Lipase catalyzed esterification of lactic acid

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Reactions between lactic acid and alcohols or carboxylic acids catalyzed by lipase from Candida antarctica were evaluated with hexane as solvent. Lactic acid was a good acyl donor and esters of both primary and secondary alcohols were effectively synthesized. No interfering dimer formation due to lactic acid acting as both nucleophile and acyl donor was observed. In agreement with this, no esterification occurred between lactic acid and carboxylic acids.

Introduction
Lipases have been successfully used as catalysts for the synthesis of esters, both in small-scale work and on an industrial scale (Miller et al., 1988). The mild reaction conditions in the enzymatic reactions make it possible to obtain products of very high purity. Another advantage of enzymes is their selectivity. The selectivity is of prime importance when reactions involving polyfunctional compounds are carried out. It is often possible to carry out highly selective enzymatic reaction without the use of protecting groups. In the present study, esters of lactic acid with different alcohols were prepared. In these reactions there is a potential risk that lactic acid will act both as acyl donor and nucleophile, thus yielding dimers and oligomers of lactic acid. To avoid this, one could either protect the hydroxyl group of the lactic acid or use an enzyme with which lactic acid is not effective as a nucleophile. Here, the second alternative was evaluated using a commercially available lipase from Candida antarctica as catalyst.

Materials and methods
Lactic acid was purchased from ICN as a 85%(w/v), racemic solution and as a 99%(w/w), L-(+)-enantiomerically pure, crystalline solid. The enzyme preparation, Novozyme SP 435, was obtained from Novo Nordisk Industri A/S, Denmark. n-Butanol, n-hexanol, n-octanol, n-decanol, 2-butanol, 2-propanol and 3-methyl-2-butanol were used as nucleophiles. All chemicals were of analytical grade.

A typical reaction contained 3–5 mg enzyme preparation with alcohol and lactic acid in equimolar amounts or an excess of one part. The reagents were diluted in hexane and a visual two-phase system was formed. The solvents and alcohols were water saturated to obtain a constant water activity. The analytical scale reactions were performed at 25°C in capped 4 mL glass vials, with reciprocal shaking at 185 rpm.

The preparative scale butyl lactate syntheses were run in two phase systems at 70°C with the alcohol dissolved in hexane mixed with an excess of lactic acid in 100 mL round-bottomed flasks, equipped with magnetic stirrer and condenser. After reaction, the enzyme particles were removed by filtration and the aqueous phase was discarded. Finally, the solvent was removed by evaporation and the product collected by distillation at 70°C, 25 mm Hg.

The analyses were performed with a Shimadzu GC-9AM equipped with a FID and a 2.1 m glass-column with a diameter of 2.6 mm, packed with 10% SP-1000 on 100/120 Chromosorb® WAW. The optical rotation of butyl lactate was measured in dry ethanol with an Optical Activity Ltd., AA-100 automatic polarimeter.

Results and discussion
Lactic acid as acyl donor
Hexane has been shown to be a good solvent for esterification reactions (Miller et al., 1988, Valivety et al., 1991). The product ester is well solubilized in this solvent and high yields can thus be obtained. The esterification of lactic acid with a few different primary alcohols was therefore attempted in hexane. The lipase used, Novozyme SP 435 (from Candida antarctica) is commercially available and known to be a good catalyst in transesterification reactions in organic media (Öhrner et al., 1994) and in the lactonisation of 16-hydroxyhexadecanoic acid (Robinson et al., 1994). In the esterification of lactic acid, the highest reaction rate was obtained with 1-butanol, while the longer alcohols resulted in...
lower rates (Figure 1). Part of this effect is most probably caused by the effective solvatisation of the long chain alcohols in the solvent, resulting in low activity coefficients and low reaction rates (Janssen and Halling, 1994). Of course, the effect can also be due to the specificity of the lipase for alcohols of different chain length.

The reactions were carried out with different molar ratios of alcohol and lactic acid. It was observed that an excess of alcohol generally caused low reaction rates while an excess of lactic acid caused high reaction rates (Table 1). The results agree with previous observations that lipases usually express higher activity towards primary alcohols (Miller et al., 1988).

No formation of lactic acid dimers or oligomers was observed in any of these reactions. It will thus be fairly easy to optimise the reactions to get high yields of several lactic acid esters.

**Lactic acid as nucleophile**

The absence of dimer formation indicates that lactic acid does not act as a nucleophile when *C. antarctica* lipase is used as catalyst. This was further tested using reaction mixtures containing lactic acid and decanoic acid. Longer fatty acids are good acyl donors for lipase catalyzed esterifications. However, no production of lactyl-decanoate was observed, verifying the low nucleophilicity of the lactic acid hydroxyl group. A possible reason for the poor reactivity in these reactions is the steric hindrance. It has previously been observed that *C. antarctica* lipase catalyses the lactonisation of 16-hydroxyhexadecanoic acid (having a primary hydroxyl group) (Robinson et al., 1994) i.e. hydroxy acids can be accepted as nucleophiles by this lipase. As discussed above, secondary alcohols are not as good nucleophiles as primary ones. In lactic acid the steric hindrance is further increased by the presence of the carboxyl group. To further test the importance of steric effects, 3-methyl-2-butanol was tested as nucleophile in the lipase-catalyzed esterification. No reaction occurred, indicating that steric hindrance is important in determining which nucleophiles are effective.

### Table 1

<table>
<thead>
<tr>
<th>Product</th>
<th>Acid 0.28 M/Alc. 0.28 M</th>
<th>Acid 0.2 M/Alc. 1.0 M</th>
<th>Acid 1.0 M/Alc. 0.2 M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Init. rate (μmol/h)</td>
<td>Conv. (%)</td>
<td>Init. rate (μmol/h)</td>
</tr>
<tr>
<td>2-propyl lactate</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>2-butyl lactate</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Butyl lactate</td>
<td>13</td>
<td>54</td>
<td>3.2</td>
</tr>
<tr>
<td>Hexyl lactate</td>
<td>6.3</td>
<td>27</td>
<td>0.77</td>
</tr>
<tr>
<td>Octyl lactate</td>
<td>1.3</td>
<td>7.7</td>
<td>0.64</td>
</tr>
<tr>
<td>Decyl lactate</td>
<td>1.2</td>
<td>2.9</td>
<td>0.59</td>
</tr>
</tbody>
</table>

**Stereoselectivity in lipase-catalyzed esterification of lactic acid**

In order to study the preference of the lipase for the different lactic acid enantiomers, preparative syntheses of 1-butyl lactate were carried out with racemic and optically pure L-lactic acid. The butyl lactate prepared from the optically pure lactic acid had an optical...