LIPASE CATALYZED ENANTIOSELECTIVE ESTERIFICATION REACTION TO PRODUCE CHIRAL PRODUCTS

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Introduction

The increasing understanding of the mechanism of drugs, pesticides and hormones interaction at the molecular level has led to the significance of chirality as the key to the efficacy and safety of many of these products (Patel et al. 1995). In many cases only one stereoisomer (enantiomer) of these substances is required for efficacy and safety, and the other stereoisomer is either inactive or may be toxic. This new development has contributed to the developing enantiomers of these products to avoid the possibility of side effects due to undesirable stereoisomers. Preparation of enantiomers by chemical methods is not only laborious and but also expensive. In this project, we are developing an alternative method of using enzymes as biocatalysts to produce enantiomers via asymmetric synthesis.

Materials and Methods

Enzymes used in this study were eight kinds of lipases from yeast, fungi and bacteria. The esterification reaction was carried out as described previously (Pan et al. 1990). The products of the reaction were isolated by column chromatography (silica gel 60, 70-230 mesh). The optical purity of remaining (S)-acid was determined from optical rotation readings using an Atago Polax-D polarimeter (Japan) at 25°C. The amounts of remaining alcohol, acid and ester produced were determined by gas chromatography (Shimadzu GC-14A, Japan) with a flame-ionisation detector using a 30 m capillary column type Restek (AT-1000). Lipase was modified with monomethoxy-polyethylene glycol of molecular weight 1900 and 5000 as described previously (Basri et al. 1995). Reductive alkylation of lipase was as described previously (Ampon et al. 1991). Lipase was immobilised to various polymer organic beads (Basri et al. 1996).

Results and Discussion

In the stereoselective screening, 8 lipases exhibited some stereobias with (R)-acid. They were lipases from *Candida rugosa, Rhizopus nivieus, Pseudomonas roqueforti* and *Aspergillus niger, Rhizopus arrhizus* and *Mucus javanicus* as well as mycelial lipases from mesophilic Rhizopus oryzae (MroML) and Rhizopus rhizopodiformis (RrML). However, lipase from *Candida rugosa* which showed the highest stereobias was selected as the enzyme to be used for further study as it also exhibited high activity as well as it is readily available.

In the modification of lipase with acetyldehyde and octyldehyde, the stereoselective activities of all the modified lipase preparations were higher than the native enzyme. Lipase that was modified with high molecular weight aldehyde showed a higher stereoselective activity as compared with those modified with the low molecular weight aldehyde at the same degree of modification. Increasing the degree of modification for both acetyl-lipase and octyl lipase increased the stereoselective activity as shown by the increase in the enantiomeric excess (%ee). In the case of the lipase modification with activated monomethoxypolyethylene glycol, similar increased in the percentage of enantiomeric excess was observed as the degree of modification of lipase was increased). As the degree of modification or the molecular weight of the modifier is increased, which is associated with the increase in hydrophobicity, the formation of the (R)-acyl enzyme was more favoured than the (S)-acyl enzyme. In our study, generally, the immobilisation procedure using the tested supports did not improve the enantioselective reaction of (RS)-2-(4-chlorophenoxy)propanoic acid with tetradecanol. This was as expected as the molecular interaction between the enzyme and the supports is through physical adsorption type which are mainly contributed by weak hydrophobic and Van der Waals interactions, thus drastic change in the molecular conformation on the enzyme molecule is not expected. However, the increase in activity is an advantage as immobilised enzymes have better characteristics in reactions in organic solvents compared to native enzymes. The native lipases from different sources and the *Candida rugosa*-immobilised lipases were most active in solvents of log P values greater than 2. However, certain lipases ie. those from *Pseudomonas roqueforti* and Mucus *javanicus* are active even in aceton. Generally, the stereoselective activity of the lipases is relatively higher in non-polar solvents (log P values equal or greater than 2). Hydrophobic organic solvents appeared to be more effective in inducing the esterification of the R-2-(4-chlorophenoxy) propanoic acid with tetradecanol compared to the S-2-(4-chlorophenoxy) propanoic acid.

Conclusions

Screening of enzymes for high stereoselective activities is an important initial study to develop an efficient stereoselective reaction. Lipase from *Candida rugosa* is the best to be used for stereoselective synthesis. We can design enzyme to acquire high stereoselectivity by either chemical modification to increase the hydrophobicity of the enzyme by using modifiers of high molecular weight or/and increase the degree of modification of the enzyme and using organic solvents of higher log P in the reaction systems. Immobilisation of the enzyme using the tested supports did not show significant increase in their stereoselectivity, however, we believed that other supports of different properties may change the stereoselectivity of the enzyme. Work is underway to study this issue.

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