Enantioselectivity of some lipases: Control and prediction

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Abstract - Different strategies for the resolution of racemates in lipase catalyzed hydrolyses, esterifications, and transesterifications are presented. The importance of the equilibrium conversion for the optimization of the enantiomeric excess is emphasized. Investigations have been carried out on esters of 1-phenylethanol and 2-methylalkanoic acids. Results are presented, which provide general information about important parameters for the enhancement of the enantiomeric excess and the reaction rates. The application of a 'matched strategy' stereochemical control for increasing the enantiomeric excess is presented, i.e. the use of a chiral acid for the resolution of chiral alcohols. Molecular modelling of lipase from Rhizomucor miehei as well as the use of a simple active site model of lipase from Candida cylindracea to obtain information for the prediction and modification of enzyme specificity is presented.

The use of lipases in organic synthesis has been the object of several investigations. Many lipases are commercially available, they accept a wide range of substrates, and they can be used for synthetic transformations in organic solvents (for ref. see 1). In this short account we shall report some recent results and general observations on parameters which enhance the enantiomeric excess in lipase catalyzed reactions. Our studies have been performed with some simple model substrates, such as esters of 1-phenylethanol, and also with esters of 2-methylalkanoic acids, which are useful building blocks for the synthesis of various biologically active natural products, e.g. pheromone constituents of pine saw-flies (for ref. see 2). The use of molecular modelling and a simple active site model as tools for predictions and modifications of enzyme action will also be discussed.

Structural data (ref. 3 – 6) confirm that lipases belong to the serine hydrolases, and that their action are most probably similar to those of other enzymes belonging to this group of hydrolytic enzymes, e.g. chymotrypsin. The kinetics of lipase-catalyzed transformations is complex, since lipases usually operate at hydrophobic interfaces and seem to require conformational changes for action. These complications have to be considered when the kinetics of lipase catalyzed reactions are studied. However, some general conclusions can be derived assuming homogeneous conditions.

Calculations of the enantiomeric excess of a reaction product as a function of the conversion show that the enantiomeric excess of the product is not very much affected by a moderate change of the equilibrium conversion (Fig. 1a). The enantiomeric excess of the remaining substrate, however, is more dependent on the equilibrium conversion (Fig. 1b). Thus, altering the reaction conditions so as to favour a high equilibrium conversion offers an opportunity to enhance the enantiomeric excess of the remaining substrate (ref. 7).
One efficient way of obtaining a high equilibrium conversion is to use vinyl acetate or other enol esters as acyl donors in irreversible transesterifications (ref. 9 – 12). In our investigations on conditions for lipase catalyzed hydrolysis and esterification in order to obtain 2-methylalkanoic acids of high enantiomeric purity we observed some alternative and simple procedures to enhance the equilibrium conversion (ref. 7). For these studies we used lipase from Candida cylindracea. It is of interest to note that the lipase catalyzed hydrolysis using these stERICALLY hindered acids proceeded satisfactorily, despite the fact the esters of these acids are very difficult to hydrolyze by conventional chemical methods.

We found that the enantiomeric excess and the equilibrium conversion in our experiments with esters of 2-methylalkanoic acids were dependent on pH and the presence of calcium chloride (Fig. 2). When octyl 2-methyldecanoate was hydrolyzed at pH 8 in presence of calcium chloride (Fig. 2, ■), the (S)-acid was formed in an enantiomeric excess of 80% (E=9), but when the hydrolysis was carried out at pH 7.5 without calcium chloride (Fig. 2, ○), no enantiomeric excess and a low equilibrium conversion were observed. The latter conditions, in fact, allowed the esterification of 2-methyldecanoic acid with octanol in an aqueous medium. The low equilibrium conversion in the absence of calcium chloride may be explained as follows. The hydrophobic substrate is hydrolyzed to hydrophobic (amphiphilic) products, which remain in the interface. In the absence of calcium ions the acid will accumulate in the interface and the acid will be a better acyl donor than the ester. 1-Octanol will be a preferred nucleophile in competition with water.

Figure 1. The enantiomeric excess of (a) the product and of (b) the substrate as a function of the conversion of the substrate. Two sets of curves are computer simulated with an equilibrium conversion of 99.9% and 90%. In each set, the curves from upper to lower represent the following E-values: 100, 50, 40, 30, 20, 15, 10, 5, and 2 (ref. 8).

Figure 2. The enantiomeric excess of (S)-2-methyldecanoic acid produced on hydrolysis of its racemic octyl (■, □, ●, ○) and ethyl (▼, Δ) esters. The reactions were catalyzed by lipase from Candida cylindracea at 25 °C and pH 7.5 (●, ○, Δ) and pH 8.0 (■, □, ▼). Filled symbols denote incubations in the presence of 0.2 M CaCl₂.
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The resolution of 2-methyldecanoic acid was more successful than that of 2-methylbutyric acid both by esterification and by hydrolysis, indicating an acyl chain length dependence. The chain length of the alcohol moiety of the ester affected the resolution of the 2-methyldecanoic acid but not that of the 2-methylbutyric acid. Using esterification with 1-heptanol, (R)-2-methyldecanoic acid was produced in an enantiomeric excess of 95% (E=40). Chiral resolution of an ester of 75% ee, prepared via an asymmetric alkylation (ref. 2a), provided the ester in more than 99.9% ee.

A simple and useful method to achieve high equilibrium conversion in transesterifications is to use reduced pressure (ref. 13). The favourable effect of reduced pressure (evaporation of volatile alcohol formed upon transesterification) is clearly demonstrated by the transesterification of ethyl octanoate with 2-octanol when catalyzed by lipase from Candida antarctica (Fig. 3). By starting with a low ratio of ester to alcohol (0.6) the remaining alcohol can be obtained in a very high enantiomeric excess. Starting with a higher ratio (3.0), an ester is obtained, which upon hydrolysis provides the enantiomeric alcohol in high enantiomeric excess. We have demonstrated this method in successful resolutions of the alcohols 1–4 (Table 1).

Table I. Transesterification of secondary alcohols in ethyl octanoate under reduced pressure catalyzed by lipase from Candida antarctica (temp. 25 °C; 15 mm Hg).

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Enantiomeric ratio (E)</th>
<th>Conversion (%)</th>
<th>Reaction time (hours)</th>
<th>ee R (%)</th>
<th>ee S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)-2-Octanol</td>
<td>40</td>
<td>45</td>
<td>7</td>
<td>97 (80)</td>
<td></td>
</tr>
<tr>
<td>(2)-1-Phenylethanol</td>
<td>&gt;100</td>
<td>52</td>
<td>20</td>
<td></td>
<td>94 (71)</td>
</tr>
<tr>
<td>(3)-1-Cyclohexylethanol</td>
<td>80</td>
<td>52</td>
<td>42</td>
<td>97 (80)</td>
<td></td>
</tr>
<tr>
<td>(4)-trans-2-Methylcyclohexanol</td>
<td>44</td>
<td>44</td>
<td>21</td>
<td>93 (72)</td>
<td></td>
</tr>
</tbody>
</table>

a Enantiomeric excess of remaining alcohol; starting ratio of ethyl octanoate / alcohol 0.6.
b Enantiomeric excess of produced ester (determined after hydrolysis); starting ratio of ethyl octanoate / alcohol 3.0.

For the resolution of alcohols or acids it should be favourable to use diastereomeric esters ('bichiral' substrates). In order to investigate this possibility of enhancement of the enantiomeric excess (stereochemical control by a 'matched strategy') we used a chiral acid moiety for the resolution of chiral
(R)-2-chloropropionic acid gave rise to a higher enantiomeric ratio in 1-phenylethanol than (S)-2-chloropropionic acid in the hydrolysis of the corresponding esters of the racemic alcohol when catalyzed by lipase from *Candida cylindracea*. Moreover, we found a cooperative effect of the diastereoselectivity in the preceding chemical synthesis of the racemic ester as outlined in Scheme 1. Thus, when a mixture of esters prepared from racemic acid and racemic alcohol was used for the resolution of the alcohol, a dual enhancement of the enantioselectivity in the resolution of the chiral alcohol was observed compared with the results when a straight chain alkanoic acid was used (ref. 14).

### Scheme 1

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Chemical step</th>
<th>Enzymatic step</th>
</tr>
</thead>
<tbody>
<tr>
<td>R + S</td>
<td>fast</td>
<td>fast</td>
</tr>
<tr>
<td>R + R</td>
<td>medium</td>
<td>medium(+), R</td>
</tr>
<tr>
<td>S + S</td>
<td>medium</td>
<td>medium(-), S</td>
</tr>
<tr>
<td>S + R</td>
<td>fast</td>
<td>slow</td>
</tr>
</tbody>
</table>

In connection with our studies of 'bichiral' effects we found a reversal of the enantioselectivity in the resolution of 2-chloropropionic acid by esterification when changing from one type of alcohol to another (ref. 15). A straight chain alcohol preferentially esterified the R-acid, whereas 2-alkanols with branching in the 3-position (e.g. 1-cyclohexylethanol or 1-phenylethanol) reacted preferentially with the S-acid (Fig. 4). This reversal of the enantioselectivity was not observed in the corresponding esterification of 2-methylalkanoic acids (ref. 14).

![Figure 4](image)

Figure 4. The enantiomeric excess of remaining 2-chloropropionic acid on esterification with different alcohols, when catalyzed by lipase of *Candida cylindracea*. 1-Heptanol (▲), 2-octanol (▼), 2-butanol (◇), 3-methyl-2-butanol (△), 3-methyl-2-pentanol (▽), cyclohexylmethanol (●), 1-cyclohexylethanol (○), phenylmethanol (■), and 1-phenylethanol (○).

A model has been proposed for the 'alcohol site' of lipase from *Candida cylindracea* (ref. 16) and it has also been suggested that this lipase is more selective to the alcohol moiety than to the acyl part (ref. 17). However, our studies show that successful resolutions of acids can be carried out with this enzyme. Moreover, structural data provide information for a model of the acyl-binding domain as shown in Figure 5 (ref. 15).
We have also worked with a highly purified lipase from *Rhizomucor miehei*, the three-dimensional structure of which has been determined (ref. 4, 5). This enzyme has the active site hidden under a lid, and cannot function unless the lid is displaced, so that the active site is exposed to the substrate. This displacement or conformational change follows from the x-ray crystal structure of an inhibitor complex (ref. 5) and also from molecular dynamics calculations (ref. 19). In the open position the lid exposes its hydrophobic side to the hydrophobic interface and to the substrate and its hydrophilic side is buried in a polar cavity of the enzyme. Molecular modelling demonstrates that the open-lid conformation is stabilized by multiple hydrogen bonds to the surface of the enzyme. Arg86 of the lid takes part in this stabilization. Chemical modifications of the arginyi residues using 1,2-cyclohexanedione or phenylglyoxal resulted in reduced enzyme activity (66% and 46%, respectively). Addition of guanidine, a structural analogue of the arginine side chain, reduced the action of the enzyme as well as that of the chemically modified enzymes. In all cases, when guanidine was added, the residual activity was 26% of that of the native enzyme.

It is rewarding and stimulating to conclude from our research and from current work in many laboratories that further studies on structure/activity relationships in enzymes in combination with molecular modelling and molecular dynamics calculations as well as enzyme modifications by selective chemical methods or protein engineering will provide specialized tools for many specific organic transformations, which are difficult to carry out or cannot be carried out by non-enzymatic procedures.

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**REFERENCES**