Enzyme Catalyzed Stereo selective Reactions to Produce Useful Products*

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Key words: enzyme, lipase, stereo selective, reactions, products.

Introduction

The increasing understanding of the mechanism of drugs, pesticides and hormones interaction on a molecular level has led to the increasing awareness of the significance of chirality's as the key to the efficacy and safety of many of these products. It is now known that in many cases only one stereo isomer (enantiomer) of these substances is required for efficacy and safety, and the other stereo isomer is either inactive or may be toxic. Enantiomers can be prepared by separation of the racemes compounds by chemical methods. However, the laborious steps and the expensive and sometimes toxic chemicals needed to produce enantiomers meant much higher production costs. In this project, we are developing an alternative method, which is using enzymes as biocatalysts to produce enantiomers via asymmetric synthesis. Besides being known for their favorable properties, such as their mild and environmentally friendly reactions, enzymes are also known for their high substrate, region- and stereo-specificities. In this work, specific chital compounds such as 2-(4-chlorophenoxy) propanoic acid esters, the R-enantiomer is an important herbicide, were synthesized by using various lipases. The enzyme was designed to acquire high enantioselectivity by chemical modification and immobilization. Changing the parameters such as organic solvents and water activity optimized the reaction systems.

Materials and Methods

Eight kinds of lipases from yeast, fungi and bacteria were used in the screening studies. (RS)-2-(4-chlorophenoxy) propanoic acids were obtained from Aldrich, Milwaukee, Wis., USA. Tetradecanol was obtained from Sigma Chemical Co., St. Louis, Missouri, and USA. All other reagents were of analytical grade. The organic solvents and substrates were dried over a molecular sieve 3°A before use (unless otherwise stated). The etherification reaction was carried out as described by Basri *et al.* (1998). The remaining acid and alcohol, and the ester formed, were isolated by column chromatography (silica gel 60, 70-230 mesh) as described by Basri *et al.*, (In press). 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 and ester produced were determined by gas chromatography (Shimadzu GC-14A, Japan) with a flame-ionization detector using a 30 m capillary column type Restek (AT-1000). Lipase was modified with monomethoxy-polyethylene glycol as described by Basri *et al.* (1991, 1995). Reductive alkylation of lipase was as described by Basri *et al.* (1997). Amid nation of lipase was carried out as describe by Basri *et al.* (1992). Lipase was immobilized to various polymer organic beads as described by Basri *et al.* (1996a, 1996b, 1999).

Results and Discussion

In the stereoselective screening, 8 lipases exhibited some stereobias with (R)-acid. They are 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 various modifiers, the stereoselective activities of all the modified lipase preparations were higher than the native enzyme. Increasing the degree of modification increased the stereo selective activity as shown by the increase in the enantiomeric excess (% ee). Immobilization procedure using various supports exhibited different enantioselective activities. The native lipases from different sources and the *Candida rugosa*-immobilized 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 enantioselective 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. The initial water activity (Aw) of the reaction systems markedly influenced the enantioselectivity of the enzyme. Both percentages of ester conversion and enantioselectivity showed dependence on Aw in the reaction mixtures in organic solvents of different polarity. Optimum Aw for the enantioselective esterification was at 0.529 for all solvents used.

Conclusions

Lipase from *candida rugosa* is the best enzyme tested for enantioselective synthesis. Enzyme can be designed to acquire high stereo selectivity by chemical modification and/or immobilization. The enantioselective reaction was enhanced when organic solvents of higher log P and optimum Aw were used. These improved enzymes and reaction systems were used in the resolution of important race mates for use in the chemical and agro industries.

Benefits from the study

Designer enzymes with improved properties were produced. This enzyme is of potential interest in the field of

applied enzymologist. They find numerous applications in the field of molecular separation and catalysis. One particular interest is in the resolution of an important race mate, a herbicide for use in the chemical and agro industries An optimized reaction system was developed.

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* *An update of the abstract published in UPM Research Report 1998*

I·R·P·A INTENSIFICATION OF RESEARCH IN PRIORITY AREAS

Research funded by the Intensification of Research in Priority areas (IRPA) - Supported by IRPA RM-7 (1996-2000), Cycle 1996, Grant (09-02-04-0025.