One-Step Chemoenzymatic Synthesis of Poly(ϵ-caprolactone-*block*-methyl methacrylate) in Supercritical CO_2

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ABSTRACT: Enzymatic ring-opening polymerization (eROP) of ϵ-caprolactone (ϵ-CL) and atom-transfer radical polymerization (ATRP) of methyl methacrylate (MMA) were integrated into a one-step chemoenzymatic route to yield poly(ϵ-CL-*block*-MMA) (PCL-*b*-PMMA) in supercritical carbon dioxide ($scCO_2$). The interaction between eROP and ATRP during the copolymerization was studied: Cu(I)Br/bpy as ATRP catalyst was compatible with eROP, while $Ni(PPh)_3Br_2$ inhibited enzyme activity; under the reaction conditions, both ϵ-CL and PCL improved the solubility of poly(MMA) (PMMA) in the reaction system, and thus the ATRP proceeded successfully. The propagation of PMMA block exhibited living kinetics during the copolymerization, ensuring the molecular weight was well controlled. PCL-*b*-PMMA copolymers with varying molecular weight were synthesized with good control of the composition of the block copolymer. The one-step chemoenzymatic polymerization provides a very simple and versatile route for the synthesis of block copolymers.

Introduction

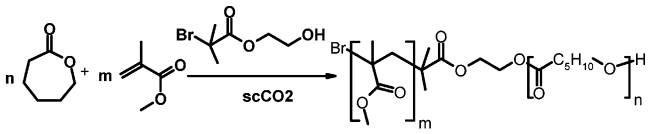
Enzymes are used as catalysts in a wide variety of reactions due to their high activity and selectivity while also being more environmentally acceptable than some conventional catalysts.[1] In the past decades, there has been an increasing interest in polymer synthesis via enzyme catalysis.[2−11] Enzyme catalysis has provided a new synthetic strategy for useful polymers, most of which are otherwise very difficult to produce by conventional chemical catalysts.[12,13]

Lipase has shown extraordinary activity for a range of polymer-forming reactions including enzymatic ring opening polymerization (eROP) of caprolactones[3,14−22] as well as copolymerization of lactones with other monomers to make copolymers.[23−29]

Research has been reported on the combination of living free radical polymerization (LFRP) with ring opening polymerization (ROP) using chemical catalysts, and block copolymers or graft copolymers have been synthesized in one or two steps.[30,31] The combination of LFRP with eROP to yield block copolymers and hyperbranched polymers in two steps has also been published.[23,24,26,29,32] However, a one-step approach combining LFRP with eROP has not been successful in organic solvents.

Supercritical CO_2, which has a critical temperature of 31.1 °C and a critical pressure of 73.8 bar, is considered a "green" solvent with low viscosity. While $scCO_2$ is widely applied in material processing,[33−35] it is also inert to free radicals and, thus, is an attractive medium for polymerization without the complication of chain transfer reactions that involve the solvent.[36−38] Although biocatalysis in $scCO_2$ has been a fertile area of research for the past decade,[39] there are few reports concerning enzyme-catalyzed polymerization in $scCO_2$. In our previous work, eROP of ϵ-caprolactone (ϵ-CL) was successfully carried out in $scCO_2$.[40] We further proposed a simple strategy for a single-step simultaneous one-pot synthesis of block copolymers by combining the eROP of ϵ-CL with the atom-transfer radical polymerization (ATRP) of methyl methacrylate (MMA) in $scCO_2$ (Scheme 1), and it was demonstrated that the enzymatic polymerization and the ATRP proceeded concurrently.[41]

Scheme 1. Simultaneous eROP of ϵ-CL and ATRP of MMA in $ScCO_2$



This follow-up paper presents further detailed experiments in which efforts were made to understand the interaction between the eROP and ATRP process. Moreover, we have investigated the kinetics of the copolymerization and used this understanding to exert some control over the composition of the copolymers produced using $scCO_2$.

Experimental Section

Materials. ϵ-Caprolactone (ϵ-CL, 99%) and methyl methacrylate (MMA, 99%) were purchased from Aldrich, dried over CaH_2 for 24 h under nitrogen, distilled under reduced pressure with three freeze−pump−thaw cycles, and stored under nitrogen until use. 2,2′-Bipyridine (bpy, 99+%) was purchased from Lancaster, copper(I) bromide (98%) and dibromobis(triphenylphosphine)-nickel(II) ($Ni(PPh)_3Br_2$, 99%) were purchased from Aldrich, and 1,4-dioxane was purchased from Fisher. Novozym-435 (10 wt % Lipase B from *Candida antarctica* on a macroporous acrylic resin) was purchased from Novozymes. SCF-grade carbon dioxide (99.99%) was purchased from BOC gases. The bifunctional initiator was synthesized as described elsewhere.[23]

Typical Block Copolymerization. (Entry 17 in Table 4) The copper(I) bromide (32 mg, 0.22 mmol), 2,2′-bipyridine (70 mg, 0.45 mmol), and Novozym-435 (0.4 g) were sealed in the autoclave (12.5 mL volume), which was heated to 35 °C while under vacuum.

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After 3 h, the autoclave was purged with CO_2 to release the vacuum. The MMA (1.5 mL, 14 mmol), ϵ-CL (5 mL, 45 mmol), and the bifunctional initiator (33 μL, 0.24 mmol) were then added to the autoclave by syringe, under a flow of CO_2, to prevent the ingress of moisture into the system. After all the liquid reagents had been added, the autoclave was sealed, the stirring started, and the pressure of CO_2 increased to 1500 psi. After 20 h of reaction, the autoclave was placed into a dry ice/acetone bath to freeze the contents. Once the pressure had fallen to atmospheric pressure, the autoclave was opened and the whole mixture in the autoclave was dissolved in chloroform. An aliquot of the solution was taken for NMR analysis to calculate the monomer conversion. The reaction mixture dissolved in chloroform was filtered to remove the Novozym-435. The resulting solution was passed through an alumina column to remove the catalyst, and the polymer was precipitated from cold methanol.

Hydrolysis of the Copolymer. To remove the PCL block, the block copolymer (1 g) was dissolved in 1,4-dioxane (28.5 mL) at 85 °C. Concentrated hydrochloric acid (1.5 mL, 37 wt % HCl in water) was added to the solution, which was left stirring for 20 h. The hydrolyzed polymer was obtained by precipitation in cold methanol. PMMA homopolymer was subjected to the same reaction conditions to determine whether the hydrolysis reaction had any effect on the ester linkage of the methacrylate. As expected, the molecular weight of the PMMA before and after the reaction remained unchanged.

Polymer Characterization. Molecular weight and molecular weight distribution of polymers were obtained by gel permeation chromatography (PL-120, Polymer Labs) with an RI detector. The columns (30 cm PLgel Mixed-C, 2 in series) were eluted by THF and calibrated with polystyrene standards. All calibration and analysis was performed at 35 °C and a flow rate of 1 mL/min. In our previous report,[40] a higher M_n and lower PDI were obtained. These previous data were collected with an evaporative light scattering detector, which tends to underestimate the low-molecular-weight fraction in the sample.

NMR spectra were recorded in $CDCl_3$ using a Bruker DPX 300 MHz spectrometer. All spectra were referenced to $CHCl_3$ at 7.26 ppm.

The MALDI-TOF mass spectrometry was carried out at the EPSRC National Mass Spectrometry Service Centre, Swansea. The samples were analyzed using a Voyager-DE-STR spectrometer, with dithranol/LiCl as the matrix.

The crystallization enthalpy of the polymers was determined using a TA MDSC 2920 system. The samples were weighed in aluminum pans, hermetically sealed, and subjected to two thermal cycles from 0 to 80 °C at 2 °C/min under 30 mL/min nitrogen flow. Crystallization enthalpy was taken as the average value of the crystallization and melting process during the second cycle.

Results and Discussion

Choice of ATRP Catalyst. For the eROP and ATRP to occur simultaneously, the ATRP catalyst was required to be compatible with the enzyme. Various metal complexes have been applied as ATRP catalysts.[42] For the ATRP of MMA, it has been reported that Cu(I)Br/bpy results in a very active system, whereas $Ni(PPh)_3Br_2$ is much less active.[43,44] The compatibility of CuBr/PMDETA and $Ni(PPh)_3Br_2$ with eROP in organic solvents has been reported previously. In organic solvents, copper did not influence the activity of the enzyme, while nickel completely inhibited the enzyme activity.[45,46]

In this study, the compatibility of Cu(I)Br/bpy and $Ni(PPh)_3Br_2$ with eROP in $scCO_2$ was investigated to determine whether similar kinetics are observed to conventional solvents. Table 1 shows that similar results to those in organic solvents are observed. $Ni(PPh)_3Br_2$ appeared to completely inhibit the enzyme activity (entry 4). The active site of Novozym-435 consists of an aspartate−histidine−serine triad, and nickel is known to deactivate this enzyme by complexing with the histidine in the triad.[46] On the other hand, apart from a slight retardation of the monomer conversion, the copper catalyst seemed not to affect the enzymatic polymerization. This is highlighted in entry 2 and 3 whereby a higher-molecular-weight polymer is formed with lower monomer conversion. The result is not surprising, as previous studies showed the molecular weight decreased and PDI increased at high conversion during the process of lipase-catalyzed ring-opening polymerization. This is due to the ability of lipases to catalyze transesterification reactions, which become much more prominent when most of the monomer is consumed, resulting in chain scission, i.e., polymer degradation.[16,17,23,47,48] Entry 2 and 3 are repeated experiments to highlight the reproducibility of the experiment.

Table 1. Enzymatic Ring-Opening Polymerization of Caprolactone: Effect of the ATRP Catalyst[a]

entry	CL(mL)	ATRP catalyst	conversion of ϵ-CL (%)	M_n[b]	PDI
1	5.0	none	89	15 500	2.60
2	5.0	0.22 mmol Cu(I)Br 0.45 mmol bpy	74	23 300	1.48
3[c]	5.0	0.22 mmol Cu(I)Br 0.45 mmol bpy	76	23 000	1.57
4	5.0	0.22 mmol $Ni(PPh)_3Br_2$	0		

[a] Novozym-435 0.4 g, no MMA, 33 μL of initiator, 12.5 mL of autoclave, 35 °C, 1500 psi, 20 h. [b] Number-average molecular weight detected by GPC with RI detector, relative to polystyrene calibrants. [c] Repeat of entry 2.

Table 2. Role of Cosolvent (ϵ-CL/PCL) in ATRP of MMA in $ScCO_2$[a]

entry	ϵ-CL(mL)	MMA (mL)	conversion of MMA (%)	M_n[b] (PMMA)	PDI (PMMA)
5	0.0	1.5	25	20 400	1.36
6[c]	0.0	1.5	21	18 600	1.32
7	1.5	1.5	36	9000	1.19
8	3.0	1.5	56	8700	1.19
9	5.0	1.5	57	6300	1.14
10	PCL 5.15 g[d,e]	1.5	15	6200	1.30
11	1.5 mL CL + 1.55 g PCL[e,f]	1.5	59	7500	1.20

[a] No enzyme, 33 μL of initiator, 32 mg of Cu(I)Br, 70 mg of bpy, 12.5 mL of autoclave, 35 °C, 1500 psi, 20 h. [b] Number-average molecular weight, relative to polystyrene calibrants. [c] Repeat of entry 5. [d] Equivalent to the mass of 5 mL of ϵ-CL monomer. [e] PCL M_n = 9000 Da, PDI = 1.5. [f] Equivalent to the mass of 3 mL of ϵ-CL in total.

On the basis of these results, Cu(I)Br/bpy was selected as ATRP catalyst in the simultaneous copolymerization of PCL-*b*-PMMA.

Cosolvent Role of ϵ-CL/PCL in ATRP of MMA in $scCO_2$. $scCO_2$ is generally accepted as a poor solvent for most polymers, hence reports on ATRP in $scCO_2$ are rare. Successful polymerization employing ATRP in $scCO_2$ has been shown when fluorinated reagents were used to produce homogeneous, controlled reactions.[49] Here, we demonstrate that ϵ-CL monomer and $scCO_2$-plasticized PCL act as very effective cosolvents when ATRP of MMA is performed in $scCO_2$. Indeed, homogeneity of the reaction mixture is achieved even in the absence of CO_2-philic fluorinated reagents.

To achieve an insight into the phase behavior during ATRP of MMA in $scCO_2$, the polymerization was carried out in a view cell. When no ϵ-CL was present, PMMA rapidly precipitated out from the monomer/CO_2 solution. With ϵ-CL as cosolvent, the reaction mixture remained homogeneous throughout the period of polymerization. Another important aspect for consideration is the solubility of the Cu(I)Br/bpy complex. This catalyst is not soluble in pure $scCO_2$. However, it readily dissolves with the assistance of ϵ-CL, hence eliminating the necessity for fluorinated reagents.

Table 3. Kinetics of Simultaneous eROP and ATRP[a]

entry	time (h)	conversion (ϵ-CL) (%)	conversion (MMA) (%)	M_n[b] (copolymer)	PDI (copolymer)	M_n[b,c] (PMMA)	PDI[c] (PMMA)
12	5	23	39	8200	1.35	6500	1.22
13	10	44	51	12 500	1.77	7400	1.31
14	15	53	60	13 600	1.95	8500	1.31
15	20	67	71	16 800	2.30	9000	1.31

[a] The reaction conditions are: 0.4 g Novozym-435, 5 mL of ϵ-CL, 3 mL of MMA, 33 μL of initiator, 32 mg of Cu(I)Br, 70 mg of bpy, 12.5 mL of autoclave, 35 °C, 1500 psi. [b] Number-average molecular weight, relative to polystyrene calibrants. [c] M_n and PDI of PMMA are measured after hydrolytic removal of PCL block from the copolymer.

Table 4. Controlling the Composition of PCL-*b*-PMMA in the Simultaneous Copolymerization

entry	CL (mL)	MMA (mL)	conversion (CL) (%)	conversion (MMA) (%)	M_n[a] (copolymer)	PDI	M_n[a,b] (PMMA)	PDI[b]	CL/MMA[c]
16[d]	5.0	0.8	72	57	17 900	1.75	3200	1.19	89/11
17[d]	5.0	1.5	77	66	23 400	1.71	5900	1.28	76/24
18[d]	5.0	3.0	67	71	16 800	2.30	9000	1.31	57/43
19[d,e]	5.0	3.0	75	65	12 800	2.66	8000	1.31	60/40
20[f]	5.0	3.0	35	68	15 000	1.62	10 100	1.31	29/71

[a] Number-average molecular weight, relative to polystyrene calibrants. [b] M_n and PDI of PMMA are measured after hydrolytic removal of PCL block from the copolymer. [c] Molar ratio of CL unit to MMA unit in the block copolymer, determined by NMR. [d] Novozym-435 0.4 g, 33 μL of initiator, 32 mg of Cu(I)Br, 70 mg of bpy, 12.5 mL of autoclave, 35 °C, 1500 psi, 20 h. [e] Repeat of entry 18. [f] Novozym-435 0.2 g. Other conditions same as d.

ATRP of MMA in $scCO_2$ was carried out under various conditions. The details are presented in Table 2.

In the absence of cosolvent, the monomer conversion is low and lack of control leads to high-molecular-weight PMMA (entries 5, 6). However, when ϵ-CL monomer was used as cosolvent (entries 7, 8, 9), the ATRP of MMA was successful in $scCO_2$ and 3 mL of ϵ-CL was sufficient to achieve over 50% yield (entry 8). In previous publications,[33,50,51] we have demonstrated that $scCO_2$ can plasticize and effectively liquefy polymers such as PCL under conditions of 35 °C and 1500 psi. We reasoned that perhaps this liquefied PCL may be able to act as the cosolvent for the ATRP polymerization of MMA. Our results show that, when PCL was used as the cosolvent, the ATRP reaction was found to be very slow (entry 10), presumably because of the high viscosity of the reaction mixture and, hence, retarded mass transfer.

To lower the apparent viscosity, we introduced a combination of ϵ-CL monomer and PCL. Qualitative observations in a view cell apparatus indicated that there was a distinct lowering of the reaction system viscosity. Hence, the combination of ϵ-CL monomer and PCL had the same cosolvent effect as pure monomer (entries 8, 11). We believe the existence of ϵ-CL monomer is advantageous for $scCO_2$ to plasticize/dissolve the PCL and then both the ϵ-CL monomer and PCL acted together as cosolvent to solubilize the propagating PMMA chain in $scCO_2$. This result showed that, in a one-step copolymerization by eROP of ϵ-CL and ATRP of MMA in $scCO_2$, the conversion of ϵ-CL to PCL does not hinder the propagation of PMMA chain.

One-Step Copolymerization by eROP and ATRP: Kinetics. To study the kinetics of the one-step copolymerization, four reactions were performed under the same conditions and stopped at 5, 10, 15, and 20 h, respectively. The copolymer was hydrolyzed to remove the PCL block, precipitated in cold methanol, and analyzed by GPC to give the molecular weight of the PMMA block. The results in Table 3 show simultaneous propagation by both eROP and ATRP. While ϵ-CL and MMA monomers were consumed during the progress of copolymerization, both the molecular weight of the copolymer and the PMMA block increased with reaction time.

If we consider the ATRP of MMA during the copolymerization, a linear increase in M_n of the PMMA block with conversion of the MMA monomer was found and the PDI during the polymerization was low (PDI ≤ 1.31), as shown in Figure 1. This suggests that the propagation of the PMMA block is a "living" polymerization, as the number of propagating radical chains remains approximately constant throughout the entire reaction. In addition, good control over the molecular weight distribution was also retained.

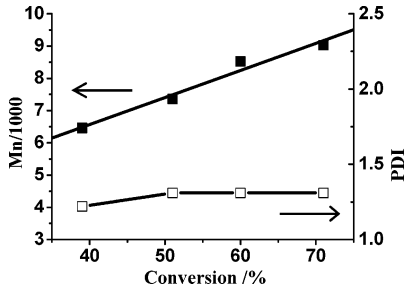

Figure 1. Evolution of M_n (■) and PDI (□) as a function of monomer conversion for ATRP of MMA during the simultaneous one-pot copolymerization of MMA and ϵ-CL. (M_n and PDI analyzed following removal of the PCL block from the copolymer by hydrolysis.)

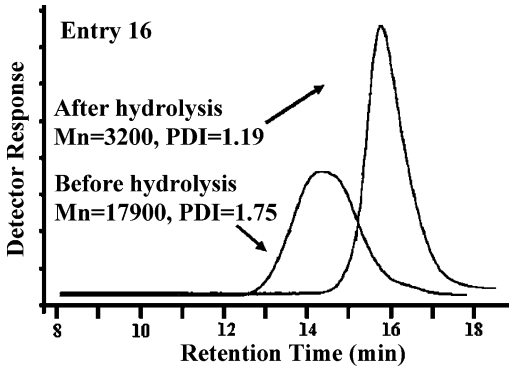

Figure 2. GPC trace of copolymer before and after hydrolysis (entry 16 in Table 4). The clean shift to lower molecular weight upon hydrolysis clearly demonstrates that block copolymers have been formed.

One Step Copolymerization of eROP and ATRP: Controlling the Composition of Copolymer. Because the PCL block and PMMA block propagate concurrently, the solubility and mobility of the propagating polymer chain is different from homopolymerization. Hence, it has proven difficult to gain absolute control over the propagation kinetics of each block

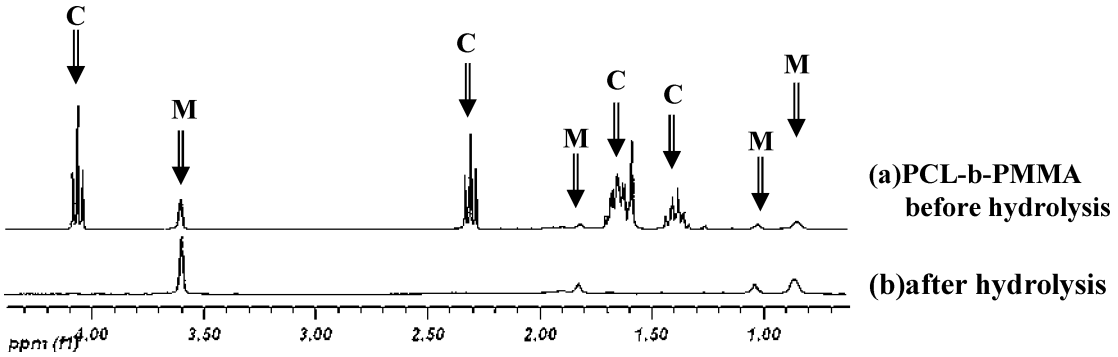

Figure 3. ^{1}H NMR spectra of (a) block copolymer with both PCL/PMMA block and (b) the remaining polymer (PMMA) after hydrolysis (^{1}H NMR, 300 MHz, $CDCl_3$, Entry 17 in Table 4.). (C: PCL; M: PMMA)

individually in a one-step copolymerization. However, through delicate control of the parameters in the simultaneous copolymerization, PCL-*b*-PMMA with various compositions (i.e., the molar ratio of ϵ-CL unit to MMA unit in the copolymer) can be synthesized. For the eROP of ϵ-CL, the molecular weight of PCL increases with higher enzyme/monomer ratio and higher monomer/initiator ratio. For the ATRP of MMA, the molecular weight of PMMA depends on the monomer/initiator ratio and monomer conversion. Therefore, in this study, the amount of ϵ-CL monomer and bi-initiator was kept constant, and the PCL block length was controlled by varying the enzyme content (enzyme/monomer ratio) and the PMMA block length was controlled by varying the MMA monomer concentration (monomer/initiator ratio). Some typical results are presented in Table 4.

In entries 16−19, MMA monomer content increased while all other parameters were kept the same (entries 18 and 19 are repeated experiments to demonstrate the reproducibility of this process.). As expected, the content of MMA units in the obtained copolymer increased from 11 to 43% (molar percentage). To further increase the MMA content in PCL-*b*-PMMA, half the amount of enzyme was used to slow the eROP (entry 20). Copolymer with 71% (molar percentage) MMA content was subsequently synthesized. Therefore, it can be seen that the effect of changing the amount of enzyme on the CL/MMA content ratio in the copolymer by comparing entry 18 and entry 20 in Table 4 (ratio CL/MMA from 57/43 to 29/71, respectively). Although the molecular weight of the copolymer and PMMA block (following hydrolysis of the copolymer) can be measured by GPC, it is extremely difficult to compare the absolute block length of PCL due to the large variation in PDI of the different copolymers. Nevertheless, precise control can be obtained over the composition of the copolymer.

Characterization of PCL-*b*-PMMA. To demonstrate block copolymer formation, the product PCL-*b*-PMMA was hydrolyzed to remove the PCL block. The GPC traces of the block copolymer before and after hydrolysis are presented in Figure 2. The peak eluting at shorter time is due to block copolymer. It corresponds to a molecular weight of 17 900 Da with a PDI of 1.75. After hydrolysis, the peak has a much lower molecular weight (3200 Da) and much narrower molecular weight distribution (PDI = 1.19). This peak is due solely to the PMMA block. The removal of the PCL from the block copolymer after hydrolysis was also confirmed by NMR analysis. In the spectrum of the block copolymer, NMR peaks at 1.40, 1.65, 2.30, and 4.05 ppm are assigned to the PCL block (Figure 3a). These peaks are not present in the spectrum of the hydrolyzed product (Figure 3b).

NMR spectra provide additional evidence that the composition of PCL-*b*-PMMA is well controlled. Figure 4 shows a series of

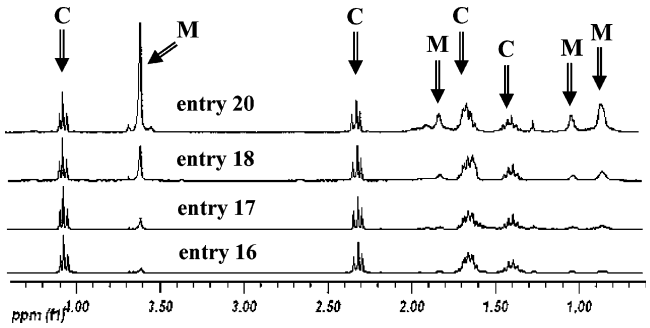

Figure 4. ^{1}H NMR spectra showing PCL-*b*-PMMA with various CL/MMA molar ratios. (C: PCL; M: PMMA). An increase in PMMA peaks intensity is observed from entries 16 to 20.

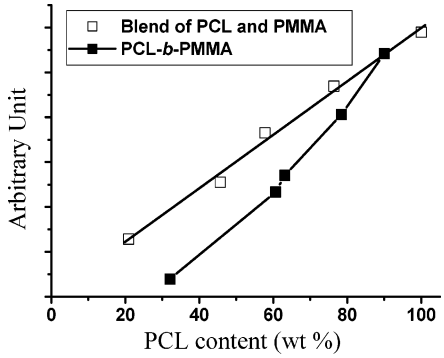

Figure 5. Plot of average enthalpy for PCL melting/crystallization transition (DSC) for a physical blend of PCL/PMMA (□) and for the PCL-*b*-PMMA copolymer (■). (Data taken as the average value of the crystallization and melting process in the second thermal cycle)

NMR spectra of the copolymer samples obtained in entries 16, 17, 18, and 20 in Table 4. The spectra are normalized to the PCL peak at 4.08 ppm, and a clear increase in PMMA intensity is observed from entries 16 to 20. This is indicative of the increasingly larger PMMA portion in the block copolymer that was targeted in this series of experiments.

For the eROP of ϵ-CL, water acts as a competitive initiator. Despite exhaustive measures to remove all the water from the system, it is still possible to find a small quantity of PCL homopolymer in the product, which is initiated by adventitious water in the enzyme beads. To further confirm that block copolymers were actually formed rather than a physical mixture of PCL and PMMA, the end group of the polymer product was analyzed by using oxalyl chloride treatment as described in the literature.[52] The amount of PCL homopolymer with chain-end carboxylic acid groups was determined by ^{1}H NMR upon the addition of oxalyl chloride to give the percentage of water-initiated PCL homopolymer (see Supporting Information for a detailed explanation and ^{1}H NMR spectra). The results of end-

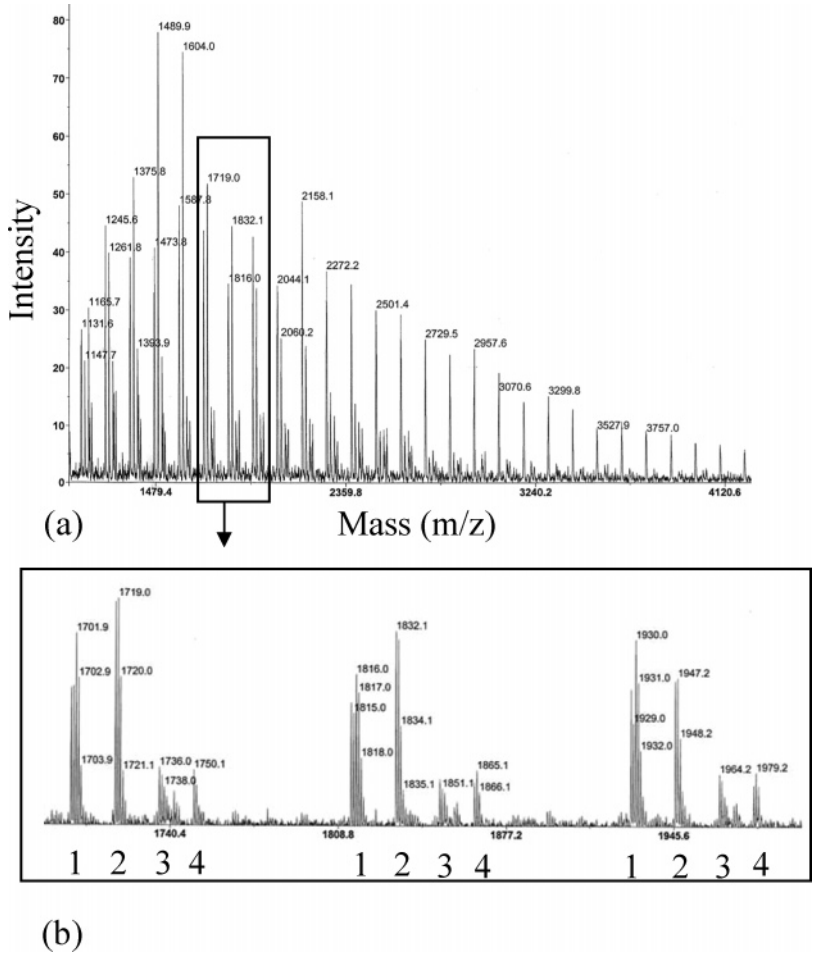

Figure 6. MALDI-TOF MS of low-molecular-weight PCL-*b*-PMMA (M_n = 5300, PDI = 1.57) (a) and an expansion of the region between 1700 and 2000 mass units (b). Four main series can be found in the spectrum. Series 1, 3, and 4 are associated with the block copolymer. Series 2 can either be due to cyclic PCL or it may be associated to block copolymer.

Table 5. Molecular Weight of PCL-*b*-PMMA and Cyclic PCL by Theoretical Calculation and MALDI-TOF MS

series	polymer chain	calculated M_w (Da)	detected M_w (Da)
1	Li + initiator + 8 MMA + 6/7/8 CL	1703.8/1817.9/1932.1	1701.9/1816.0/1930.0
2(a)	Li + initiator + 7 MMA + 7/8/9 CL	1717.8/1832.0/1946.1	1719.0/1832.1/1947.2
2(b)	Li + 15/16/17 CL (cyclic PCL)	1719.1/1833.2/1947.4	
3	Li + initiator + 6 MMA + 8/9/10 CL	1731.8/1846.0/1960.1	1736.0/1851.1/1964.2
4	Li + initiator + 5 MMA + 9/10/11 CL	1745.9/1860.0/1974.1	1750.1/1865.1/1979.2

group analysis confirm that block copolymers were actually formed, and the product consisted predominantly of PCL-*b*-PMMA, with water initiated PCL typically less than 10%.

Differential scanning calorimetry (DSC) was used to investigate the enthalpy of crystallization of physical blend of PCL and PMMA for comparison with the block copolymer. For the blend of PCL and PMMA, the PCL product in entry 1 (M_n = 15 500, PDI = 2.6) and the PMMA product in entry 8 (M_n = 8700, PDI = 1.19) were weighed into an aluminum pan with various PCL contents. For the block copolymer, PCL-*b*-PMMA products from entries 16 to 20 were analyzed. The samples were subjected to two thermal cycles from 0 to 80 °C at 2 °C/min. The crystallization enthalpy was taken as the average value of the crystallization and melting process during the second cycle. The results are shown in Figure 5. A physical blend of PMMA and PCL does not alter the crystallization degree of the PCL, as the enthalpy of crystallization increases linearly with PCL content (□ in Figure 5). However, the block copolymer (■ in the plot) with PCL content < 90% has a lower crystallization enthalpy than the blend of PCL and PMMA at the same PCL content. Indeed, the more PMMA incorporated into the block copolymer, the larger the deviation from the result of the blend. This indicates a lower degree of crystallinity of the PCL block than in the blend of PCL and PMMA homopolymers, which is due to the inhibiting effect PMMA has on effective packing of the PCL chains in the crystallites. This effect becomes less apparent as the PCL content increases. When the PCL block becomes predominant, a PCL chain in the block copolymer exists in an environment very similar to the PCL homopolymer, and thus crystallite formation is much as it would be in the homopolymer. These results are in good accordance with the published literature, where the crystallinity degree of PCL in PCL/PMMA blend is nearly the same as in PCL homopolymer when the PCL content is greater than 20%.[53] However, the crystallinity of PCL block is lower in the block copolymer.[54] The DSC results again confirm that block copolymers were actually formed rather than a mixture of PCL and PMMA homopolymers.

MALDI-TOF mass spectrometry was also used to analyze the block copolymers, and a typical spectrum is shown in Figure 6a, with an expansion in Figure 6b. As is well-known, MALDI-TOF is particularly sensitive to low-molecular-weight polymers,

hence the data tend to highlight any low-molecular-weight impurities in the sample. One type of low-molecular-weight side product of eROP with Lipase B from *Candida Antarctica* are the cyclic polyesters.[47,55] The spectrum in Figure 6 contains four major series, and these clearly show the presence of copolymer products, very clearly and uniquely identified by molecular masses. However, there is an ambiguity with series 2 (Figure 6b), which can be associated with either cyclic PCL (series 2b) or a block copolymer (series 2a) in Table 5. Thus, it would not be surprising if a small amount of cyclic material is present. Further experiments reporting a detailed investigation of cyclic production will be reported in a future publication. At higher masses (>3000 atomic mass unit), one would not expect to see any evidence for the presence of any cyclic material; such high-molecular-weight cyclics become too large to enter the active site of the enzyme. Detailed analysis of the MALDI series at higher molecular weights shows no evidence for the formation of cyclics and copolymer species predominating. Thus, the MALDI-TOF data complement NMR, GPC, and DSC results and can also be used qualitatively to demonstrate that, in this work, we have produced block-copolymer PCL-*b*-PMMA, and not homopolymer mixtures.

Conclusion

Enzymatic ring-opening polymerization of ϵ-caprolactone and atom-transfer radical polymerization of methyl methacrylate were integrated into a one-step chemoenzymatic route to yield PCL-*b*-PMMA in $scCO_2$. The interaction between eROP and ATRP during the copolymerization was studied in detail. We found that Cu(I)Br/bpy as ATRP catalyst was compatible with the enzyme while $Ni(PPh)_3Br_2$ clearly inhibited enzyme activity. Under the reaction conditions, both ϵ-CL and added PCL improved the solubility of PMMA in the reaction system and allowed ATRP to proceed successfully. The propagation of the PMMA block exhibited living kinetics during the copolymerization, ensuring the molecular weight of the PMMA block was well controlled. The block PCL block length was manipulated by control of the enzyme concentration. Overall, the use of $scCO_2$ allows controlled one-pot synthesis of PCL-*b*-PMMA copolymers with varying molecular weight and good control of the composition. Careful analysis of the samples using GPC, NMR, DSC, and MALDI-TOF MS confirmed the formation of block copolymers and not simple homopolymer mixtures. This one-step chemoenzymatic polymerization provides a simple and versatile route to synthesis of block copolymers. This technique can be extended to produce a wide range of block copolymers incorporating monomers with very different physical and chemical properties.[56]

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Supporting Information Available: Analysis of the end group by treatment with oxalyl chloride: structure of the PCL homopolymer and the PCL-*b*-PMMA block copolymer before and after addition of oxalyl chloride; ^{1}H-NMR spectra of product before and after addition of oxalyl chloride. This material is available free of charge via the Internet at http://pubs.acs.org.

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