Catalytic processes in the chemistry of lactic acid and PLLA: enzymatic stereoselective alcoholysis of rac-lactide

Abstract: The preparation of the enantiomerically pure (R,R)-lactide (>99%ee) on the gram scale by alcoholysis of rac-lactide in the presence of Amano lipase PS is described. The synthesis of enantiopure lactide by this method is advantageous over traditional preparation via thermal tin-catalysed cyclisation of corresponding oligolactic acids, since the reaction temperature are much lower. That results that no meso-lactide is formed. The alcoholysis of rac-lactide with n-BuOH was studied in the presence of various enzymes in different solvent systems. The kinetic study of the alcoholysis of rac-lactide in the presence of CALB was performed.

Keywords: asymmetric synthesis; enzyme catalysis; ICGC-6; kinetic resolution; lactic acid; lactides.

Introduction

Lactides are important building blocks for the synthesis of polylactic acids (PLA) via ring-opening polymerization [1–9]. The polymer derived from natural L-(S)-lactic acid namely poly(L-lactide) (PLLA) is of particular interest. PLLA is a bioabsorbable and biodegradable material [10–12]. These properties grant it a large potential for applications with special focus on ecological aspects. The fermentation of sugar feedstocks is most common method of large-scale production of L-lactic acid. D-(R)-(−)-lactic acid is also present in nature, but it is less abundant and occurs mostly as a part of racemic mixture. Since a couple of years, there is a large need for chirally pure (R,R)-lactide especially as a monomer for PDLA or as a co-monomer for polymerization with L-lactide since stereo block copolymers show some special material properties in comparison to homochiral PLLA [7, 10, 13, 14]. Currently, PLLA is dominating the market [15].

(R,R)-Lactide is produced traditionally from (R)-lactic acid by the same procedure as (S,S)-lactide. Besides numerous biochemical methods [16–21] there are also some chemical approaches for the production
of enantiopure (R)-lactic acid with excellent stereoselectivities >99 %ee [22, 23]. Inversion of (S)-lactic acid could be an interesting alternative for the production of (R)-lactic acid [24]. Nevertheless (R)-lactic acid remains expensive starting material up to now. It should be also noted that production of enantiopure lactides via thermal tin-catalyzed cyclisation of corresponding oligolactic acids has certain drawback. The epimerisation of stereocenter takes place under these conditions that leads to the formation of meso-compound and even significant decrease of enantiopurity [25].

More advantageous seems to be the use of rac-lactide as a starting material. Since this compound is readily available in bulk and to the fair price. rac-lactide is also available via base or frustrated Lewis pair mediated racemisation of corresponding meso-lactide [26, 27].

The enzymatic alcoholysis of rac-lactide leading to the mixture of (R)-butyl lactate and (S)-butyl lactoyllactate was described by Jeon et al. [28]. (R)-Butyl lactate could be separated and converted into (R,R)-lactide, but a series of additional chemical steps are necessary [29–31]. The kinetic resolution of rac-lactide to the (R,R)-lactide and (S,S)-alkyl lactoyllactate is much more attractive, since D-lactide could be obtained directly by this reaction without any additional transformations.

In this paper we report the kinetic resolution of rac-lactide by alcoholysis in the presence of lipases. We will demonstrate that enantiomerically pure D-lactide (>99 %ee) is accessible by lipase catalyzed alcoholysis of rac-lactide by tuning of reaction medium on a gram-scale. The kinetics of Candida Antarctica lipase B (CALB) – catalyzed alcoholysis was studied in detail.

Results

Kinetic resolution of rac-lactide by alcoholysis with n-BuOH was studied in the presence of two immobilized lipases namely CALB on acrylic resin, Amano lipase PS on diatomite and set of cross-linked enzyme aggregates lipases and proteases from CLEA Tech. Only in case of Bacillus subtilis protease the alcoholysis of (R,R)-lactide in hexane/THF 4:1 was slightly faster than (S,S)-lactide. Alcoholysis of (S,S)-lactide takes place preferentially over alcoholysis of (R,R)-lactide with all other tested enzymes. Stereoselectivities of alcoholysis with Rhizopus niveus, Thermomyces lanuginosus, Penicillium expansum, Porcine pancreas phospholipase, Porcine pancreas, Aspergillus niger, Penicillium camamberti, Penicillium roquefuerti, Pseudomonas cepacia, Mucor Javanicus, Candida rugosa, Pseudomonas stutzeri CLEA-lipases and Aspergillus oryzae acidic and Aspergillus oryzae basic CLEA-proteases were low in hexane/THF 4:1 or CH₂Cl₂ at 40 °C and does not exceed 60 % ee at low conversions. Also slow epimerization of rac-lactide that leads to the formation of meso-lactide was observed in some cases (Scheme 1).

CLEA Rhizomucor miehei lipase was the most effective among the tested CLEA-lipases. The alcoholysis in the presence of this lipase was studied in detail in three solvents namely toluene, hexane/THF and CH₂Cl₂ (Table 1). The reaction in the presence of CLEA R. miehei lipase in toluene proceeded absolutely unselectively and very slow (Run 1–2). Good stereoselectivities for (R,R)-lactide were observed in hexane/THF 4:1 (Runs 3–5). The alcoholysis is slow in this solvent. The reaction mixture is heterogeneous at the beginning,
because of the bad solubility of lactide in hexane/THF. That makes the determination of the concentrations of lactide by GC impossible at lower conversion in this solvent system (Run 3, 4). \((R,R)\)-Lactide with 88 %ee was observed at very high conversion of 85 % (Run 5). The solubility of lactide is excellent in \(\text{CH}_2\text{Cl}_2\). The alcoholysis in this medium is much faster compare to other tested solvents. Still the observed enantiomeric purities of lactide was at a high level (Runs 6–7).

**CALB lipase** is one of the most important lipases in organic synthesis [32, 33]. The alcoholysis in the presence of this lipase was studied in detail in different solvents (Table 2). Application of mixtures of hexane or \(\text{CH}_2\text{Cl}_2\) with DMF as a medium led to very low enantioselectivities (Run 1, 2). Good ee-values for \((R,R)\)-lactide were observed in hexane/2,2,2-trifluoroethanol (TFE) 4:1 as a solvent (Run 3). Mixtures of dichloromethane with TFE and DMSO provided only low stereoselection and low rate of alcoholysis (Runs 4, 5). Alcoholysis in hexane/DMSO 4:1 was fast in comparison to other binary solvent systems. Still the observed enantiomeric purity of lactide at 52 % conversion was only 23 %ee (Run 6). The transformation is very fast in toluene. Seventy-five percent conversion was observed after 2 h (Run 7). Unreacted \((R,R)\)-lactide remained in the solution with a purity of 90 %ee. Also pure \(\text{CH}_2\text{Cl}_2\) is an appropriate solvent for alcoholysis in the presence of immobilized CALB (Runs 8–10). Stereoselection of alcoholysis remained high up to high conversion.

**Table 1:** Alcoholysis with CLEA R. miehei lipase.

<table>
<thead>
<tr>
<th>Run</th>
<th>Time (h)</th>
<th>Solvent</th>
<th>Conversion (%)</th>
<th>Lactide ee in (%)</th>
<th>(n)-Butyl-lactoyllactate ee in (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>Toluene</td>
<td>9</td>
<td>0</td>
<td>2 ((S,S))</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>Toluene</td>
<td>13</td>
<td>0</td>
<td>2 ((S,S))</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>Hexane/THF 4:1</td>
<td>n.d.(^a)</td>
<td>56 ((R,R))</td>
<td>40 ((S,S))</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>Hexane/THF 4:1</td>
<td>n.d.(^a)</td>
<td>70 ((R,R))</td>
<td>26 ((S,S))</td>
</tr>
<tr>
<td>5</td>
<td>84</td>
<td>Hexane/THF 4:1</td>
<td>85</td>
<td>88 ((R,R))</td>
<td>20 ((S,S))</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>(\text{CH}_2\text{Cl}_2)</td>
<td>12</td>
<td>10 ((R,R))</td>
<td>84 ((S,S))</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>(\text{CH}_2\text{Cl}_2)</td>
<td>28</td>
<td>25 ((R,R))</td>
<td>75 ((S,S))</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>(\text{CH}_2\text{Cl}_2)</td>
<td>39</td>
<td>40 ((R,R))</td>
<td>55 ((S,S))</td>
</tr>
</tbody>
</table>

\(^a\)10 mL Solvent, 40 °C; 400 units lipase, 10 mmol \(n\)-BuOH, 1.1 mmol rac-lactide.

**Table 2:** Alcoholysis with CALB in different solvents.

<table>
<thead>
<tr>
<th>Run</th>
<th>Time (h)</th>
<th>Solvent</th>
<th>Conversion (%)</th>
<th>Lactide ee in (%)</th>
<th>(n)-Butyl lactoyllactate ee in (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>Hexane/DMF 4:1</td>
<td>25</td>
<td>2 ((R,R))</td>
<td>17 ((S,S))</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>(\text{CH}_2\text{Cl}_2)/DMF 4:1</td>
<td>3</td>
<td>0</td>
<td>11 ((S,S))</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>Hexane/TFE 4:1</td>
<td>50</td>
<td>67 ((R,R))</td>
<td>63 ((S,S))</td>
</tr>
<tr>
<td>4</td>
<td>44</td>
<td>(\text{CH}_2\text{Cl}_2)/TFE 4:1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>(\text{CH}_2\text{Cl}_2)/DMSO 4:1</td>
<td>8</td>
<td>2 ((R,R))</td>
<td>30 ((S,S))</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>Hexane/DMSO 4:1</td>
<td>52</td>
<td>23 ((R,R))</td>
<td>50 ((S,S))</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>Toluene</td>
<td>75</td>
<td>90 ((R,R))</td>
<td>44 ((S,S))</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>(\text{CH}_2\text{Cl}_2)</td>
<td>10</td>
<td>35 ((R,R))</td>
<td>73 ((S,S))</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>(\text{CH}_2\text{Cl}_2)</td>
<td>31</td>
<td>72 ((R,R))</td>
<td>60 ((S,S))</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>(\text{CH}_2\text{Cl}_2)</td>
<td>67</td>
<td>84 ((R,R))</td>
<td>60 ((S,S))</td>
</tr>
</tbody>
</table>

\(^a\)10 mL Solvent, 40 °C, 400 units lipase, 10 mmol \(n\)-BuOH, 1.1 mmol rac-lactide.
Due to the promising results with CALB in alcoholysis, this system was studied more in detail at different temperatures. The stereoselectivity in dependence on the conversion at 25 °C and 40 °C, respectively, is illustrated in the Fig. 1. Enantiomeric excess up to 93% were achieved for (R,R)-lactide at high conversion (83%) at 40 °C (Fig. 1). A lowering of the temperature to 25 °C led to similar stereoselectivities, but longer reaction times were required.

Likewise, the dependency of the stereoisomeric composition of all three components namely lactide, butyl lactoyllactate and butyl lactate were studied. The results for the alcoholysis at 40 °C are depicted in Fig. 2. The enantiomeric excess of (R,R)-lactide increased and the ee-value of (S,S)-butyl lactoyllactate decreased when the reaction progressed. The ee-value of (R)-butyl lactate is quite high (94%) even at the beginning. It increases up to 98%ee with the progress of the reaction.

The following kinetic scheme was proposed for the acquired kinetic data:

\[
\begin{align*}
A_R & \xrightarrow{K_{1R}} B_R & \xrightarrow{K_{2R}} 2C_R \\
A_S & \xrightarrow{K_{1S}} B_S & \xrightarrow{K_{2S}} 2C_S 
\end{align*}
\]  

(1)  

(2)

Here, \(K_{1R}\), \(K_{2R}\) are rate constants for the alcoholysis reactions of \(R\)-stereoisomers namely (R,R)-lactide and (R,R)-butyl lactoyllactate. \(K_{1S}\), \(K_{2S}\) correspond to rate constants for the reactions of corresponding \(S\)-stereoisomers. Epimerisation between (R,R)- and (S,S)-lactides is considered to be very slow and it could be therefore
neglected in our kinetic model. All reactions in Schemes 1 and 2 could be treated as first order since \(n\)-butanol is present in a large excess. Reverse reactions could be neglected.

Molar fractions of lactides (A), butyl lactoyllactate (B) and butyl lactates (C) were determined from experimental data. The volume of the reaction mixture remains constant, therefore the concentrations of all components are proportional to the number of molecules \(N_A\), \(N_B\), \(N_C\). From the condition of mass balance by \(x_C < 1\) follows that:

\[
N_A = N_{A0} \times x_A / (1 - x_A / 2) = N_{A0} \times x_A^-; \\
N_B = N_{B0} \times x_B / (1 - x_B / 2) = N_{B0} \times x_B^-; \\
N_C = N_{C0} \times x_C / (1 - x_C / 2) = N_{C0} \times x_C^-; \\
\]

\(N_{A0}\) is the initial amount of lactide molecules.

It could be assumed that concentrations of stereoisomers are proportional to the molar fraction of corresponding compounds. Furthermore, the concentrations of all stereoisomeric compounds are considered to be proportional:

\[
x_{AS} = \left(\frac{1 + ee}{2}\right) \times x_A^-; \\
x_{SR} = \left(\frac{1 - ee}{2}\right) \times x_B^-; \\
x_{CR} = \left(\frac{1 + ee}{2}\right) \times x_C^-;
\]

Herein, \(ee\) is the enantiomeric excess of the \(S\) form in case of butyl lactoyllactate (B), and of the \(R\)-stereoisomers in case of lactide (A) and butyl lactate (C).

Butyl lactoyllactate (B) exist mostly in form of the \(S\)-stereoisomer and lactide (A) enriches with \(R\)-stereoisomer with progress of the alcoholysis. This observation indicates that reaction (1S) proceeds with a faster rate. On the other hand, butyl lactate (C) forms preliminary as \(R\)-enantiomer, that means that \(K_2\) constant is very small and could not be determined from the obtained experimental data. Two linear regressions were generated from kinetic data on the first part of kinetic curve in \(\ln x_{AS}, t\) and \(\ln x_{AR}, t\) coordinates and rate constants were determined.

\[
t = 40^\circ\text{C}, K_{1S} = 1.14 \text{ h}^{-1} \\
t = 25^\circ\text{C}, K_{1S} = 0.24 \text{ h}^{-1} \\
t = 40^\circ\text{C}, K_{1R} = 0.5 \text{ h}^{-1} \\
t = 25^\circ\text{C}, K_{1R} = 0.073 \text{ h}^{-1}
\]
The linear regression correlation coefficient is over 0.95. Since the rates of reactions were determined at the different temperatures the energy of activation could be estimated. For the reaction (1R) it is about 10 kcal/mol and 7.5 kcal/mol for the reaction (1S), respectively. It should be considered that theses values gave raw approximation, nevertheless it is certain that rate of the reaction (1R) increases faster than for the reaction 1S by increasing of the temperature.

It could be shown, that by $x_C < 1$

$$X_C(t) = 2k_{1R}k_{2R}t^2$$

since $k_{1R}$ is known, therefore $k_{2R}$ could be estimated from the acquired data. The constants by two temperatures are:

$t = 40^\circ C$, $K_{1R} = 0.02 \text{ h}^{-1}$

$t = 25^\circ C$, $K_{2R} = 0.0084 \text{ h}^{-1}$

The energy of activation of the reaction 2R could be determined from these data:

$$E_{\text{act}} (2R) \approx 5 \text{ kcal / mol}$$

Suggested kinetic Schemes 1 and 2 demonstrates a good agreement with obtained experimental data.

The alcoholysis in the presence of Amano lipase PS was studied in detail in four solvents (Table 3). In contrast to CALB-catalysed reaction the following alcoholysis of $n$-butyl lactoyllactate is a quite fast reaction. Significant amounts of $n$-butyl lactate are formed, before the entire amount of lactide is consumed. Hexane/iPr$_2$O 4:1 as a reaction medium provides only moderate stereoselectivity (Runs 1, 2). Similar activity and selectivity were observed in iPr$_2$O (Run 3). High stereoselection was observed in toluene and dichloromethane (Runs 4–9). The alcoholysis in toluene is faster than that in CH$_2$Cl$_2$.

The observed stereoselection of the alcoholysis in toluene remained high during the entire process. The optimisation of the amount of $n$-butanol should allow performing the reaction until full consumption of alcohol without GC-control (Table 4). The enantioselectivity over 97 % was observed at 73 % conversion with three equivalents of $n$-BuOH (Run 3). Reducing the amount of alcohol to two equivalents afforded 99.5 %ee (Run 6). These data indicates that the optical purity of (R,R)-lactide over 98 can be reached at ¾ conversion. Indeed, the alcoholysis of rac-lactide with 1.5 equivalents $n$-butanol gives (R,R)-lactide with 99.3 %ee at 74 % conversion (Run 7). This result allows to run the transformation without GC-control and achieve highest possible stereoselectivity and yield for (R,R)-lactide.

The (R,R)-lactide with 99 %ee could be isolated from this reaction mixture by simple crystallization from pentane.

**Table 3: Alcoholysis with Amano lipase PS.**

<table>
<thead>
<tr>
<th>Run*</th>
<th>Solvent</th>
<th>Time (h)</th>
<th>Lactide ee (%)</th>
<th>n-Butyl lactoyllactate ee (%)</th>
<th>n-Butyl lactate ee (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hexane/iPr$_2$O 4:1</td>
<td>1.5</td>
<td>57 (R,R)</td>
<td>64 (S,S)</td>
<td>43 (S)</td>
</tr>
<tr>
<td>2</td>
<td>Hexane/iPr$_2$O 4:1</td>
<td>12</td>
<td>49 (R,R)</td>
<td>33 (S,S)</td>
<td>30 (S)</td>
</tr>
<tr>
<td>3</td>
<td>iPr$_2$O</td>
<td>1.5</td>
<td>50 (R,R)</td>
<td>52 (S,S)</td>
<td>36 (S)</td>
</tr>
<tr>
<td>4</td>
<td>Toluene</td>
<td>1.5</td>
<td>12 (R,R)</td>
<td>80 (S,S)</td>
<td>19 (S)</td>
</tr>
<tr>
<td>5</td>
<td>Toluene</td>
<td>3.5</td>
<td>28 (R,R)</td>
<td>71 (S,S)</td>
<td>25 (S)</td>
</tr>
<tr>
<td>6</td>
<td>Toluene</td>
<td>12</td>
<td>73 (R,R)</td>
<td>43 (S,S)</td>
<td>37 (S)</td>
</tr>
<tr>
<td>7</td>
<td>CH$_2$Cl$_2$</td>
<td>1.5</td>
<td>4 (R,R)</td>
<td>90 (S,S)</td>
<td>10 (S)</td>
</tr>
<tr>
<td>8</td>
<td>CH$_2$Cl$_2$</td>
<td>20</td>
<td>55 (R,R)</td>
<td>63 (S,S)</td>
<td>33 (S)</td>
</tr>
<tr>
<td>9</td>
<td>CH$_2$Cl$_2$</td>
<td>40</td>
<td>67 (R,R)</td>
<td>47 (S,S)</td>
<td>46 (S)</td>
</tr>
</tbody>
</table>

*10 mL Solvent, 40 °C; 400 mg Amano lipase PS (200 units), 10 mmol $n$-BuOH, 3.5 mmol rac-lactide.
Conclusion

Herein we report for the first time the synthesis of enantiomerically pure (R,R)-lactide (>99 %ee) by alcoholysis of rac-lactide in the presence of Amano lipase PS on a gram scale. This procedure is superior in comparison to a traditional way for the synthesis of enantiopure lactides via thermal tin-catalyzed cyclisation of corresponding oligolactic acids, since the reaction temperature of our approach is much lower. Moreover, no meso-lactide is formed in our approach. Likewise, the alcoholysis of rac-lactide with n-BuOH in the presence of various enzymes in different solvents was studied. In addition, the kinetics of the CALB catalyzed alcoholysis of rac-lactide is given.

Experimental part

All reagents unless otherwise mentioned were purchased from commercial sources and used without additional purification. Solvents were dried and freshly distilled under argon before use. Amano lipase PS immobilized on diatomite and CALB on acrylic resin were purchased from Aldrich. For the determination of the enantiomeric ratio of lactide and butyl lactate was used GC at Lipodex E column with chiral stationary phases from Macherey–Nagel GmbH. GC with Chiralpak β-PM column from Astec Tech. Co. was used for the determination of the enantiomeric ratio of butyl lactoyllactate.

Synthesis of (R,R)-lactide by alcoholysis of rac-lactide.

To the solution of 5.0 g (0.035 mol) rac-lactide and 5 mL (0.053 mol) n-BuOH in 100 mL abs. toluene was added 4 g. Amano lipase PS immobilized on diatomite. The reaction mixture was stirred for 72 h at 40 °C and followed by GC. The analysis has shown 74 % conversion. The enzyme was removed by filtration. The reaction mixture was concentrated at 35 °C and 20 mbar. The resulted solution was diluted with 30 mL of pentane. The precipitated product was filtered off and washed 2×10 mL pentane. (R,R)-Lactide 1.1 g (44 % yield, 99.3 %ee) was isolated as white plates.

Acknowledgements: We acknowledge financial support by ThyssenKrupp Industrial Solutions GmbH Leuna. The authors thank Dr. C. Fischer, S. Buchholz, and S. Schareina for excellent analytical support.

References

[15] For example since 2007 BASF sells PLA-blend under the trade name Ecovio®: http://www.plasticsportal.net/wa/plasticsEU-de_DE/portal/show/content/literature/ecovio.