

The Efficient and Selective Catalytic Oxidation of Terpenoids and Aromatic Hydrocarbons by the P450 Monooxygenase CYP101B1

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Abstract

CYP101B1, from the bacterium *Novosphingobium aromaticivorans*, has been shown to bind and oxidise β -ionone to 3-hydroxy- β -ionone and camphor to 5-*exo*-hydroxycamphor.¹ Whole-cell reactions of CYP101B1 have been observed to turn blue, which suggests indole oxidation to indigo.¹⁻³ Therefore, CYP101B1 has the potential to act as a biocatalyst for the oxidation of a broad range of substrates.

β -Ionone and other similar norisoprenoids were initially tested to determine which structural features were important for binding to CYP101B1. Small adjustments to the β -ionone structure indicated the butenone side chain was important for tight substrate binding to CYP101B1. The cyclohexene component of β -ionone is also a better fit for the active site than linear or aromatic analogues. Further testing of aromatic substrates, such as indole, phenylcyclohexane and *p*-cymene, indicated that CYP101B1 binds substituted aromatics such as phenylcyclohexane and could produce products in a reasonable yield. Smaller aromatics, such as *p*-cymene, could still bind and react with CYP101B1, but the activities and product levels generated were lower than the larger two-ring aromatics.

CYP101B1 oxidation of camphor and other terpenoid based substrates was studied. However, these substrates had large dissociation constants and low product formation rates due to a larger proportion of the NADH being used to reduce oxygen to hydrogen peroxide or water instead of products. Multiple oxidation products were formed with these substrates, which was most likely due to their poor fit in the CYP101B1 active site. This was not an ideal outcome, so the substrates or enzyme require modification to improve the reactivity and selectivity of the biocatalyst.

Terpenoid substrates were modified to include an acetate side chain, resulting in a structure more similar to the norisoprenoids. This greatly improved the binding and activity with CYP101B1, and resulted in production of a single oxidation product in the *in vitro* turnovers. The ketone moiety of the acetate group leads to better binding to CYP101B1 and results in more desirable catalytic properties.

The affinity of CYP101B1 for aromatic structures was determined using a range of biphenyl and naphthalene derivatives. These structures exhibited reasonably tight binding; however, moderate NADH consumption rates, product formation rates and coupling between the substrate and CYP101B1 were observed. Therefore, the CYP101B1 active site favours more polar sub-

strates. Activity for hydrophobic substrates could be increased by modifying CYP101B1 to remove specific contact between a hydrophilic amino acid side chain and the ketone group, which is important in the binding of norisoprenoids and monoterpenoid acetate substrates. The turnovers had high product selectivity and, in most cases, only a single product was generated. The drug, diclofenac, was reacted with CYP101B producing the metabolite, 4'-hydroxydiclofenac, in reasonable yield.

CYP101B1 is a useful biocatalyst for selective C–H bond oxidation of norisoprenoids and terpenoid acetates. It also shows potential for other substrates if CYP101B1 is modified or with the addition of an ester protecting group onto the target molecule.

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