Enzymatic Ring-Opening Polymerization and Atom Transfer Radical Polymerization from a Bifunctional Initiator

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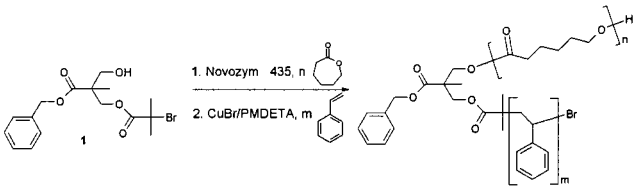
Introduction. This paper reports the first combination of an enzymatic polymerization with a "chemical" polymerization technique, i.e., lipase-catalyzed ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). We employ a bifunctional initiator that allows the synthesis of block copolymers from two dissimilar monomers, i.e., vinyl and lactone, without further transformation of the polymer end groups.

Enzymatic ROP is a promising alternative technique to the "chemical" ROP employing organometallic compounds as a catalyst. Intensive studies over recent years indicate that various polyesters and polycarbonates can be synthesized by the choice of monomer and lipase.[1] While not strictly a controlled or living polymerization, this new procedure has attributes comparable to traditional controlled polymerization techniques. It allows, for example, reasonable control of molecular weight and chain end functionality.[2] Therefore, it may be a valuable alternative synthetic tool to access copolymers and even more complex polymer architectures. From the industrial point of view, enzymatic reactions/polymerizations are significant due to technological advantages such as regio- and stereoselectivity, product purity (no residual catalyst), and the fact that both batch and continuous operation are possible.[3,4] In addition, the environmental tolerance of enzymatic procedures makes them attractive in industrial processes.[4] However, to fully integrate enzymatic polymerizations as an additional technique into polymer synthesis and eventually the preparation of novel materials, its versatility and compatibility with other techniques must be proven.

This study combines enzymatic ROP of ϵ-caprolactone (ϵ-CL) and ATRP of styrene because the latter is a very robust technique providing excellent control over molecular weight, polydispersity, and polymer end groups.[5] Furthermore, the compatibility of ATRP with ROP mediated by organometallic compounds was already reported in the literature.[6]

Results and Discussion. The bifunctional initiator **1** was prepared from the benzyl ester of bis(hydroxy)-propionic acid (bis-MPA).[7] One hydroxy group was esterified with 2-bromo-2-methylpropionyl bromide. The initiator contains a single primary alcohol group to initiate enzymatic ROP and an activated bromide, which is an effective initiator for ATRP (Scheme 1). The design of the bifunctional initiator was chosen carefully since the benzyl ester moiety provides a tag for ^{1}H NMR analysis as well as a chromophore for UV detection in the gel permeation chromatography (GPC) analysis of the block copolymer. To prove the efficiency of the initiator, a block copolymer was synthesized in two consecutive steps starting with the enzymatic ROP of ϵ-CL, followed by the ATRP macroinitiation of styrene.

Scheme 1. Enzymatic ROP and ATRP from a Bifunctional Initiator



This order was chosen because ATRP macroinitiation[8] is more efficient and straightforward than enzymatic macroinitiation due to its controlled character and the sterical demands of the latter.[9]

All enzymatic polymerizations were conducted employing Novozym 435 from Novo Nordisk (lipase CALB immobilized on an acrylic resin) and the initiator **1** in dry toluene under nitrogen with a constant target molecular weight around 5.0×10^3 g/mol.[10] Since water is an effective initiator in the enzymatic ROP, it can compete with the hydroxy function of **1** in the initiation step. This would result in PCL that is not end-functionalized with the ATRP initiator but with a carboxylic acid end group.[11] Therefore, it is important to thoroughly dry the reaction components in order to minimize the water initiation. Equally important is the optimization of the reaction time of the enzymatic ROP since lipases also catalyze transesterification reactions.[12] This side reaction will become more prominent when most of the monomer is consumed and will result in chain scission, i.e., polymer degradation. To identify the optimum reaction time, initiator **1** was employed in an ROP of ϵ-CL, and samples were withdrawn from the polymerization reaction after 3 and 5 h. The samples were analyzed by ^{1}H NMR and GPC with triple detection (UV, RI, viscosity) without prior purification. Figure 1 shows the GPC traces of the UV detection of the samples taken before starting the reaction ($t = 0$) and at $t = 3$ and 5 h. The GPC trace of the pure initiator shows that the peak at about 40 min retention time is the unreacted initiator. During the course of the polymerization, initiator is consumed as indicated by the decrease of that signal. After 3 h the initiator concentration reaches its minimum (95% consumed as calculated from signal integration). Apparently, prolonged reaction time does not result in further consumption of initiator. This corresponds to results obtained from the respective ^{1}H NMR measurements; it shows that all ϵ-CL is consumed after 3 h under the applied reaction conditions.

At the same time evolving polymer traces with very weak UV activity—attributed to the attached initiator—can be detected in the GPC. (A "conventionally" obtained PCL showed no UV activity.) Since the UV traces are not discernible in Figure 1, the corresponding traces of the RI detection are shown as an inset. Comparison of the GPC traces from RI and viscosity detection of the polymers obtained after 3 and 5 h indicates a shift of the peak to lower molecular weight as well as a broadening of the peak with longer reaction time. This can be rationalized by enzyme-catalyzed transesterification reactions becoming dominant at low monomer concentration as described above.

To synthesize block copolymers, the enzymatically polymerized PCL was precipitated and subsequently

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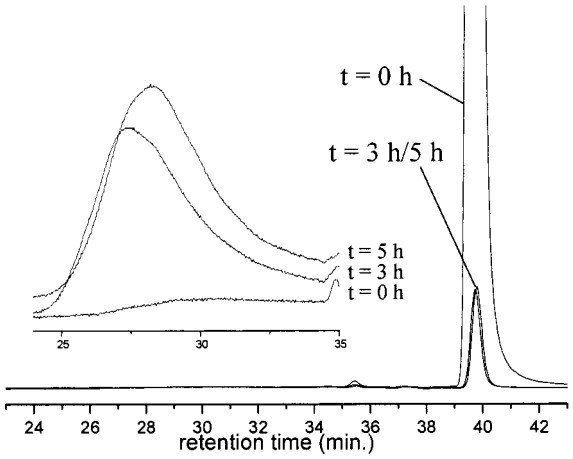

Figure 1. GPC traces of enzymatic ring-opening polymerization employing initiator **1** and ϵ-caprolactone. UV detection of crude samples taken at various reaction times shows the consumption of the initiator. The inset shows the development of the polymer traces by RI detection of the respective polymer region (t = 3 h: $M_n = 7.5 \times 10^3$ g/mol; polydispersity = 1.6; t = 5 h: $M_n = 4.0 \times 10^3$ g/mol; polydispersity = 2.3; values determined by GPC calibrated with PS).

used to macroinitiate styrene.[13] To determine whether the initiator is attached to the PCL, ^{1}H NMR spectra were carefully examined (Figure 2). Beside the dominant polymer signal, the resonances of the initiator group can be assigned in the ^{1}H NMR spectrum of the PCL. All initiator signals experience a shift upon polymerization, which is highest for the methylene group **c** shifting from 3.75 ppm in the initiator to about 4.2 ppm in the PCL due to the ester formation of the adjacent hydroxy function. Moreover, the signal assigned to the methyl groups **f** close to the activated bromide (1.9 ppm) can also be detected in the PCL, suggesting an intact ATRP initiator group. This indicates that the presence of the ATRP initiator group does not interfere with the activity of the enzyme. To quantify the amount of water initiated PCL, oxalyl chloride was added to the polymer solution directly in the NMR tube, according to a literature procedure.[14] Because of the reaction of the carboxylic acid end group of the water-initiated PCL with the oxalyl chloride, the proton NMR signal of the methylene group adjacent to the carboxylic acid group shifts into an uncrowded region of the spectrum at 2.9 ppm and can be quantified by integration. Although all components, i.e., solvent and monomers, were carefully dried prior to polymerization, the proportion of water initiation can be as high as 40% in some cases. This is close to the theoretically expected initiation by water attached to the enzyme beads as determined by Karl Fischer titration. When Novozym 435 beads were dried over P_2O_5 prior to polymerization, the amount of water-initiated PCL could be reduced to about 5% as determined by ^{1}H NMR.[15] Unlike PCL obtained without drying the enzyme, PCL obtained under dry conditions showed a symmetrical trace in the GPC with a polydispersity of 1.6 (Figure 3).[16]

To obtain the block copolymer, the PCL macroinitiator was then used for the ATRP of styrene. The attempted block length of PS block was about 3 times that of the PCL block as calculated from the macroinitiator/styrene feed ratio. The SEC traces of both the macroinitiator and the block copolymer are shown in Figure 3 with the expected increase in molecular weight and decrease in polydispersity of the block copolymer due to the con-

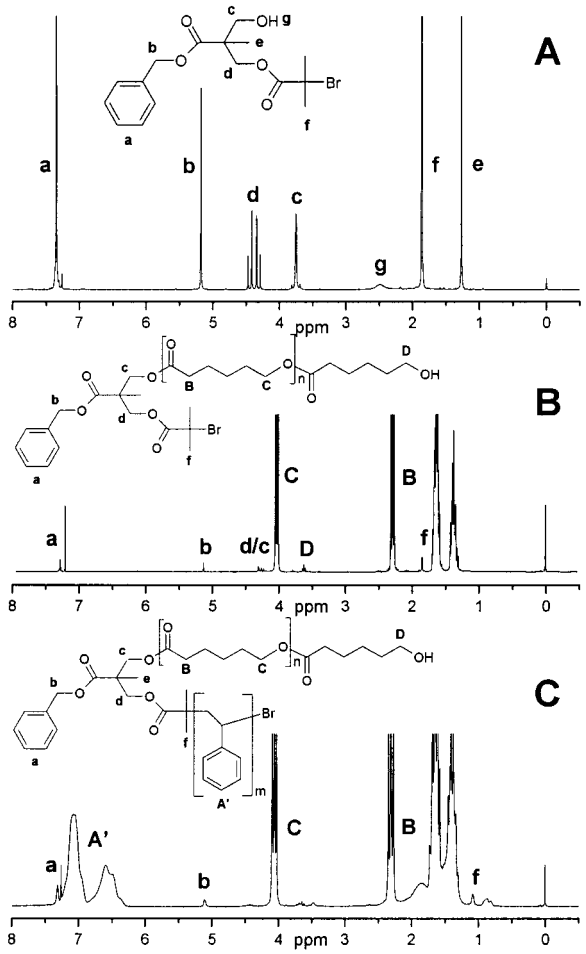

Figure 2. ^{1}H NMR ($CDCl_3$) spectra of the initiator **1** (A); poly(caprolactone) (5.8×10^3 g/mol; polydispersity: 1.7) (B) and poly(styrene-*b*-caprolactone) (PS-block 5.1×10^3 g/mol; polydispersity block copolymer: 1.4) (C). Molecular weights were calculated from the ^{1}H NMR integrated peak areas of the initiator peak **a** and polymer peaks **C** and **A**. The polydispersity was determined by GPC calibrated with PS.

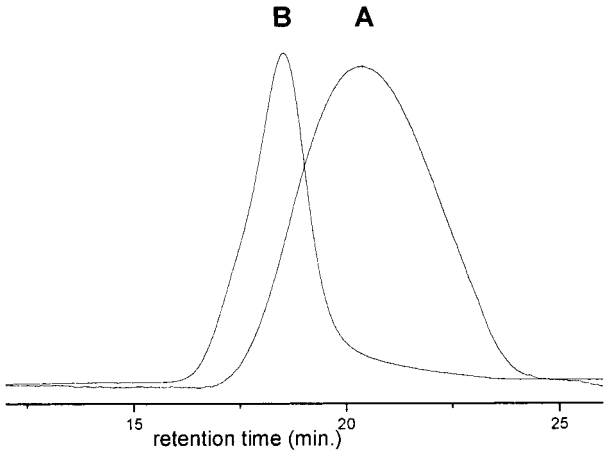

Figure 3. (A) GPC (RI detection) of enzymatically polymerized poly(caprolactone) (5.8×10^3 g/mol, polydispersity 1.7) employing initiator **1** and (B) poly(styrene-*b*-caprolactone) (PS block, 15×10^3 g/mol; polydispersity block copolymer, 1.2). Molecular weight and polydispersity were determined as described in Figure 2.

trolled character of the ATRP. To further confirm the block copolymer structure, it was dissolved in 1,4-dioxane (85 °C) and hydrolyzed with aqueous hydrochloric acid solution to give the cleaved polystyrene

block. Analysis of the product by ^{1}H NMR shows the disappearance of the all PCL resonances and comparison of the GPC traces show a shift to lower molecular weight with low polydispersity (1.2).

Figure 2C shows for better clarity the ^{1}H NMR spectrum of a block copolymer with about equal block length with resonances for both blocks assigned. Noteworthy is the chemical shift from 1.9 to 1.1 ppm of the methyl protons **f** of the ATRP initiator unit upon styrene polymerization due to the replacement of the activated bromide by the PS.[17] This proves the feasibility of the bifunctional initiator in the block copolymer synthesis employing enzymatic ROP and ATRP in two consecutive polymerizations.

Conclusion. In conclusion, we described a versatile route for the synthesis of block copolymers combining enzymatic ROP and ATRP. A PCL end-capped with the initiator was obtained in high selectivity by enzymatic ROP using a bifunctional initiator bearing initiator groups for both techniques. Sequential macroinitiation of styrene (or other vinyl monomers) by ATRP results in block copolymers in high yield (90−95%) without requiring an intermediate transformation step.

We are currently investigating whether both polymerizations can be conducted in one pot. Therefore, we have to examine the reaction kinetics in more detail and evaluate how well the enzyme works under ATRP conditions. The results of this study will be reported in a forthcoming paper.

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(10) Novozym 435 (0.5 g) and **1** (0.5 g, 1.3 mmol) were weighed into a flame-dried round-bottom flask. Then ϵ-CL (5.0 g, 43 mmol) and toluene (5 mL) were added under nitrogen, and the mixture was heated to 60 °C. After 3 h the polymer was precipitated in cold methanol to yield 3.1 g of polymer.

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(13) The PCL macroinitiator (0.5 g, ca. 0.1 mmol) and CuBr (15 mg, 0.1 mmol) were weighed into a flame-dried round-bottom flask equipped with a three-way stopcock. Several cycles of evacuation and subsequent purging with nitrogen were conducted to remove most of the dissolved oxygen. Finally, styrene (1.5 g, 14 mmol) and PMDETA (17 mg, 0.1 mmol) were added under nitrogen by a syringe, and the mixture was heated to 95 °C for 4 h. The solidified polymer was dissolved in THF and precipitated in methanol, yielding 1.2 g of polymer.

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(15) The water content of the immobilized enzyme determined by Karl Fischer titration is 2.9 mass % before drying. For the applied reaction conditions the molar amount of water is almost equal to the amount of initiator; i.e., roughly half of the polymer could be initiated by water. On the other hand, the dried enzyme contains 0.15 mass % water, which can theoretically result in 3−4% of water initiated polymer provided all other components are dry.

(16) Comparison of GPC traces before and after precipitation in methanol shows that low molecular weight fractions were cut out due to the better solubility compared to the high molecular weight fraction.

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