Investigation of Factors Influencing the Chemoenzymatic Synthesis of Block Copolymers

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ABSTRACT: The chemoenzymatic synthesis of block copolymers from a bifunctional initiator using enzymatic ring-opening polymerization (eROP) and ATRP in two consecutive steps was investigated. First, a polycaprolactone (PCL) macroinitiator was obtained via an enzymatic ring-opening polymerization initiated by the bifunctional initiator. By carefully managing the water activity in the system, the amount of PCL not initiated by the bifunctional initiator was reduced to <5%. Moreover, comparison of the results from ^{1}H NMR and MALDI-ToF of PCL obtained from different bifunctional initiators revealed an influence of the initiator structure on the initiation behavior in the enzymatic reaction. Block copolymers were obtained in a subsequent ATRP. The combination of various characterization techniques such as GPC, GPEC, and DSC provided clear evidence of the block structure of the polymers.

Introduction

Biocatalytic approaches in polymer science are expected to further increase the diversity of polymeric materials. Major progress has been achieved over the past years in applying enzyme catalysis in polymer science.[1,2] The application of enzymes in polymer synthesis and transformation is attractive due to their ability to function under mild conditions with high enantio- and regioselectivity. The stability of lipases in organic media and their ability to promote transesterification and condensation reactions on a broad range of low and high molar mass substrates have been shown in many examples. In particular, immobilized Lipase B from *Candida Antarctica* (Novozym 435) has shown exceptional activity for a range of polymer forming reactions, including the ring-opening polymerization of cyclic monomers (e.g., lactones, carbonates).[3]

The application of enzymes as catalysts in a nonnatural environment was until now predominantly the domain of organic chemistry.[4] The development of biocatalytic methods in this field on both academic and industrial levels, however, offers interesting opportunities for the use of these technologies in polymer chemistry.[5] However, the full exploitation of biocatalysis in polymer science will require the development of mutually compatible chemo- and biocatalytic methods.[6−9] In our laboratory, we therefore explore the integration of biocatalytic and traditional polymer synthesis. Our goal is the development of chemoenzymatic cascade polymerization reactions, i.e., combined catalytic reactions without an intermediate recovery step. We believe that this new concept can eventually lead to the development of new materials and a sustainable technology, once its versatility has been demonstrated on the fundamental level.

The synthesis of block copolymers is particularly suited to investigate the combination of two fundamentally different synthetic techniques, since the marriage of two chemically different building blocks often requires a considerable synthetic effort. Block copolymers with building blocks, based on two intrinsically different polymerization mechanisms, e.g., polyester and polymethacrylate, can be obtained either by chemically linking two preformed polymer blocks or, alternatively, in two consecutive polymerizations using macroinitiators. With respect to the latter case, the majority requires an intermediate transformation step in order to convert the end group of the first block into an active initiator for the second polymerization. A very powerful and elegant synthetic pathway to block copolymers is the use of an initiator combining two fundamentally different initiating groups in one molecule. This allows two consecutive polymerizations without an intermediate transformation step. The feasibility of this approach has been successfully demonstrated for the combination of various chemical polymerization techniques.[10−15] Moreover, we recently reported the first example of a one-pot chemoenzymatic cascade polymerization combining nitroxide-mediated radical polymerization (NMP) and enzymatic ring-opening polymerization (eROP).[16]

Here, we report the synthesis of block copolymers combining atom transfer radical polymerization (ATRP) and eROP from a bifunctional initiator. In previous studies, we found that block copolymers can be obtained using this strategy.[17,18] In this paper we report the findings from our detailed investigation into the key factors for obtaining a high block copolymer yield in a chemoenzymatic ATRP−eROP procedure. While this paper is focused on the optimization of the enzymatic polymerization and the synthesis of block copolymers in two consecutive steps, a subsequent paper will report the simultaneous polymerization with special view on the compatibility in a one-pot approach (Scheme 1). With respect to the first point, our goal was to obtain a well-controlled lipase-catalyzed polymerization by (i) carefully managing the water activity in the system, (ii) selecting a bifunctional initiator that shows fast and complete initiator consumption with fast polymerization

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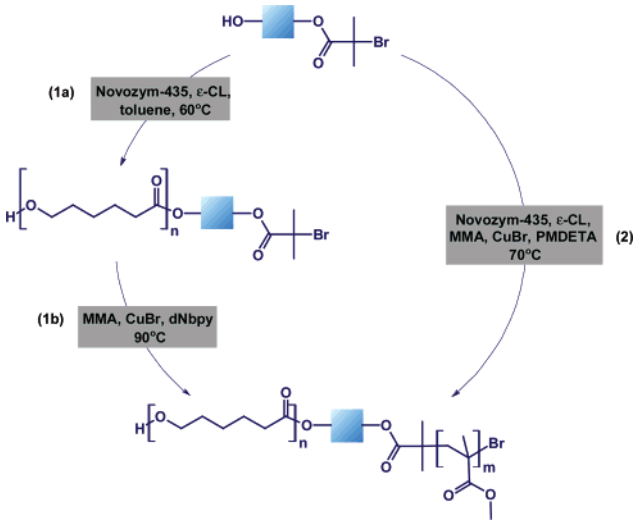
Scheme 1. Chemoenzymatic Polymerization Combining ATRP and eROP in Two Consecutive Steps (1) and in a One-Pot Cascade Approach (2)



kinetics of CL, and (iii) combining different characterization techniques that will provide clear evidence of block copolymers produced with an eROP/ATRP cascade approach. The ambition was to interpret the results obtained in this part of the study in order to ultimately design a one-pot chemoenzymatic cascade reaction.

Experimental Part

Materials. All chemicals were purchased from Aldrich, stored over molecular sieves, and used without further purification unless otherwise noted. Toluene was dried over alumina. The bifunctional initiators were synthesized and characterized according to literature procedures.[17,18] Novozym 435 was obtained from Novozymes and stored over P_2O_5 in a desiccator. Molecular sieves (3 Å) were dried in an oven at 420 °C prior to use. MMA was filtered through neutral aluminum oxide to remove the inhibitor and stored over molecular sieves at 4 °C.

Methods. The water concentrations of the reaction media were determined by Karl Fischer coulometry using a Mettler Toledo DL32 coulometer and Apura CombiCoulometer fritless (Merck) as electrolyte. Size exclusion chromatography (SEC) was performed on a Waters model 510 pump and Waters 712 WISP, using PL-gel mix D columns (300 × 7.5 mm, Polymer Laboratories) at 40 °C. THF was used as eluent with a flow rate of 1.0 mL/min. All samples were diluted to 1.0 mg/mL and filtered using 0.2 μm syringe filters. Molecular weights of PCL were calculated from a universal calibration curve using $K = 0.001\ 09$ dL/g and $\alpha = 0.6$. The molecular weights of the block copolymers were calculated on the basis of polystyrene standards. ^{1}H NMR spectroscopy was performed using a Varian 400 NMR. Data were acquired using VNMR-software. MALDI-ToF-MS analysis was carried out on a Voyager DE-STR from Applied Biosystems using *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]−malononitrile (DCTB) as matrix material. All spectra were recorded in the reflector mode. Gradient polymer elution chromatography (GPEC) was performed on an Agilent 1100 series HPLC with autosampler and an ELSD 2000 detector. A Zorbax RX-Sil column (150 × 4.6 mm) was used at 25 °C at an eluent flow rate of 1.0 mL/min. An eluent gradient going from pure hexane to pure THF in a period of 10 min was used, followed by elution with pure THF (2 min). Data were acquired with HP Chemstation software. Differential scanning calorimetry (DSC) was performed on a TA Q100 DSC. Two heating and cooling cycles were applied over a temperature range from −90 to 150 °C at a rate of 20 K/min. Data were acquired using TA Universal Analysis software.

Enzyme Pretreatment. An exact amount of Novozym 435 was weighed into a Schlenk flask together with a magnetic stirrer bar. The enzyme was then dried according to the following procedures: (1) Reference sample; the enzyme was used as received. (2) The Schlenk flask was placed in a desiccator with P_2O_5 under vacuum (1 mbar) for 16 h. (3) The Schlenk flask was placed in a desiccator with P_2O_5 for 16 h at 130 and 160 °C. (4) The Schlenk flask was placed in a desiccator with P_2O_5 under vacuum (1 mbar) for 16 h at 50 and 100 °C. (5) Activated molecular sieves were added to the Schlenk flask before it was placed in a desiccator with P_2O_5 for 16 h at 50 °C under vacuum (1 mbar).

Model Polymerization. For all model polymerizations the amount of Novozym 435 with respect to monomer was kept constant at 10% (w/w). A solution of benzyl alcohol in toluene (56 mg, 3.78 M) was added to the Schlenk tube with dried enzyme, and the tube was heated in an oil bath to 60 °C. The reaction was started by adding a solution of ϵ-caprolactone in toluene (3.28 g, 4.81 M) to the tube. Immediately after the start of the reaction a sample was withdrawn from the reaction mixture and analyzed using Karl Fischer coulometry. At specified time intervals further samples (∼0.4 mL) were withdrawn from the reaction mixture with a syringe and analyzed by ^{1}H NMR spectroscopy to determine monomer conversion. After 60 min, the reaction mixture was dissolved in dichloromethane and the Novozym 435 removed by filtration. The polymer was precipitated in cold methanol and dried overnight under vacuum at 50 °C, resulting in a final yield of 0.89 g (70%).

Initiator Kinetics. All bifunctional initiators (**1**−**3**) were used in polymerization reactions according to the model reaction; i.e., solutions of the appropriate initiator and monomer in toluene were used in the same molar ratios. Novozym 435 was dried according to method 5. During the reaction, samples were withdrawn at specified time intervals and analyzed with ^{1}H NMR in order to monitor monomer and initiator conversion and with SEC to follow the molecular weight development. After 1 h, dichloromethane was added to the reaction mixture and the Novozym 435 removed by filtration. The crude polymer was then precipitated in cold methanol and analyzed for water initiation and molecular weight as described above.

Block Copolymerization. A copolymer of P(CL-*b*-MMA) was synthesized in a two-step approach. PCL obtained from initiator **2**, synthesized according to the model polymerization, was used as a macroinitiator in a subsequent ATRP. The PCL (0.80 g, M_n = 6800 g/mol, PDI = 2.0) was weighed into a 10 mL two-necked round-bottom flask with a magnetic stirrer bar wherein CuBr (16.7 mg, 0.116 mmol) and dNbpy (101.8 mg, 0.249 mmol) were subsequently added (1:1:2.1 molar ratio). The flask, containing all solid reaction compounds, was then deoxygenated by five consecutive argon/vacuum cycles and kept under continuous argon flow. MMA (2.29 g, 22.9 mmol) was degassed with argon for 15 min and added to the flask by a syringe. To dissolve all components, the mixture was stirred for 30 min at room temperature after which the reaction was started by placing the flask in an oil bath at 90 °C. At the start and the end of the reaction, samples were taken for ^{1}H NMR and SEC analysis. The reaction was stopped when the polymerization mixture solidified (after ≈1 h). The polymer was dissolved in dichloromethane and the solution passed through a column filled with Al_2O_3 to remove the ATRP catalyst. The obtained polymer was then precipitated in cold methanol and analyzed by GPEC, SEC, and DSC. The yield of block copolymer was typically 1.5 g (50%).

Hydrolysis of the Block Copolymer. The PCL block was hydrolyzed by dissolving the block copolymer (0.60 g) in a 20 mL mixture of 1,4-dioxane/hydrochloric acid (37%) (20:1 (v/v)). The mixture was then stirred in a 50 mL round-bottom flask with a magnetic stirrer for 24 h at 85 °C. After hydrolysis, the solvents were removed under vacuum, and the crude product was precipitated in cold methanol. The resulting polymer (yield: 0.40 g, 67%) was analyzed with SEC, GPEC, and ^{1}H NMR.

Table 1. Effect of Different Enzyme Drying Methods in an eROP (Initiator: Benzyl Alcohol) on the Amount of Water in the Polymerization Mixture, the Monomer Conversion, and the Amount of Water Initiated Chains in the Final Polymer

entry	drying method[a]	water in reaction mixture[b] [mg/g]	water initiated polymer[c] [%]	monomer conversion[d] [%]
1	1	0.271	52	95
2	2	0.076	21	90
3	3 (130 °C)	0.145	33	51
4	3 (160 °C)	0.154		
5	4 (50 °C)	0.080	30	90
6	4 (100 °C)	0.110	9	81
7	5 (RT, 1 atm)	0.024	29	84
8	5 (50 °C, vacuum)	0.037	<5	95

[a] The entries correspond to the drying procedures described in the Experimental Part. [b] Determined by Karl Fischer titration. [c] Determined by ^{1}H NMR after precipitation.[26] [d] Determined by ^{1}H NMR by comparison of the $-CH_2-CO-$ signal in the monomer (4.25 ppm) and the polymer (4.1 ppm).

Results and Discussion

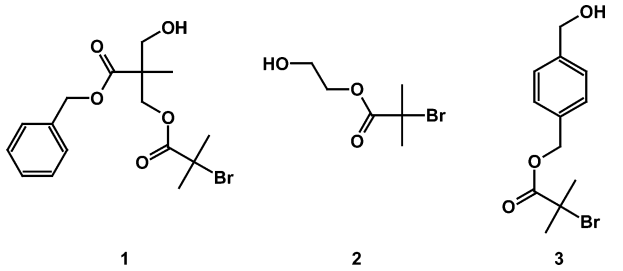
As outlined in Scheme 1, the combination of ATRP and eROP for the synthesis of block copolymers can be conducted either in two consecutive steps or in a one-pot cascade approach. The latter provides, without doubt, the higher synthetic and analytical challenge. To obtain high block copolymer yields, it is of paramount importance to fine-tune the reaction kinetics and minimize all side reactions leading to homopolymer formation. We therefore carefully investigated the governing factors to minimize the extent of undesired side reactions in order to obtain a maximum level of control over the polymerization and hence the polymer structure. Only the use of a bifunctional initiator equipped with an activated bromide group for ATRP and a primary hydroxy-group for the eROP allows the synthesis of block copolymers without an intermediate transformation step. The key to obtain high block copolymer yields is (i) high initiation efficiency from both initiator groups and (ii) exclusively initiation from the bifunctional initiator. Low initiator efficiency in either one of the two polymerizations or initiation by a species other than the bifunctional initiator inevitably results in homopolymer formation. Since the efficiency of ATRP is generally known to be high, we primarily focused our effort on the optimization of the enzymatic ROP, i.e., the conditions that lead to a high initiator efficiency in the enzymatic reaction.

Water Activity. It is generally accepted that the eROP of CL is initiated by a nucleophilic attack of a hydroxy compound on the enzyme activated monomer.[19−21] Various hydroxy-functionalized (macro)initiators have been reported in the past for the enzymatic synthesis of functionalized polymers and block copolymers, respectively.[20−22] The control of the polymer structure strongly depends on the frequency of side-reactions caused by the water activity, i.e., when water is either the acyl acceptor that initiates the chain growth or water reacts with intrachain esters causing chain degradation.[23] Both reactions can broaden the molecular weight distribution and will ultimately alter the composition of groups at the PCL chain ends. These competitive reactions, caused by water, results in polymers with carboxylic acid end groups, i.e., polymer chains that lack functionality to initiate a subsequent radical polymerization. In our study, it was important to control the latter since the degree of incorporation of the bifunctional initiator eventually determines the block copolymer yield. Consequently, the water concentration in the reaction medium has to be reduced as much as possible in order to obtain a high yield of initiator-functionalized PCL.

According to a literature report, one can distinguish different sources of water with respect to immobilized enzymes:[24] First, a layer of tightly bound water which is necessary to maintain the tertiary structure of the enzyme and hence its activity. (This water does not act as a competing nucleophile in the eROP, and removal results in enzyme denaturation.) In contrast, there is water that is loosely bound either to the enzyme itself or the carrier. This water is in equilibrium with free water in the reaction medium and can therefore act as a competing nucleophile. Any applied drying technique must only remove the free and loosely bound water but leave the essential enzyme bound water layer intact. We therefore screened a variety of different enzyme drying methods in a model reaction using benzyl alcohol as an initiator for the eROP of CL in dry toluene. Upon addition of both initiator and monomer to an exact amount of dried enzyme, the initial amount of water in the reaction mixture was determined by Karl Fischer titration. In addition, the final monomer conversion and the amount of water initiated polymer of the final product were determined by ^{1}H NMR (Table 1).

An experiment with the untreated enzyme, i.e., enzyme stored under ambient conditions, serves as a reference (entry 1, Table 1). The concentration of water titrated in the reference reaction mixture is 0.271 mg/g. After 1 h of polymerization, a monomer conversion of 95% was observed, confirming the activity of the enzyme. The amount of polymers with carboxylic acid groups determined by ^{1}H NMR was 52%. If the Novozym 435 was dried over P_2O_5 under vacuum (entry 2), a far lower water concentration (0.076 mg/g) was titrated. This results in a lower amount of carboxylic acid end groups in the polymer (21%) at comparable monomer conversion (90%). Drying at higher temperatures (entries 3 and 4) resulted in a significant decline of enzyme activity up to the expected total deactivation at 160 °C as evidenced from the decline in monomer conversion. Better results were obtained when the enzyme was dried at 50 °C under vacuum (entry 5: water concentration 0.08 mg/g). No significant decrease of activity was observed; however, the amount of carboxylic acid functionalized polymer was still 30%. This could be further reduced by the addition of molecular sieves to the reaction mixture. In this case, both the amount of water in the system (0.037 mg/g) and the amount of carboxylic end groups in the polymer were considerably decreased to <5%, while the enzyme activity was retained (entry 8). This drying method effectively removes most of the free and loosely bound water without deactivating the enzyme, thereby reducing the nucleophilic initiation of the eROP by water

Scheme 2. Structures of Bifunctional Initiators



considerably. All further experiments were conducted according to this successful drying protocol.

Initiator Structure. Three different bifunctional initiators (Scheme 2) were synthesized for the block copolymerization. Since all initiators possess a relatively bulky structure, their feasibility in a lipase-catalyzed CL homopolymerization was investigated first. The goal was to identify the structure with the highest initiating efficiency that leads to a high degree of end-functionalized PCL according to route 1a in Scheme 1. Benzyl alcohol was chosen as a reference because of its high initiator efficiency in this reaction. All reactions were conducted at 60 °C for 1 h in triplicate with Novozym 435 dried according to method 5.

Figure 1A,B shows the consumption of the different initiators during the polymerization. Inspection of Figure 1A illustrates the rapid conversion of benzyl alcohol, being >90% after 15 min. Similar results were found for initiator **2** and **3**, the latter resembling the structure

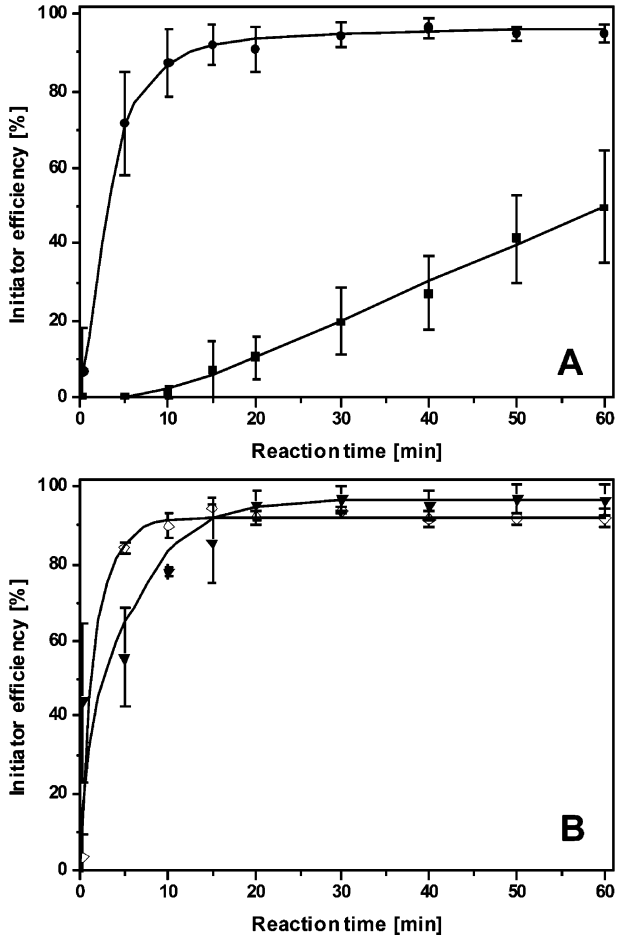


Figure 1. Initiator conversion in enzymatic polymerizations of CL, employing (A) (●) benzyl alcohol and initiator **1** (■) and (B) initiators **2** (◇) and **3** (▼).

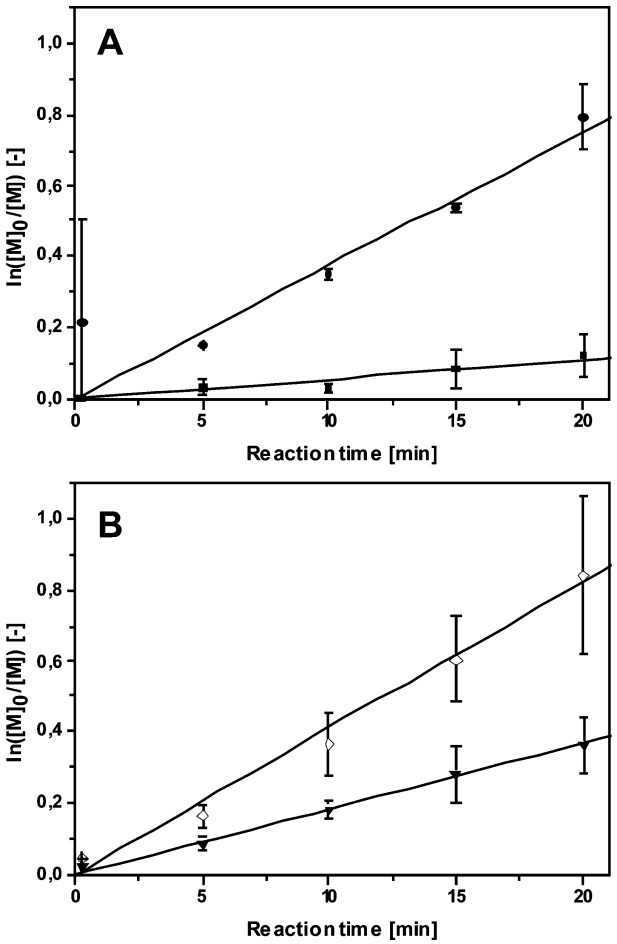


Figure 2. Kinetic plots of monomer conversion in the enzymatic polymerization of CL, determined by ^{1}H NMR on samples withdrawn from the reaction: (A) (●) benzyl alcohol and initiator **1** (■); (B) initiators **2** (◇) and **3** (▼).

of benzyl alcohol. Both bifunctional initiators are consumed at a similarly fast rate. Furthermore, ^{1}H NMR analysis of the products shows a complete shift of the benzylic proton signal of **3** from 4.65 to 5.1 ppm.[18] This is consistent with the initiators linked by an ester to the PCL chain. Initiator **1**, on the other hand, is a poor initiator in this polymerization as evidenced from the slow initiator consumption and monomer conversion after 1 h (Figures 1A and Figure 2A). This is surprising since we have applied this initiator in an earlier study for a similar synthesis of block copolymers without noticing significant amounts of nonfunctionalized PCL.[17] It can be speculated that the observed inhibition period in the polymerization using **1** implies that small amounts of water initiate the polymerization, causing very low monomer and initiator conversion. In addition, **1** is a mixture of enantiomers, which in conjunction with the enantioselectivity of the enzyme may influence the initiation kinetics. These results further suggest that the incorporation of benzyl alcohol as well as **2** and **3** to the growing polymer chain is predominantly through initiation. In contrast, **1** might very well be built into the chain by transesterification during the course of the reaction. As a consequence, high end group functionalization with **1** can only be achieved at longer reaction times like those applied for the synthesis of ATRP initiator end-capped PCL in our earlier report.

The kinetics of eROP was found to be first order with respect to monomer consumption, which agrees with previous literature reports.[19−21] Although the monomer

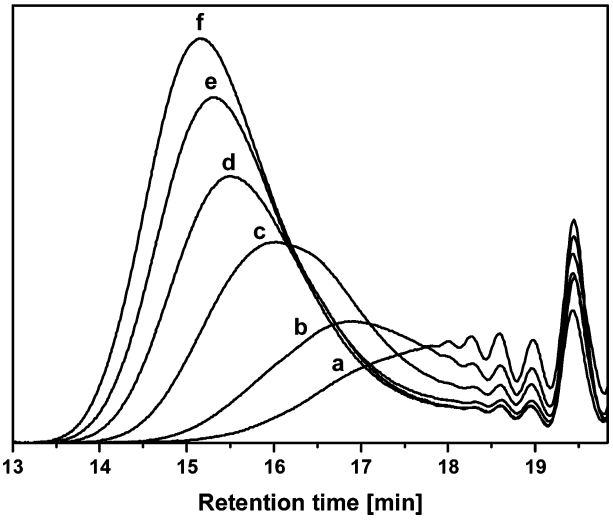


Figure 3. SEC traces of PCL samples withdrawn from the eROP of ϵ-caprolactone with initiator **2** at 60 °C in toluene after (a) 5, (b) 10, (c) 20, (d) 30, (e) 40, and (f) 60 min.

consumption in eROP is supposed to be independent of the nucleophile (i.e., initiator), we clearly observe a relationship (Figure 2). Reason for this discrepancy might be the actual low amount of water present in our reaction while performing the kinetic experiments. If the water concentration is far higher than the amount of initiator used, differences in reaction kinetics between the initiators might not be observed.

Block Copolymer Synthesis. Based on the optimized reaction conditions resulting in a high degree of incorporation of the bifunctional initiator, PCL macroinitiators were synthesized using initiator **2**. The development of the molecular weight was followed by SEC analysis of samples withdrawn at time intervals from the reaction mixture. It becomes evident from the chromatograms of Figure 3 that the polymerization proceeds in a well-defined fashion. PCL with a molecular weight of 6800 g/mol and a polydispersity (PDI) of 2.0 was obtained after 120 min. Furthermore, ^{1}H NMR analysis of the polymer confirms a low concentration of carboxylic acid end-capped polymers (<2%), implying a high degree of incorporation of **2** into the polymer.

A useful technique to confirm the incorporation of the initiator is MALDI-ToF-MS. Figure 4 shows a spectrum of a macroinitiator obtained from a typical enzymatic polymerization. Three distributions of peaks can be identified in this spectrum. The signals of every series are separated by 114 Da, which corresponds to the mass of the CL repeating unit. The distributions on the low molecular weight end of the spectrum (A in Figure 4) can be assigned to cyclic polymers, whereas the desired bromine-functional polymers are more prominent in the high molecular weight region of the spectrum (C in Figure 4). In agreement with the work of Hult, we observe that the relative amount of cyclic polymers depends on the reaction time and the monomer concentration.[21] The third distribution of peaks can be assigned to carboxylic acid end-functionalized polymer chains generated by side reactions caused by water (B in Figure 4). In agreement with the results from ^{1}H NMR analysis, these polymers are present as a minor component. Unfortunately, MALDI-ToF-MS analysis does not provide reliable quantitative data. Therefore, we applied liquid chromatography under critical conditions (LCCC) to separate and quantify the three major polymer

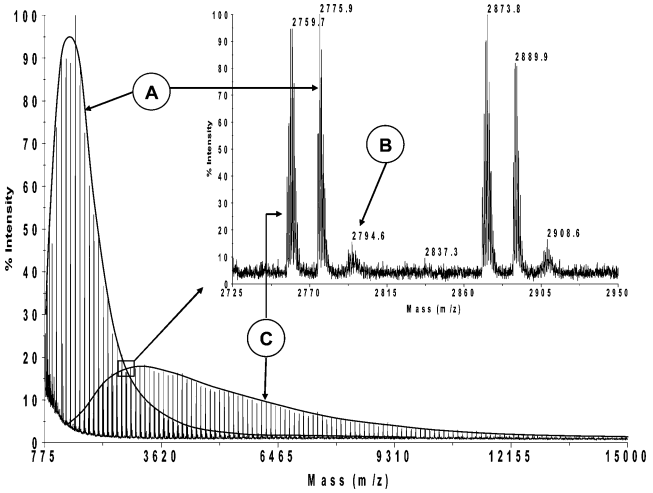


Figure 4. MALDI-ToF-MS spectra of PCL obtained from eROP using initiator **2**: (A) cyclic polymers, (B) carboxylic acid end-functionalized polymers, and (C) polymers end-functionalized with **2**.

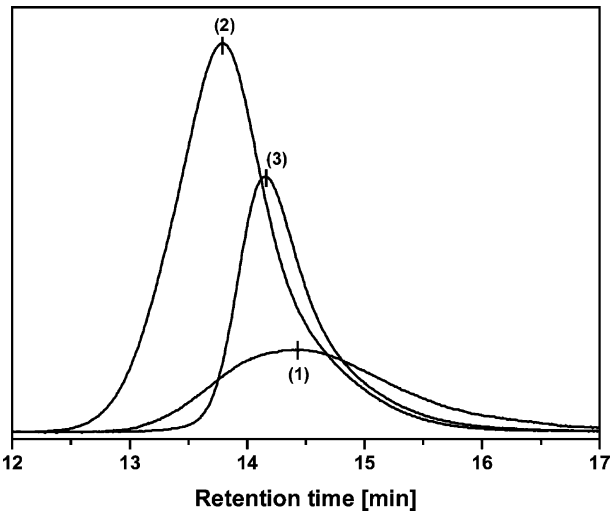


Figure 5. SEC traces of the macroinitiator (1), the block copolymer (2), and the PMMA block after hydrolysis (3).

components. Preliminary results point to a good agreement between the quantification of the water initiated polymer chains by ^{1}H NMR and LCCC. A detailed comparison between NMR, MALDI-ToF, and LCCC will be discussed in a subsequent paper.

The precipitated PCL macroinitiator was subsequently used for ATRP of MMA using CuBr/dNbpy as catalyst. An increase in the molecular weight from 13 400 to 36 900 g/mol was observed by SEC (Figure 5) calibration on polystyrene standards. From ^{1}H NMR, a block length ratio of PMMA to PCL of 4:1 was determined by comparison of the integrated peak area of the methoxy protons of the MMA units ($\delta = 3.62$ ppm) with the integrated peak area of the methylene ester protons in the PCL backbone. This is in good agreement with the monomer feed ratio. To directly analyze the PMMA block of the P(CL-*b*-MMA), the PCL block was hydrolyzed according to a literature procedure.[13] Comparison of the SEC trace of the remaining PMMA block with that of P(CL-*b*-MMA) reveals a shift to lower molecular weight, namely to 24 400 g/mol, due to the removal of the PCL block. Although this provides strong evidence for the block structure, the overlapping traces do not allow a final conclusion on the composition of the block copolymer yield, exclusively based on the SEC results.

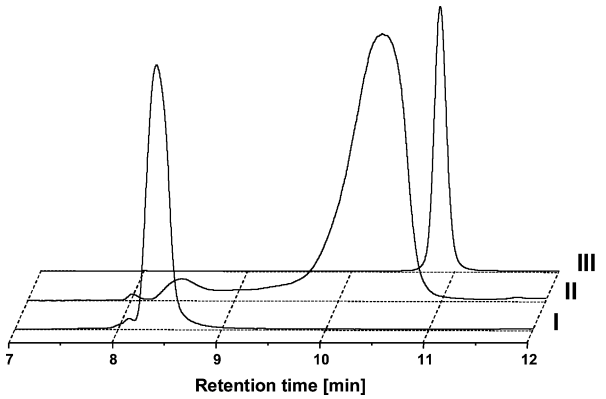


Figure 6. GPEC traces of homopolymer PCL (**I**; M_n = 13 000 g/mol), P(MMA-*b*-PCL) obtained in the chemoenzymatic reaction (**II**; M_n = 37 000 g/mol), and homopolymer PMMA (**III**; M_n = 15 000 g/mol).

To provide additional evidence for the block structure of the isolated polymers, these were further investigated by gradient polymer elution chromatography (GPEC).[25] The separation in GPEC is based on both the interaction between the polymer and the stationary phase (adsorption chromatography) and on the solubility of the copolymer in the eluent mixture. Within certain molecular weight limits it allows the separation of block and homopolymers solely on the basis of their chemical nature. In our case THF/heptane was used as solvent/nonsolvent system. It was found that, by applying a gradient from 100% heptane to 100% THF in 10 min, a proper separation of a blend of the two homopolymers could be achieved. Whereas pure PCL elutes at a retention time of 8.3 min, pure PMMA elutes at 10.8 min (Figure 6). The GPEC trace of the block copolymer, centered at a retention time of 10.4 min, is clearly separated from the traces of the two homopolymers. Further inspection of the chromatogram reveals only a small trace of PCL homopolymer present in the isolated block copolymer product, which most probably stems from the unfunctionalized and cyclic PCL.

Conclusions

We investigated the chemoenzymatic synthesis of block copolymers combining eROP and ATRP in two consecutive steps using a bifunctional initiator. A detailed analysis of the reaction condition revealed that a high block copolymer yield can be realized under optimized reaction conditions. Side reactions, such as the formation of PCL homopolymer, in the enzymatic polymerization of CL could be minimized to <5% by an optimized enzyme drying procedure. Moreover, the structure of the bifunctional initiator was found to play a major role in the initiation behavior and, hence, the yield of PCL macroinitiator. Block copolymers were obtained in a consecutive ATRP. Detailed analysis of the obtained polymer confirmed the presence of predominantly block copolymer structures. In a forthcoming paper we will report on the combination of both polymerization techniques in one pot.

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References and Notes

(1) Gross, R. A.; Kumar, A.; Kalra, B. *Chem. Rev.* **2001**, *101*, 2097.
(2) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793.
(3) Anderson, E. M.; Larsson, K. M.; Kirk, O. *Biocatal. Biotransform.* **1998**, *16*, 181.
(4) Faber, K. *Biotransformations in Organic Chemistry*, 4th ed.; Springer-Verlag: Berlin, 2000.
(5) Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Wiltholt, B. *Nature (London)* **2001**, *409*, 258.
(6) Huerta, F. F.; Minidis, A. B. E.; Bäckvall, J.-E. *Chem. Soc. Rev.* **2001**, *30*, 321.
(7) Bruggink, A.; Schoevaart, R.; Kieboom, T. *Org. Process Res. Dev.* **2003**, *7*, 622.
(8) Carrea, G.; Riva, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 2226.
(9) Faber, K. *Chem.—Eur. J.* **2001**, *7*, 5004.
(10) Harada, A.; Cammas, S.; Kataoka, K. *Macromolecules* **1996**, *29*, 6183.
(11) Bernaerts, K. V.; Schacht, E. H.; Goethals, E. J.; Du Prez, F. E. *J. Polym. Sci., Polym. Chem.* **2003**, *41*, 3206.
(12) Tunca, U.; Erdogan, T.; Hizal, G. *J. Polym. Sci., Polym. Chem.* **2002**, *40*, 2025.
(13) Mecerreyes, D.; Moineau, G.; Dubois, P.; Jerome, R.; Hawker, C. J.; Hedrick, J. L.; Malmström, E.; Trollsås, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 1274.
(14) Klaerner, G.; Trollsås, M.; Heise, A.; Husemann, M.; Atthoff, B.; Hawker, C. J.; Hedrick, J. L.; Miller, R. D. *Macromolecules* **1999**, *32*, 8227.
(15) Weimer, M. W.; Scherman, O. A.; Sogah, D. Y. *Macromolecules* **1998**, *31*, 8425.
(16) van As, B. A. C.; Thomassen, P.; Kalra, B.; Gross, R. A.; Meijer, E. W.; Palmans, A. R. A.; Heise, A. *Macromolecules* **2004**, *37*, 8973.
(17) Meyer, U.; Palmans, A. R. A.; Loontjens, T.; Heise, A. *Macromolecules* **2002**, *35*, 2873.
(18) Peeters, J.; Palmans, A. R. A.; Veld, M.; Scheijen, F.; Heise, A.; Meijer, E. W. *Biomacromolecules* **2004**, *5*, 1862.
(19) Henderson, L. A.; Svirkin, Y. Y.; Gross, R. A.; Kaplan, D. L.; Swift, G. *Macromolecules* **1996**, *29*, 7759.
(20) Uyama, H.; Suda, S.; Kobayashi, S. *Acta Polym.* **1998**, *49*, 700.
(21) Cordova, A.; Iversen, T.; Hult, K. *Polymer* **1999**, *40*, 6709.
(22) Kumar, A.; Gross, R. A.; Wang, Y. B.; Hillmeyer, M. A. *Macromolecules* **2002**, *35*, 7606.
(23) Kumar, A.; Gross, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 11767.
(24) Lee, C. S.; Ru, M. T.; Haake, M.; Dordick, J. S.; Reimer, J. A.; Clark, D. S. *Biotechnol. Bioeng.* **1998**, *57*, 686.
(25) Philipsen, H. J. A. *J. Chromatogr., A* **2004**, *1037*, 329.
(26) Mahapatro, A.; Kalra, B.; Kumar, A.; Gross, R. A. *Biomacromolecules* **2003**, *4*, 544.

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