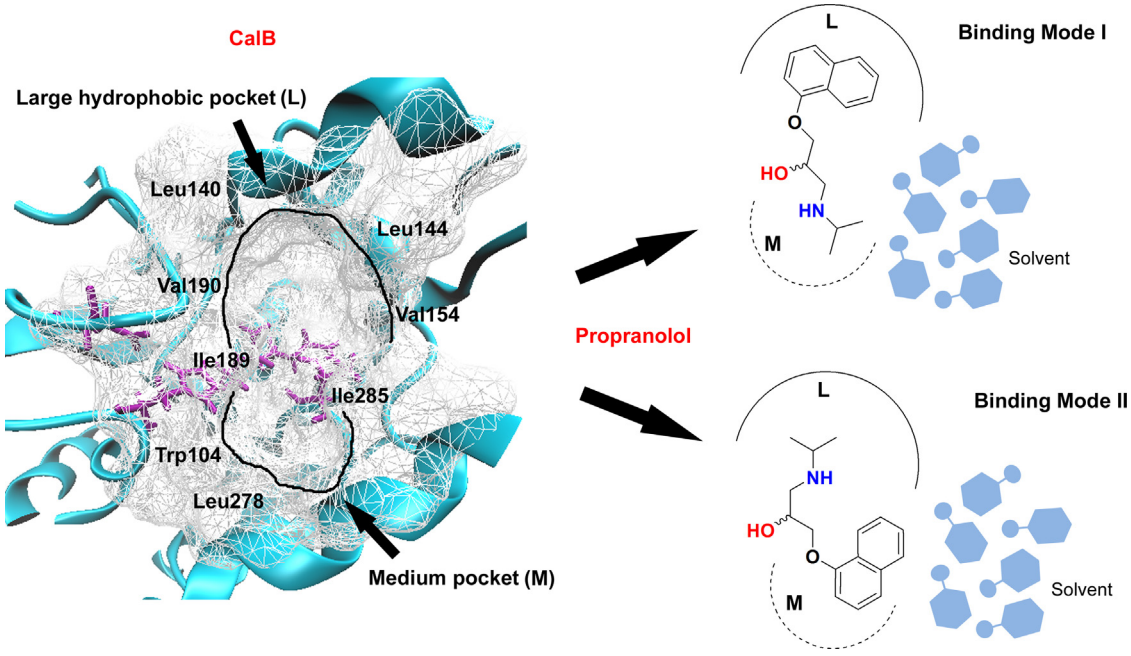

Fig. 1. Characteristic binding modes of propranolol in the CalB binding pocket. The structure of acetyl-CalB (AcCalB) is shown on the left with the catalytic triad Asp-His-Ser oriented from left to right. The binding pocket of CalB is constituted by a large hydrophobic pocket above the catalytic triad and a medium pocket below it. The large pocket is lined by Ile189 and Val190 on the left, Val154 on the far right, as well as Leu140 and Leu144 at the top. Deep in this pocket, Asp134 is on the left and Gln157 on the right. The medium pocket is below the catalytic Ser105 and is crowded by Trp104 below it and the Leu278–Ala287 helix (helix α10) to the right. In binding mode I (up to the right) the naphthoxy side chain of propranolol lies in the large hydrophobic pocket of CalB while its isopropylamine side chain in the medium pocket and may extends to toward the entrance of the binding pocket. Conversely, in binding mode II (down to the right) the former lies in the medium pocket and the latter in the large pocket.