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Functionalization of Polymers Using Candida Antarctica Lipase B (CALB) Catalysis

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ABSTRACT

The objective of this research was to investigate the enzyme-catalyzed functionalization of polymers. For this purpose, first, model small molecules were employed in Candida antarctica lipase B (CALB)-catalyzed transesterification and Michael addition reactions(1).Lipase B is more active towards a broad range of esters, amides and thiols(2). CALB-catalyzed transesterification of ethyl acetate and vinyl acetate with 2-phenyl-1-propanol (2PPOH), the model compound for primary hydroxyl-functionalized polyisobutylene (PIB) prepared from the a-methylstyrene epoxide (a-MSE)/TiCl4 initiator system, indicated that the latter was a more effective acyl donor as it formed unstable vinyl alcohol which instantly tautomerized to acetaldehyde and thus rendered the reaction irreversible. The comparison of the catalytic activity of CALB with that of a commercially available transesterification catalyst, bis[dibutylchlorotin(IV)] oxide, revealed that in the transesterification of vinyl acetate with 2PPOH, CALB was the more reactive catalyst.

Keywords : Candida antarctica lipase B (CALB), transesterification, methylstyrene epoxide (a-MSE), polyisobutylene (PIB), tautomerized

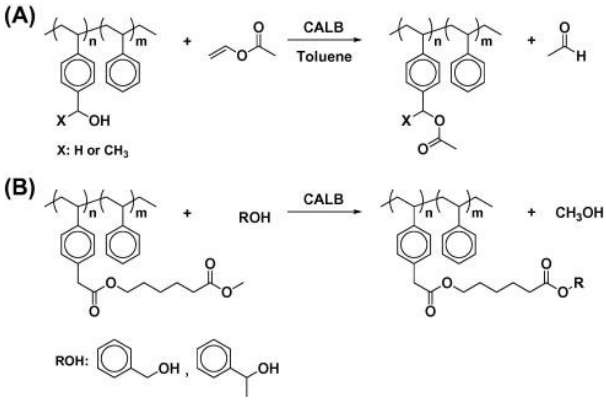
I. INTRODUCTION

LIPASE CATALYSED TRANSESTERIFICATION REACTIONS

Enzyme-catalyzed transesterification reactions are especially useful for the preparation of optically active compounds by asymmetrization or resolution of racemic or enzymes that hydrolyze peptide bonds in vivo, have been used as transesterification catalysts, the latter found limited application due to high substrate selectivity. Several parameters such as the structure of acyl donor, the enzyme source, the structure of alcohol and the reaction stoichiometry must be taken into account for a successful transesterification.

II. EXPERIMENTAL

Transesterification reactions are generally reversible. In order to change the reversible nature of the reaction into an irreversible type, the nucleophilicity of the leaving group of the acyl donor should be depleted by the introduction of electron-withdrawing groups such as trifluoroethyl- or trichloroethyl- into the ester. Alternatively, the use of oxime esters, thioesters, and anhydrides as activated acyl donors have been proposed. The use of enol esters(3) such as vinyl or isopropenyl esters appears to be the most useful since they liberate unstable enols as by-products which rapidly tautomerize to give the corresponding aldehydes or ketones.



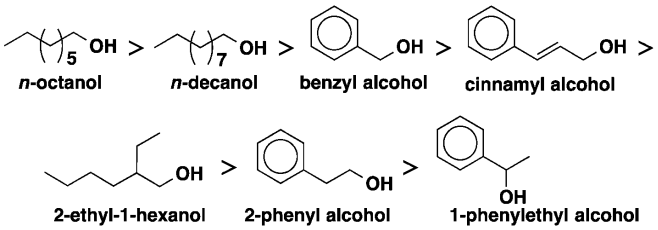
Therefore, the reaction becomes completely irreversible. It was shown that acyl transfer reactions using enol esters are 100 to 1000 times faster than the reactions using non-activated esters such as ethyl acetate.(3) Vinyl esters are favored over isopropenyl esters because of less steric hindrance and thus higher reaction rates.(4) Acetaldehyde, which forms during the reactions with vinyl esters, is known to inactivate the lipases from Candida rugosa and Geotrichum candidum by forming a Schiffs base with the lysine residues of the protein; however most lipases, including CALB, tolerate the liberated acetaldehyde.

[5]On comparing the catalytic activity of various commercially available lipases in transesterification of vinyl acetate with n-octanol ;CALB, Mucor meihei lipase and Pseudomonas lipase were immobilized on macroporous polyacrylic resin beads, anionic resin and diatomite, respectively, whereas Candida rugosa lipase and porcine pancreatic lipase were in free form. It was observed that CALB was the most efficient lipase (Table)

ENZYME	ACTIVITY (μmol.min^{-1}.mg^{-1})
Candida antartica lipase B	11.3
Mucor meihei lipase	1.30
Pseudomonas lipase	0.93
Candida rugosa lipase	0.00
Porcine pancreatic lipase	0.00

It gave 82% conversion, whereas Mucor meihei lipase and Pseudomonas lipase gave only 18% and 8% conversions, respectively, within 90 min. The inactivity of Candida rugosa lipase and porcine pancreatic lipase was attributed to denaturation by the acetaldehyde by-product.

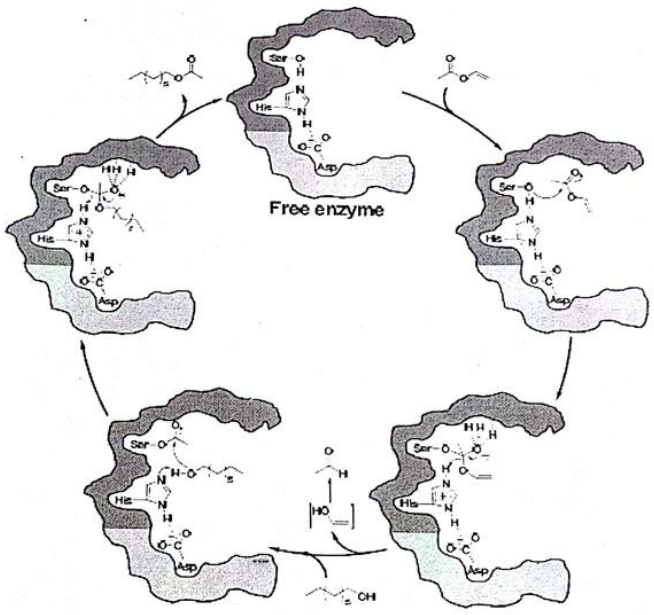
The structure of the alcohol is another important parameter affecting the initial rate and overall conversions in enzymatic transesterification. It was observed that straight-chain alcohols gave better conversion compared to aromatic and branched-chain alcohols in the CALB-catalyzed transesterification of vinyl acetate due to less steric hindrance around the hydroxyl group .



[5]Furthermore, aromatic alcohols with saturated shorter side chains (e.g. benzyl alcohol) were more reactive than those with longer but unsaturated side chains (e.g. cinnamyl alcohol).

Although an increase in ester concentration was shown to increase the rate and conversion of transesterification, an increase in alcohol concentration might cause reduced rates and conversions due to competitive inhibition by the alcohol which can bind reversibly to the enzyme active site and prevent the binding of the ester substrate[6]. The driving force for alcohol binding might be the high polarity of the region around the active serine site of the enzyme[7]. Varying the concentration of n-octanol while keeping the amount of vinyl acetate (1 mol/L) constant in CALB-catalyzed transesterification, an increase in the reaction rate when «-octanol concentration was increased from 0.25 mol/L to 1 mol/L; however, upon further increase the rate decreased.

The mechanism of transesterification is similar to the mechanism of lipase-catalyzed hydrolysis except that the incoming nucleophile attacking the acyl-enzyme complex is an alcohol rather than water.[8] The CALB-catalyzed transesterification of vinyl acetate with «-octanol .



The reaction involves four sequential steps. First, the nucleophilic serine (Ser105) residue attacks the carbonyl group of the vinyl acetate, forming a tetrahedral intermediate which is stabilized by the oxyanion hole of the enzyme via three hydrogen bonds: one from glutamine (Gln106) and two from threonine (Thr40) units. In the second step, the ester bond is cleaved to form the first product, vinyl alcohol which will tautomerize to acetaldehyde, and the acyl-enzyme complex. In the third step, the reactant alcohol, n-octanol, attacks the acyl-enzyme complex to form a second tetrahedral intermediate which is again stabilized by the oxyanion hole. In the last step, the enzyme is deacylated to form the desired product, octyl acetate. The nucleophilic attack by the Ser105 is mediated by the His224-Asp187 pair. Similarly to many other lipases, the last step, i.e. the deacylation, was shown to be the rate limiting step in CALB-catalyzed transesterification. It was demostrated [9] that the reaction rates for ester substrates differing only in the leaving group were the same. Similarly [8] the same reaction rates were observed using vinyl- and ethyl octanoate as the acyl donors and (R)-2-octanol as the acyl acceptor in the presence of CALB.

III. REFERENCES

[1]. Uppenberg, J.; Oehrner, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthonsen, T.; Jones, T. A. Crystallographic and molecular-modeling studies of lipase B from Candida antarctica reveal a stereospecificity pocket for secondary alcohols. Biochemistry 1995, 34, 16838-16851.

[2]. Kirk, O.; Christensen, M. W. Lipases from Candida antarctica: Unique Biocatalysts from a Unique Origin. Org. Process Res. Dev. 2002, 6, 446-451.

[3]. Wang, Y. F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C. H. Lipase-catalyzed irreversible transesterifications using enol esters as acylating reagents: preparative enantio- and regioselective syntheses of alcohols, glycerol derivatives, sugars and organometallics. J. Am. Chem. Soc. 1988, 770, 7200-7205.

[4]. Faber, K.; Riva, S. Enzyme-catalyzed irreversible acyl transfer. Synthesis 1992, 895-910.Weber, H. K.; Stecher, H.; Faber, K. Sensitivity of microbial lipases to acetaldehyde formed by acyl-transfer reactions from vinyl esters. Biotechnol. Lett.1995, 77, 803-808.

[5]. Yadav, G. D.; Trivedi, A. H. Kinetic modeling of immobilized-lipase catalyzed transesterification of n-octanol with vinyl acetate in non-aqueous media. Enzyme Microb. Technol. 2003, 32, 783-789.

[6]. Yadav, G. D.; Lathi, P. S. Lipase catalyzed transesterification of methyl acetoacetate with n-butanol. J. Mol. Catal. B: Enzym. 2005, 32, 107-113.

[7]. Rizzi, M.; Stylos, P.; Riek, A.; Reuss, M. A kinetic study of immobilized lipase catalyzing the synthesis of isoamyl acetate by transesterification in n-hexane. Enzyme Microb. Technol. 1992, 74, 709-714.

[8]. Martinelle, M.; Hult, K. Kinetics of acyl transfer reactions in organic media catalysed by Candida antarctica lipase B. Biochim. Biophys. Acta 1995, 7257, 191-197.

[9]. Garcia-Alles, L. F.; Gotor, V. Lipase-catalyzed transesterification in organic media: solvent effects on equilibrium and individual rate constants. Biotechnol. Bioeng.1998, 59, 684-694.