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Lipase Catalyzed Chemoselective Ring-Opening Polymerization of ε-Caprolactone in Presence of γ-Hydroxy-α-methylenebutyric Acid

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The ring-opening polymerization (ROP) of ε-caprolactone (CL) using γ-hydroxy-α-methylenebutyric acid (HMBA) as the initiator was studied. The use of stannous octoate ($Sn(Oct)_2$) and Novozym 435 (Lipase B from *Candida Antarctica*) as catalysts was compared. Under enzymatic conditions, poly(ε-caprolactone) (PCL) with well-defined end group functionalization was obtained. In contrast, under $Sn(Oct)_2$ catalysis, the lactonization of HMBA was observed as a side reaction which lead to an insufficient end group functionalization of the prepared polyesters.

Keywords: catalysis; kinetics (polym.); macromonomers; poly(ε-caprolactone); ring-opening polymerization

1 Introduction

Poly(ε-caprolactone) (PCL) attracts much interest due to its biocompatibility (1, 2), thermal properties and its chemical functionalization potential. PCL has therefore been used for the preparation of various materials and complex polymer architectures (3–6). PCL with controlled molecular weight and well-defined end groups can be prepared by ring-opening polymerization (ROP) of ε-caprolactone (CL) using primary alcohols as initiators. Stannous octoate ($Sn(Oct)_2$) is known as one of the most common transesterification catalyst for the ROP of lactones (3, 7–10). However, to obtain materials for biomedical applications, a high purity of polyesters is required. Therefore, the application of nonmetal catalysts from natural resources provides a suitable alternative (11, 12). Novozym 435 (an immobilized Lipase B from *Candida Antarctica*) can be easily used for enzymatic catalyzed ROP of lactones to produce polyesters (13–15). For Novozym 435, the application of toluene as solvent in a temperature range between 80 and 90°C has been described to provide optimal conditions for the successful ROP of CL (16). Both catalytic procedures ($Sn(Oct)_2$ and Lipase) provide a useful method for the preparation of polyesters with well-defined end groups and controlled molecular weight. But beside the absence of partially toxic metal ions, the enzymatic ROP shows further advantages like chemo- and enantionselectivities. Macrocyclic lactones for instance, show a much lower polymerizability than CL under metal catalyzed conditions. However, they can be polymerized enzymatically much faster than CL (13, 17, 18). Furthermore, the enantioselective enzymatic ROP of racemic substituted lactones has yielded optically active polyesters which are not accessible by metal catalyzed ROP (19).

In this contribution, we present the ROP of CL with γ-hydroxy-α-methylenebutyric acid (HMBA) as the initiator. We focused our study on the comparison between lipase and $Sn(Oct)_2$ catalysis with respect to the polymerization kinetics and the structure of the obtained polyesters.

2 Experimental

2.1 Materials and Instrumentation

α-Methylene-γ-butyrolactone was prepared as reported by Jenkins et al. (20). Stannous octoate (Aldrich) was distilled in vacuum before use. ε-Caprolactone (Fluka) was distilled

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over calcium hydride and stored under nitrogen. Novozym 435 (Aldrich) was stored over phosphorus pentoxide at 4°C. Toluene (extra dry grade, water <30 ppm, Acros) was used as received. Polymerization experiments were carried out in an inert nitrogen atmosphere.

The structures of the synthesized compounds were proven by ^{1}H- and ^{13}C-NMR spectroscopy using a Bruker Avance DRX 500 spectrometer at 500 MHz for hydrogen nuclei and 125 MHz for carbon nuclei, using $CDCl_3$ or DMSO-d_6 as solvent. The $\delta-$scale was calibrated to TMS. Infrared spectra were recorded on a Nicolet 5SXB FT-IR spectrometer. Matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF) was performed on a Bruker Ultraflex TOF mass spectrometer using the reflector mode. The samples were prepared from a solution in chloroform with addition of dithranol and sodium trifluoro acetate.

2.2Synthesis

2.2.1 Synthesis of γ-hydroxy-α-methylenebutyric acid (HMBA)

A solution of 19.6 g (0.2 mmol) α-methylene-γ-butyrolactone in 400 ml of a 1 M sodium hydroxide solution was stirred for one hour. The mixture was acidified with hydrochloric acid to pH = 3 and saturated with sodium chloride. The aqueous solution was continuously extracted with 1 l diethylether for 2 h. The organic layer was dried over magnesium sulfate and the solvent was evaporated. The obtained solid was recrystallized from chloroform yielding 17.9 g (77%) of HMBA.

C, H, N: Calcd. C 51.72, H 6.94; Found C 51.46 H 7.21, FT-IR: $\tilde{\nu}$ = 3363 (vs, OH), 2984, 2966, 2927, 2905 (s, CH_2), 1695 (vs, C=O), 1629 (s, C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ = 6.06 (s, 1H, vinyl), 5.62 (s, 1H, vinyl), 3.49 (t, $^{3}J_{H,H}$ = 6.9 Hz, 2H, OC$\underline{H}_2$-), 2.37 (t, $^{3}J_{H,H}$ = 6.9 Hz, 2H, allyl) ppm. ^{13}C-NMR (DMSO-d_6): δ = 168 (carboxyl), 139 (-$\underline{C}$(=CH_2)-), 126 (=$\underline{C}H_2$), 60 (HO$\underline{C}H_2$-), 35 (HOC$H_2\underline{C}H_2$-) ppm. MS (EI): m/z = 98 (M^+-H_2O), 86 (M^+-CH_2O), 68 (M^+-H_2O, -CH_2O).

2.2.2 Procedure for lipase catalyzed ROP of CL in solution

A mixture of HMBA (68 mg, 0.55 mmol), CL (0.93 ml, 1.0 g, 8.8 mmol), toluene (1 ml) and Novozym 435 (50 mg) was placed in a dried glass tube that was sealed with a septum. The tube was kept under stirring at 80°C for 6 h. For kinetic investigations samples from this mixture were taken every hour.

2.2.3 Procedure for Sn(Oct)$_2$ catalyzed ROP of CL in bulk

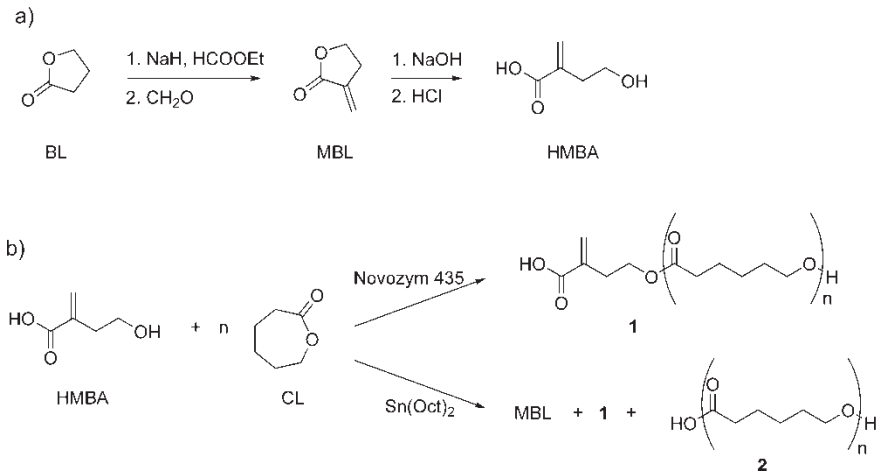
A mixture of HMBA (68 mg, 0.55 mmol), CL (0.93 ml, 1.0 g, 8.8 mmol) and $Sn(Oct)_2$ (3.6 mg, $8.8\cdot10^{-3}$ mmol) was placed in a dried glass tube that was sealed with a septum. The tube was kept under stirring at 130°C for 5 h. For kinetic investigations samples from this mixture were taken every hour.

2.2.3 Procedure for Sn(Oct)$_2$ catalyzed ROP of CL in solution

A mixture of HMBA (68 mg, 0.55 mmol), CL (0.93 ml, 1.0 g, 8.8 mmol), toluene (1 ml) and $Sn(Oct)_2$ (3.6 mg, $8.8\cdot10^{-3}$ mmol) was placed in a dried glass tube that was sealed with a septum. The tube was kept under stirring at 80°C for 5 h. For kinetic investigations, samples from this mixture were taken every hour.

3 Results and Discussion

γ-Hydroxy-α-methylenebutyric acid (HMBA) was synthesized in three steps (Scheme 1a). Therefore, γ-butyrolactone (BL) was methylenated by formylation, followed by conversion with paraformaldehyde under condensation of the formiate anion to yield α-methylene-γ-butyrolactone (MBL) as described in the literature (20). To the best of our knowledge, there is only one report from Hutchinson et al. about the synthesis of HMBA via hydrolysis of MBL.



Sch. 1. a) Preparation of HMBA and b) ROP of CL initiated with HMBA using Novozym 435 or $Sn(Oct)_2$.

The authors describe the use barium hydroxide for the saponification of MBL (21). In this contribution, it was possible to improve the hydrolysis by using sodium hydroxide, though HMBA was obtained in good yields of about 80%.

HMBA was used as the initiator for the ROP of CL. For comparison, Novozym 435 and $Sn(Oct)_2$ were evaluated as catalysts (Scheme 1b). It was found that under the applied transesterification conditions, HMBA can either initiate the desired polymerization or recyclize by elimination of water to give MBL. In the latter case, the resulting water initiates the ROP of CL and the unfunctionalized PCL **2** is obtained. In order to investigate the conditions that enable the desired quantitative end group functionalization of the polyester with a radically polymerizable methylen group, we have investigated the polymerization kinetics in presence of both catalysts. According to the literature, we used a system consisting of CL/HMBA = 15:1 with Novozym 435 (5 wt% of CL) in toluene as solvent (CL/toluene = 1/1 wt./vol.) at 80°C (16). In contrast to that, typically the polymerization with $Sn(Oct)_2$ as catalyst is carried out at higher temperatures of about 130°C in bulk (8). Thus, we also polymerized with $Sn(Oct)_2$ at solvent free conditions. The time dependent concentrations of CL, HMBA, MBL and PCL **1** and **2** in the reaction mixture were determined by ^{1}H-NMR spectroscopy. The separated signals of ε-methylene protons in CL (4.25 ppm), ε-methylene protons in PCL **1** and **2** (4.07 ppm), ε-methylene protons of the hydroxy end group in PCL **1** and **2** (3.58 ppm), β-methylene protons of MBL (3.00 ppm) and the vinylic protons of HMBA (6.43 and 5.82 ppm), MBL (6.24 and 5.68) and PCL **1** (6.35 and 5.72 ppm) were used to determine the conversions.

It turned out that, in the case of Novozym 435 catalysis (Figure 1a), the conversion of CL is nearly quantitative (98%) after 300 min of reaction time. It is interesting to note that HMBA is not incorporated quantitatively, but reaches an equilibrium concentration of about 80% of conversion after 60 min. This may be due to the mechanism of lipase catalyzed ROP of lactones (Scheme 2a). Initiation and propagation are thereby competitive reactions pathways of the enzyme activated monomer, which differ regarding the attacking OH-compound. Due to the low polarity of growing polyester chain, it is more rapidly incorporated by lipase than the hydrophilic HMBA. Consequently, after a certain concentration of the growing polymer, the propagation is favored compared with the initiation and a residual concentration of HMBA remains. A similar behavior was previously shown by Heise et al. (22), where bifunctional initiators were used for the enzymatic ROP of CL. Even though the described initiators where less hydrophilic compared to HMBA, they already showed a non-quantitative conversion. A minor formation of MBL by lactonization of HMBA can be observed as a side reaction. The MBL amount is slightly increasing during the reaction time and reaches a value of 5% after 360 min.

In comparison, by using $Sn(Oct)_2$ as catalyst (Figure 1b) the conversion of CL is nearly quantitative (95%) after 240 min and a complete conversion of HMBA is reached after 30 min. But in contrast to the enzymatic ROP, a strong increase of MBL amount can be observed in the beginning of the polymerization. The amount of MBA was determined at 36% after 60 min and then slightly decreased during the course of reaction. From the ^{1}H-NMR analysis, it was possible to conclude that both catalysts do achieve a complete monomer conversion after about 6 h, but discrepancies can be found in the strong tendency of lactonization of the initiator HMBA only in the case of $Sn(Oct)_2$ catalysis.

In order to exclude that the different behaviors of the catalysts are attributed to temperature or solvent effects, we applied the same reaction conditions (toluene as solvent at 80°C), as used for the lipase experiments, for further kinetic investigation of $Sn(Oct)_2$ catalysis (Figure 2). As expected, the $Sn(Oct)_2$ catalyzed conversions of CL and HMBA are relatively slow. But, even at these mild conditions, the lactonization of HMBA to MBL is relatively high. After 300 min, 16% of MBL were determined. Thus, we can conclude that the degree of lactonization of HMBA is clearly influenced by the different catalysts rather than by the reaction conditions.

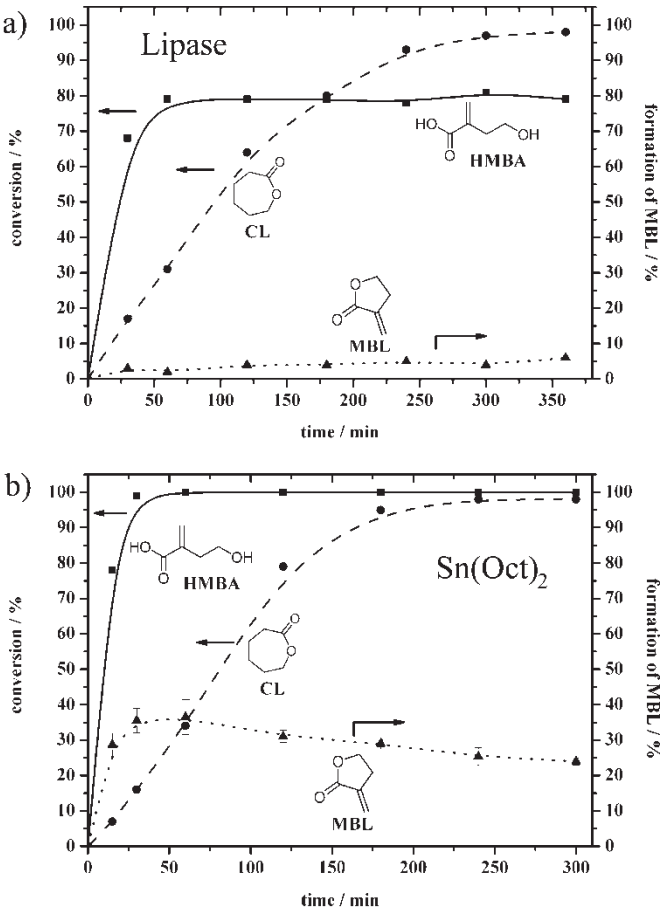
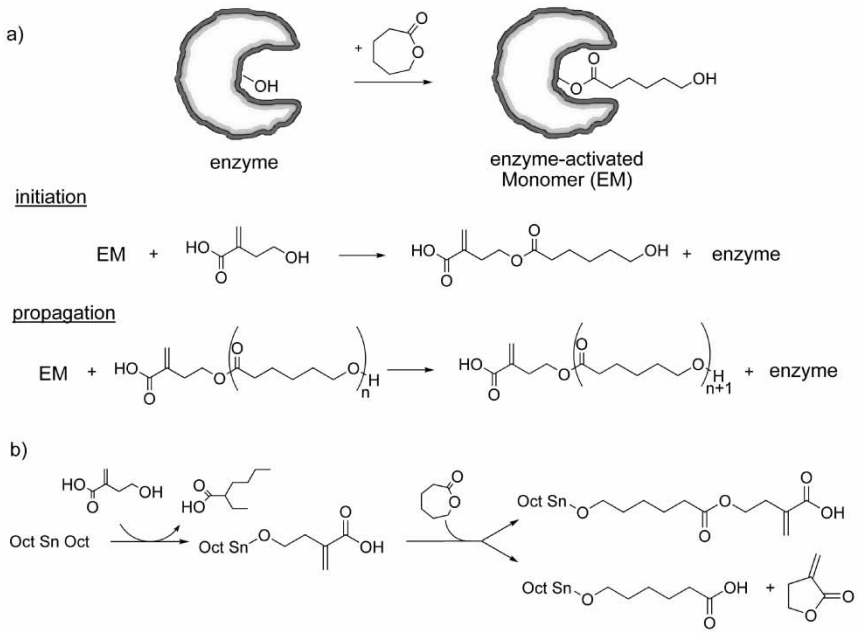


Fig. 1. Conversion of CL and HMBA and formation of MBL vs. reaction time for a) ROP (Novozym 435) of CL with HMBA carried out at 80°C in toluene (CL/toluene, 1:1 wt./vol.) and b) ROP ($Sn(Oct)_2$) of CL with HMBA carried out at 130°C in bulk.



Sch. 2. ROP of CL with HMBA via a) Enzymatic "monomer-activated" mechanism and b) "coordination-insertion" mechanism of $Sn(Oct)_2$.

The number average molecular weight of polymer samples from $Sn(Oct)_2$ polymerization (130°C, bulk) and Lipase polymerization (80°C, solution) was determined via ^{1}H-NMR end group analysis. In Figure 3, the obtained values are plotted vs. the conversion of CL. The values of samples polymerized by $Sn(Oct)_2$ catalysis run nearly along the dashed line which was calculated from monomer/initiator ratio of 15 considering a complete initiator conversion. In comparison, the M_n values of samples from Lipase polymerization are slightly higher. This increase is based on the fact that only 80% conversion of the initiator HMBA takes place as mentioned above. The values correspond with the dotted line which was calculated considering 80% of initiator conversion.

To investigate the influence of the different reaction pathways on the structure of the obtained polyesters we compared MALDI-TOF mass spectra of the polymers after complete polymerization. Figures 4(a,b) shows the MALDI-TOF spectrum of PCL prepared with Novozym 435 catalysis. In the complete spectrum, a series of signals dominate which can be assigned to the different lengths of HMBA functionalized PCL **1** cationized with Na^+ (Figure 4a). A more detailed picture (Figure 4b) shows the 1560–1700 m/z-region of the

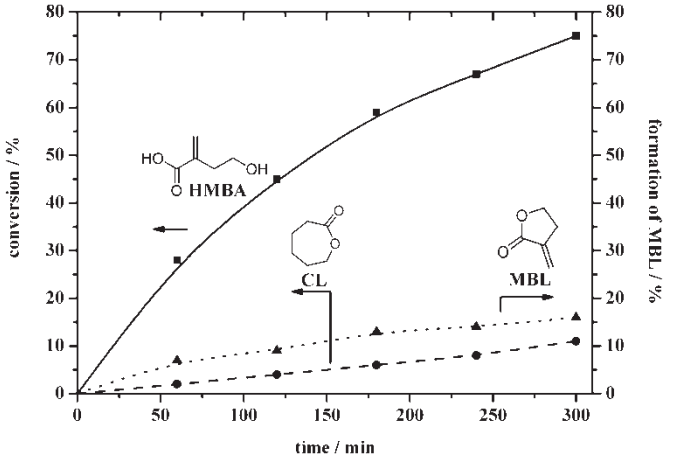


Fig. 2. Conversion of CL and HMBA ([CL]:[HMBA] = 15) and formation of MBL vs. reaction time for Sn(Oct)2 catalysis of CL under initiation of HMBA carried out at 80°C in toluene (CL/toluene, 1:1 wt/vol).

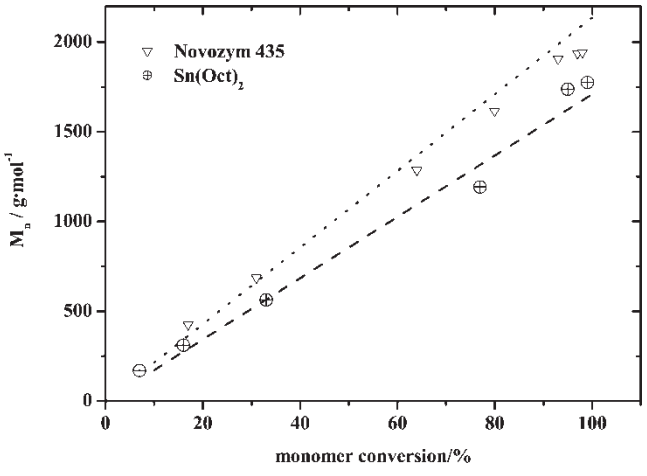


Fig. 3. Number average molecular weight vs. conversion of CL for the ROP catalyzed with Novozym 435 (80°C, solution) and $Sn(Oct)_2$ (130°C, bulk), respectively. The dashed and dotted lines represent the calculated course considering a full and 80% conversion of the initiator, respectively.

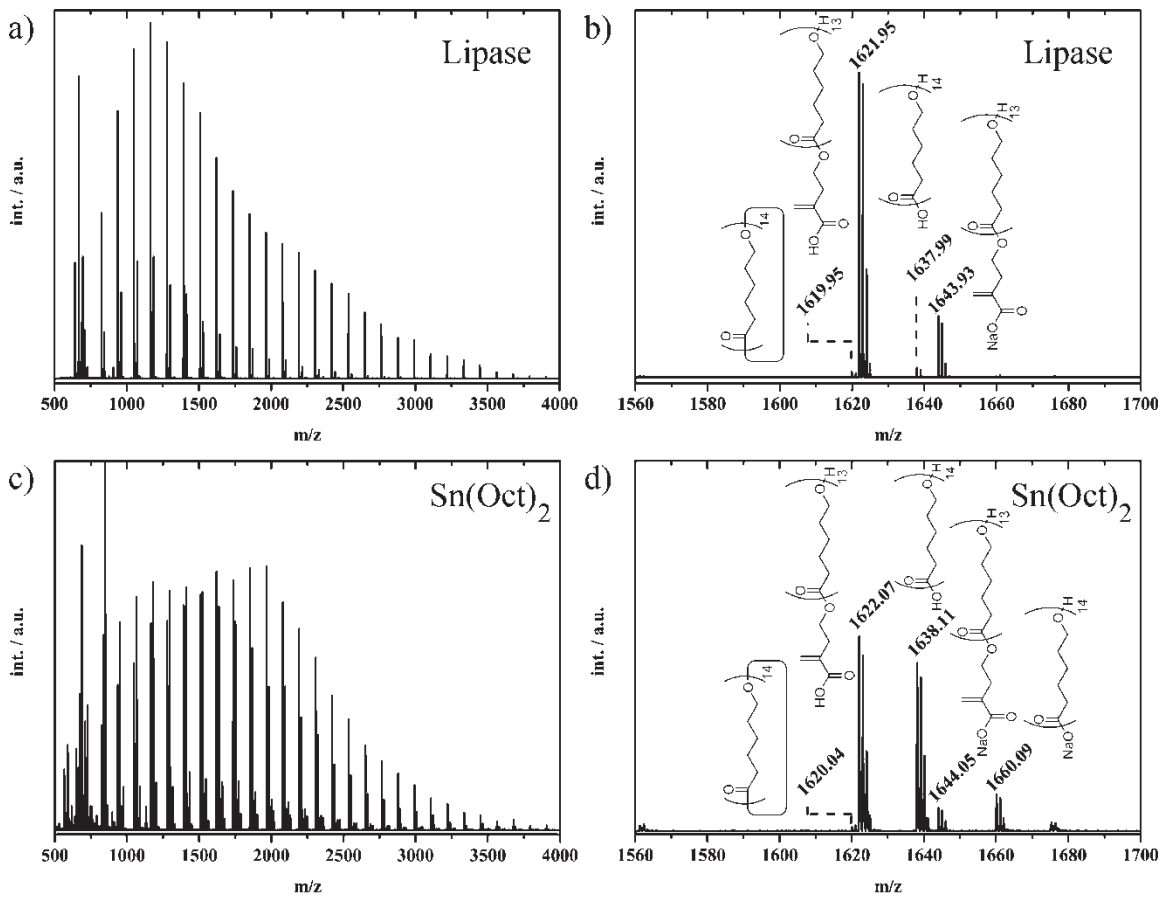


Fig. 4. MALDI-TOF spectra of PCL (cationized with Na^+) polymerized by ROP of CL using Novozym 435 under initiation of HMBA carried out at 80°C in toluene (CL/toluene, 1:1 wt./vol.); a) full spectrum; b) 1560–1700 m/z region; and using $Sn(Oct)_2$ carried out at 130°C in bulk; c) full spectrum; d) 1560–1700 m/z region.

spectrum. It is possible to assign the signals as HMBA functionalized PCL **1**, unfunctionalized PCL **2** and cyclic PCL. PCL **1** occurs as two different species (carboxylic acid and sodium carboxylate) which is attributable to sample preparation. The distribution of signals shown in the 1560–1700 m/z-region is nearly representative for the complete m/z range. Though it should be noted that the signals of cyclic polyesters are more prominent in the lower m/z range and become negligible after $m/z = 2000$. Finally, from the MALDI-TOF analysis it is possible to argue that PCL **1** is the most abundant polymer.

In comparison, Figures 4(c,d) shows the MALDI-TOF spectrum of PCL prepared with $Sn(Oct)_2$ catalysis. In the complete spectrum (Figure 4(c)), two series of signals dominate which can be assigned to the different lengths of HMBA functionalized PCL **1** and unfunctionalized PCL **2**; all cationized with Na^+. In the 1560–1700 m/z region of the spectrum (Figure 4(d)), it is possible to assign the signals to cyclic polymers and functionalized PCL **1** and unfunctionalized PCL **2** (both as free acid and sodium carboxylate). But, in contrast to the distribution obtained after Lipase catalyzed polymerization, the signal of the PCL **2** has almost the same intensity. Over the complete m/z range, the distribution of signals slightly changes though the signals of the desired PCL **1** are more prominent in the higher m/z range after 1500 and the signals of PCL **2** accordingly before $m/z = 1500$. Although MALDI-TOF analysis does not provide reliable quantitative data, we can conclude in agreement with the results from ^{1}H-NMR that only by using the $Sn(Oct)_2$ as catalyst the formation of both PCL structures **1** and **2** occurs. Kinetics and the well defined end group functionalization obtained under lipase catalysis confirm the chemoselectivity of the Novozym 435 catalysis.

The different behaviors of metal and lipase catalysis are a result of the different polymerization mechanisms. The enzymatic ROP of lactones is assumed to proceed through a "monomer-activated" mechanism (Scheme 2a) (14). The reaction between the enzyme and CL to form an acyl-enzyme intermediate (EM) is the rate-determining step. The EM can either react with an initiator molecule (initiation) or with a hydroxy-terminated PCL chain (propagation). In contrast to that, the ROP with $Sn(Oct)_2$ catalysis is described to proceed through a "coordination-insertion" mechanism (8) as shown in Scheme 2b. The OH-group of the initiator and $Sn(Oct)_2$ form a stannous alkoxide species as first step. After complexation of CL onto the stannous alkoxide, a

rearrangement generates the active propagating chain with a stannous alkoxide end group. We can assume that through the formation of the stannous alkoxide of HMBA the recyclization becomes a more prominent side reaction. Thus, only in presence of $Sn(Oct)_2$ a high amount of MBL was found.

4 Conclusion

It can be confirmed from the above described results that the Novozym 435 catalyzed ROP of CL under HMBA initiation yields PCL **1** with well defined end group functionalization. However, under $Sn(Oct)_2$ catalysis, the lactonization of HMBA as side reaction can be observed and the polymerization yields a mixture of functionalized and unfunctionalized PCL **1** and **2**. This clearly shows a new example of chemoselectivity of the enzymatic ring-opening polymerization of lactones. The obtained polyesters bearing a radically polymerizable group under conservation of the COOH- and OH-end groups represent a new type of macromonomers. They may be used for the formation of polymeric brushes via homopolymerization or as comonomer to generate graft copolymers.

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