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Lipase-Catalyzed Ring-Opening Copolymerization of ϵ -Caprolactone and β -Lactam

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S Supporting Information

ABSTRACT: The enzymatic ring-opening copolymerization of ϵ -caprolactone (ϵ -CL) and β -lactam by using *Candida antarctica* lipase B (CAL-B) as catalyst was studied. Variation of the feed ratios of 25:75, 50:50, and 75:25 of ϵ -CL/ β -lactam was performed. The products contain poly(ϵ -CL-*co*- β -lactam) and the homopolymers of poly(ϵ -CL) and poly(β -lactam). The structure of the copolymers was determined by MALDI-ToF MS. Poly(ϵ -CL-*co*- β -lactam) has an alternating and random structure consisting of alternating repeating units with oligo(ϵ -CL) or oligo(β -lactam). The highest fraction of the alternating copolymers resulted from the reaction with a feed ratio 50:50. The copolymer is a semicrystalline polymer with a T_m at 124 °C and T_g s at −15 and 50 °C. Interestingly, the copolymer also demonstrated cold crystallization at 29 and 74 °C, after quenching the sample from the melt in liquid nitrogen.

INTRODUCTION

Enzymes, also known as biocatalysts, are proteins with catalytic activity that control the rates of metabolic reactions in living cells.¹ Apart from their function in living cells, since ancient times, enzymes have been used in preparation of food products such as cheese, beer, wine, vinegar, and in manufacture commodities such as linen, leather, and indigo.² Due to increasing progress in enzyme discovery, enzyme engineering, and biotechnology, enzymes have been used more often for in vitro applications, for example, as catalyst in enzymatic polymerizations. The use of enzymes as an in vitro catalyst offers many advantages such as high selectivity and specificity, preventing complicated step reactions, and reducing undesired byproducts.¹ Furthermore, enzymes can operate in organic solvents, which is beneficial for new types of reactions (synthesis of novel monomers, oligomers, or polymers).³

The most commonly used biocatalyst in polymerization reactions is immobilized *Candida antarctica* lipase B (CAL-B) on acrylic resin, commercially available as N435. CAL-B is used due to its stability at elevated temperature and its acceptance of various substrates.^{4,5} Furthermore, enzymes have been used in many polymerization reactions to catalyze the formation of ester or amide bonds, like in the synthesis of polyesters,^{6–9} polyamides,^{10–17} and polypeptides.^{17–19}

Poly(ϵ -caprolactone), or poly(ϵ -CL), is a well-known semicrystalline aliphatic polyester due to its biocompatibility and biodegradability.^{20,21} It has been utilized in different fields, for instance, in scaffolds for tissue engineering, sutures, orthopedics, microelectronics, drug delivery systems, as

adhesive, and in packaging.^{21,22} Poly(ϵ -CL) displays miscibility with other polymers, such as poly(vinyl chloride), poly(styrene-*co*-acrylonitrile), poly(acrylonitrile-*co*-butadiene-*co*-styrene), poly(bisphenol A), polycarbonates, nitrocellulose, and cellulose butyrate, and also displays mechanical compatibility with polyethylene, natural rubber, and poly(vinyl acetate).²² Therefore, copolymerization of ϵ -caprolactone will be an interesting approach to synthesize new materials with potentially improved thermal or physical properties compared to poly(ϵ -CL) homopolymer.

The synthesis of poly(ϵ -CL) can be carried out by two methods: (1) polycondensation of 6-hydroxyhexanoic acid and (2) ring-opening polymerization (ROP) of ϵ -CL. Developments in enzymatic polycondensation of 6-hydroxyhexanoic acid²³ and ROP of ϵ -CL^{24–28} have been reported. Ring-opening copolymerization of ϵ -CL with other lactone rings using enzymes as catalyst has been carried out previously.^{6,29–32} Besides the copolymerization with other lactone rings, enzymatic copolymerization has been performed for the synthesis of copolymers of ϵ -CL and D,L-lactide,²⁰ copolymers of ϵ -CL and linear hydroxyesters,³² block copolymers of ϵ -CL and (R)-3-hydroxybutyrate,³³ and block copolymers of ϵ -CL and dimethylsiloxane.³⁴ However, to the best of our knowledge, enzymatic ring-opening copolymerization of ϵ -CL and lactams is never reported before.

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The enzymatic ring-opening polymerization (eROP) of β -propiolactam (β -lactam) and mechanistic insight of this eROP have been established by our group and our collaborators.^{10,11,35} It has been reported previously that unbranched poly(β -lactam) with a maximal degree of polymerization ($DP_{\max}$) up to 18 resulted from eROP of β -lactam by using immobilized CAL-B as catalyst.¹⁰ On the other hand, synthesis of poly(β -lactam) by anionic polymerization of acrylamide using a metal-based compound as a catalyst resulted in branched poly(β -lactam).³⁶ Therefore, enzymes can act as alternative for metal catalysts, being more environmentally friendly.

Here, we present a new type of polyesteramide that is synthesized by enzymatic ring-opening copolymerization of ϵ -CL and β -lactam, as depicted in Scheme 1. Variation of the feed

Scheme 1. Enzymatic Ring-Opening Copolymerization of ϵ -CL and β -Lactam

ratios 25:75, 50:50, and 75:25 of ϵ -CL/ β -lactam are performed in order to determine differences in structure of the copolymer. Homopolymers of poly(ϵ -CL) and poly(β -lactam) are also synthesized as a reference for the copolymers. All the reactions are carried out enzymatically using CAL-B as catalyst.

EXPERIMENTAL METHODS

Materials. Lipase acrylic resin from *Candida antarctica* lipase B $\geq$ 5000 U/g (CAL-B commercially available as N435), ϵ -caprolactone (ϵ -CL), sodium trifluoroacetate (NaTFA), molecular sieves 4 Å, 2,5-dihydroxybenzoic acid (DHB), deuterium oxide (D_2O), chloroform- d ($CDCl_3$), phosphorus pentoxide (P_2O_5), trifluoroacetic acid- d (TFA- d), and chloroform (CHROMASOLV plus for HPLC) were purchased from Sigma-Aldrich. Cross-linked enzyme aggregates (CLEA), Lipase A from *Candida antarctica* (CLEA CAL-A), formic acid, ethyl acetate, ethanol, and calcium hydride were purchased from Merck. Dithranol was purchased from Fluka. Tetrahydrofuran (THF), methanol, and chloroform were purchased from Acros-Organics. Except for toluene, ϵ -caprolactone, 2-azetidinone (β -propiolactam or β -lactam), all chemicals were used in highest chemical grade and without further purification. Toluene was dried by a solvent purification system (SPS). β -Lactam was purified by Kugelrohr distillation. ϵ -Caprolactone was dried using calcium hydride for 3 days and vacuum distilled. *Fusarium solani pisi* cutinase (Novozym 51032) was kindly provided by Novozymes, Denmark. Immobilization of cutinase by CLEA was reported previously.¹⁵

Procedures of Enzymatic Ring-Opening Copolymerization of ϵ -CL and β -Lactam. A total of 5 mL of dried toluene was added to a mixture of ϵ -CL (1.4 mmol), β -lactam (1.4 mmol), and 0.3 g of dried molecular sieves. The mixture was stirred for 15 min at 90 °C and 100 rpm in a N_2 atmosphere. After 15 min, 50 mg of CAL-B (CAL-B was dried over P_2O_5 at 55 °C in vacuum for 24 h) was added to the

reaction mixture and stirred at 90 °C, 100 rpm for the next 72 h in a N_2 atmosphere. After cooling to room temperature, toluene was removed by rotary evaporation. An aliquot from the residue was analyzed by 1H NMR in $CDCl_3$ for conversion determination. Chloroform (10 mL) was added to the rest of the residue to dissolve the product. In the next step, the product was separated from the enzyme beads by filtration. The filtrate (chloroform fraction) that contains the product was collected. Formic acid (10 mL) was added to the residue, stirred, and filtered. The second filtrate (formic acid fraction), which contains the side reaction product, was collected as well. Subsequently, the chloroform fraction and formic acid fraction were precipitated separately in cold ethyl acetate and centrifugation at 4500 rpm for 20 min was performed to isolate both products. Finally, the products were collected and dried overnight in a vacuum oven at 55 °C.

Other reactions with different feed ratios of ϵ -CL/ β -lactam were also performed. Feed ratios of ϵ -CL/ β -lactam of 75:25 or 25:75 were carried out under the same reaction conditions. The purification process was performed as described for the reaction with 50:50 ratio of ϵ -CL/ β -lactam.

Enzymatic Ring-Opening Polymerization of ϵ -CL. Polymerization of ϵ -CL was performed under the same reaction condition as the synthesis of the copolymer. After 72 h, toluene was removed by rotary evaporation. For purification, chloroform (10 mL) was added to the residue of the reaction mixture and stirred to extract the poly(ϵ -CL). Subsequently, filtration was performed to separate the product from the beads. The next step was the precipitation of poly(ϵ -CL) in cold methanol, where after centrifugation was carried out to isolate poly(ϵ -CL). Finally, poly(ϵ -CL) was collected and dried overnight in a vacuum oven at 55 °C.

Enzymatic Ring-Opening Polymerization of β -Lactam. Polymerization of β -lactam was performed using similar procedures as reported by Schwab and co-workers, which were carried out in toluene at 55 °C for 96 h.¹⁰ Another reaction was also performed at 90 °C for 72 h with the same reaction conditions as the synthesis of the copolymers. For purification of poly(β -lactam), after removal of toluene, ethanol (10 mL) was added to the residue of the reaction mixture and stirred for 15 min, and subsequently filtration was performed. The filtrate (ethanol fraction) contains β -alanine and unreacted β -lactam. Hot water (5 mL) was added to the remaining residue, and then filtered. This first water fraction was collected. Subsequently, cold water (5 mL) was added to the residue and filtered. The filtrate was combined with the first water fraction and then freeze-dried. Finally, poly(β -lactam) was collected and dried overnight in a vacuum oven at 55 °C.

Poly(ϵ -caprolactone-co- β -propiolactam) [Poly(ϵ -CL-co- β -lactam)]. 1H NMR (400 MHz, $CDCl_3$ - d): δ 6.40 (s, H, NH), 4.08 (m, 2H, CH_2), 3.64 (m, 2H, CH_2), 3.50 (m, 2H, CH_2), 2.54 (m, 2H, CH_2), 2.41 (m, 2H, CH_2), 2.32 (m, 2H, CH_2), 2.18 (m, 2H, CH_2), 1.65 (m, 4H, CH_2), 1.37 (m, 2H, CH_2).

Poly(ϵ -caprolactone) [Poly(ϵ -CL)]. 1H NMR (400 MHz, $CDCl_3$ - d): δ 4.06 (t, J = 6.68 Hz, 2H, CH_2), 3.65 (m, 2H, CH_2), 2.31 (t, J = 7.49 Hz, 2H, CH_2), 1.64 (m, 4H, CH_2), 1.38 (m, 2H, CH_2). Melting temperature (T_m) at 52 °C (lit. 57 °C) and glass transition temperature (T_g) at -61 °C (lit. -60 °C).

Poly(β -lactam). 1H NMR (400 MHz, D_2O): δ 3.43 (m, 2H, CH_2), 3.25 (t, J = 6.72 Hz, 2H, CH_2), 2.65 (t, J = 6.68 Hz, 2H, CH_2), 2.41 (m, 2H, CH_2). Melting temperature (T_m) at 320 °C (lit. 324 °C).

Control Reactions for the Enzymatic Copolymerization. Control reactions were performed without the addition of enzyme. The control reactions were carried out using the same equimolar amounts of ϵ -CL and β -lactam as in the ring-opening copolymerization procedures.

INSTRUMENTAL METHODS

Attenuated Total Reflection-Fourier Transform Infrared (ATR FT-IR) measurements were carried out on a Bruker IFS88 FT-IR spectrometer. 1H NMR measurements were performed on a 400 MHz Varian VXR apparatus with $CDCl_3$, D_2O , or TFA- d

as solvents. The signals were referenced to tetramethylsilane ($\delta = 0.00$ ppm). MALDI-ToF MS measurements were performed on a Biosystems Voyager-DE PRO spectrometer, in positive and linear mode, by accelerating the voltage to 20 kV. For sample preparation, 20 mg/mL of 2,5-dihydroxybenzoic acid (DHB) was used as a matrix, and 1 mg/mL of sodium trifluoroacetate as the salt for cationization was mixed with 6–7 mg/mL of poly(β -lactam) or poly(ϵ -CL-co- β -lactam) in acetonitrile/H₂O (50:50) with 0.5% formic acid as the solvent. For the MALDI-ToF measurement of poly(ϵ -CL), 20 mg/mL of dithranol was used as a matrix, 1 mg/mL of sodium trifluoroacetate as the salt for cationization was mixed with 6–7 mg/mL of poly(ϵ -CL) in THF as solvent. Thermal stabilities of the respective polymers were measured by thermogravimetric analysis (TGA) using a Perkin-Elmer TGA7 under nitrogen and with a heating rate of 10 °C/min. The melting points of the respective polymers were measured by differential scanning calorimetry (DSC) using a TA-Instruments Q1000 DSC. The heating rate was 10 °C/min in N₂ atmosphere. For the T_g determination, the copolymer was quenched in liquid N₂ from the melt prior to the DSC measurement. Gel permeation chromatography (GPC) measurements were performed in chloroform at a flow rate of 1.0 mL/min on a Viscotek GPC operated with RI, VS, and LS detectors, using a guard column of PLgel 5 μ m Guard 50 mm, and two columns of PLgel 5 μ m MIXED-C 300 mm (from Agilent Technologies) at 30 °C. Samples were filtered over a 0.45 μ m PTFE filter before injection. Molecular weights were determined based on a universal calibration curve generated from narrow molecular weight polystyrene standards. Wide-angle X-ray diffraction (WAXD) was performed using a Bruker D8 Advance and Cu K α radiation, with a wavelength of $\lambda = 0.154$ nm.

RESULTS AND DISCUSSION

Poly(ϵ -CL-co- β -lactam) was synthesized via enzymatic ring-opening copolymerization of ϵ -CL and β -lactam. The reactions were performed at 90, 80, and 55 °C in toluene. For the reactions at 80 and 55 °C, the conversions were lower compared to the reaction at 90 °C. During the reaction, the product precipitated as a sticky compound. Due to the stickiness, the reaction mixture could not be stirred after 72 h. Other type of enzymes (CLEA cutinase and CLEA CAL-A) were also employed to perform this enzymatic copolymerization; however, they did not catalyze the formation of the copolymer. Therefore, in this study, all reactions were performed at 90 °C in toluene for 72 h using CAL-B as catalyst.

Studies considering the activity and thermal stability of CAL-B have been reported previously.^{10,37} We have demonstrated that CAL-B maintains its activity and shows a good thermal stability after 72 h incubation in toluene. Therefore, in this study, we focus on the synthesis and characterization of poly(ϵ -CL-co- β -lactam).

Control reactions were also performed without addition of CAL-B under the same reaction conditions. No formation of ester or amide bonds was observed, which means that no polymerization reaction occurs without the addition of CAL-B. Consequently, in the ring-opening copolymerization of ϵ -CL and β -lactam with CAL-B, the formation of ester and amide bonds is due to the catalytic activity of CAL-B.

In the ring-opening copolymerization of ϵ -CL and β -lactam, feed ratios were varied between 25:75, 50:50, and 75:25 of ϵ -CL/ β -lactam. The variation of feed ratios between monomer and comonomer were carried out in order to determine

possible differences in the copolymer composition when analyzing its structures. In addition, the homopolymers of poly(ϵ -CL) and poly(β -lactam) were also synthesized via eROP to compare the thermal properties of the copolymers with the respective homopolymers. Conversion and yield of poly(ϵ -CL), poly(β -lactam), and the copolymer are depicted in Table 1.

Table 1. Conversion and Yield from the Synthesis of Poly(ϵ -CL), Poly(β -lactam), and the Copolymer, From the Reaction Carried out at 90 °C for 72 h

feed ratio		conversion (%) ^a		yield (%)
ϵ -CL	β -lactam	ϵ -CL	β -lactam	
0	100			37 ^b
25	75	72	57	12 ^c
50	50	73	90	50 ^c
75	25	78	95	44 ^c
100	0			65

^aResult from duplicated experiments and calculated from ¹H NMR spectra in CDCl₃. ^bHigher yield up to 53% was observed when the reaction was carried at 55 °C for 96 h. ^cResult from the chloroform fraction after purification.

Ring-opening copolymerization of ϵ -CL and β -lactam resulted in a product mixture containing unreacted monomers (ϵ -CL and β -lactam), homopolymers of poly(ϵ -CL) and poly(β -lactam), and the corresponding copolymer, as shown in Scheme 1. Poly(ϵ -CL-co- β -lactam) and poly(ϵ -CL) dissolve in chloroform. Another side product, poly(β -lactam) dissolves in formic acid, whereas poly(ϵ -CL-co- β -lactam) and poly(ϵ -CL) dissolve both in chloroform, the copolymer can be purified from poly(ϵ -CL) by precipitation in cold ethyl acetate, because poly(ϵ -CL) remains dissolved in the latter solvent. In the ¹H NMR spectra of the remaining product after evaporation of the ethyl acetate fraction (Figure S2 in Supporting Information), unreacted monomers, small amounts of copolymer, and poly(ϵ -CL) were clearly observed. A high amount of poly(ϵ -CL) was observed in the ¹H NMR spectrum of the evaporated ethyl acetate fraction from the reaction with a ratio of 75:25 of ϵ -CL/ β -lactam.

As pointed out by Table 1, by varying the feed ratios from 25:75 and 50:50 to 75:25, the conversion of ϵ -CL remains constant around 70%, which means that by increasing the amount of ϵ -CL, the conversion of this monomer also increases. Concerning the conversion of β -lactam, by increasing the feed ratio of this monomer in the reaction mixture, the conversion decreases. For the ϵ -CL/ β -lactam feed ratio of 25:75, the conversion of β -lactam was lower compared to the conversion of ϵ -CL in the reaction with the feed ratio of 75:25 of ϵ -CL/ β -lactam. This is probably caused by the fact that poly(ϵ -CL) is soluble in toluene, while poly(β -lactam) and poly(ϵ -CL-co- β -lactam) demonstrate poor solubility in that solvent. Due to the poor solubility of poly(β -lactam) and poly(ϵ -CL-co- β -lactam) in toluene, precipitation from the reaction mixture occurred and the growth of the polymer chain consequently stopped. With increasing time, CAL-B also aggregated

with the poly(ϵ -CL-co- β -lactam) and poly(β -lactam) due to the sticky precipitate. In the reaction of 25:75 ϵ -CL/ β -lactam, a higher concentration of β -lactam compared to ϵ -CL leads to faster precipitation of poly(β -lactam) and poly(ϵ -CL-co- β -lactam) together with CAL-B. Therefore, when CAL-B

aggregates with the product in the reaction mixture, the polymerization reaction stopped and as a consequence we observe low monomer conversion.

ATR-FTIR spectra of the products resulting from the ring-opening copolymerization of ϵ -CL and β -lactam are depicted in Figure 1. By comparing the spectra of poly(β -lactam) and

Figure 1. ATR-FTIR spectra of (a) product that dissolves in formic acid from reaction with 50:50 ratio of ϵ -CL and β -lactam, (b) product that dissolves in chloroform from reaction with 50:50 ratio of ϵ -CL and β -lactam, (c) poly(β -lactam), and (d) poly(ϵ -CL).

poly(ϵ -CL) (c and d, respectively) with the spectra of the products (a and b), it clearly demonstrates that in spectrum (b), there is a combination of both ester (at 1727 cm^{-1}) and amide bands (at 1634 and 1546 cm^{-1}) belonging to poly(ϵ -CL) and poly(β -lactam), respectively. In spectrum (a), the peaks corresponding to the amide have a higher intensity than the peak attributed to the ester. The product with more amide bonds dissolves in formic acid and contains more homopolymer of poly(β -lactam) and copolymer with longer oligo(β -lactam) segments. In spectrum (b) in Figure 1, the product displays a combination of ester and amide signals with a high intensity and can be dissolved in chloroform.

Further structure analysis of the product that dissolves in chloroform (from the reaction with 50:50 feed ratio of ϵ -CL/ β -lactam) was performed with 2D COSY NMR. From the COSY NMR spectrum, presented in Figure 2, we propose a structure for the product which is a copolymer composed of alternating repeating units composed of hexanoate and β -alanine. It was reported previously that lipase-catalyzed copolymerization of lactone and comonomers lead to the formation of random, block like, and nonrandom copolymers.^{8,20} However, in our case, the resulted copolymer showed a high fraction of alternating copolymer, due to the 1:1 ratio of ester and amide bonds in the copolymer. Upcoming results from MALDI-ToF and TGA analysis further support the observed alternating structure.

^1H NMR analysis of the copolymers obtained from the different feed ratios of ϵ -CL/ β -lactam is shown in Figure 3. The same peak ratios of amide versus ester bonds were observed for the copolymer resulted from the reactions with feed ratios 25:75 and 50:50 of ϵ -CL/ β -lactam. On the other hand, by increasing ϵ -CL in the reaction mixture (for 75:25 of ϵ -CL/ β -lactam), the copolymer backbone contains twice as much ester

Figure 2. 2D COSY NMR spectrum of poly(ϵ -CL-co- β -lactam) in CDCl_3 .

Figure 3. ^1H NMR spectra of copolymer obtained from the reactions with different feed ratios of ϵ -CL/ β -lactam (a) 25:75, (b) 50:50, and (c) 75:25.

bonds compared to amide bonds. The observed equal peak ratio of amide and ester bonds for the feed ratios 25:75 and 50:50 of ϵ -CL/ β -lactam is probably caused by the fact that copolymers that contain more amide bonds cannot dissolve in chloroform, but only in formic acid. Because we extracted the copolymer with chloroform, the product that contains a high extent of amide segments was not extracted. Therefore, the ^1H NMR spectrum of the copolymer from the reaction with 25:75 of ϵ -CL/ β -lactam only demonstrated a 1:1 ratio of ester/amide bonds.

Structure analysis of the homopolymers and copolymers was carried out by MALDI-ToF MS. Two different structures for poly(ϵ -CL) and poly(β -lactam) were detected by MALDI-ToF MS, which are cyclic and linear, as shown in Figures S5 and S6 in the Supporting Information. For the MALDI-ToF measurements of both homopolymers, the addition of a cationization agent was crucial to obtain decent spectra. For poly(ϵ -CL), the most abundant structure was the cyclic form, while poly(β -lactam) on the other hand showed the linear form as the most abundant structure. For the synthesis of poly(ϵ -CL), diluted solutions of 1.4 mmol in 5 mL (0.28 M) were prepared, which supported the formation of the cyclic structure. As reported in

our previous study,³⁷ CAL-B is able to catalyze the formation of cyclic lactam via intramolecular reactions in a diluted system. Due to the diluted conditions, CAL-B catalyzed the intramolecular reactions, which resulted in the cyclic poly(ϵ -CL).

From the obtained MALDI-ToF spectra, the maximum degree of polymerization ($DP_{\max}$) can be determined. The $DP_{\max}$ for poly(ϵ -CL) was 78 and the most abundant degree of polymerization ($\overline{DP}$) was 29. Dithranol as matrix agent and THF as solvent were used. For poly(β -lactam), the $DP_{\max}$ showed values up to 40 and the $\overline{DP}$ was 12 (as shown in the MALDI-ToF spectra in Figure S6 in the Supporting Information). While the same method of β -lactam eROP was performed as reported previously ($DP_{\max} = 18$ and $\overline{DP} = 8$),¹⁰ we found a slightly higher DP in our recent study. This is due to the use of a different matrix (DHB) and the addition of salt (NaTFA) as cationization agent for the MALDI-ToF MS measurements. As a consequence, longer chains of poly(β -lactam) could be detected by MALDI-ToF MS.

Structure analysis of side product dissolved in formic acid was carried out by MALDI-ToF MS as well. From the MALDI-ToF spectrum (see Figure S7 in the Supporting Information), the side product contains a high fraction of poly(β -lactam). In the MALDI-ToF spectrum of the copolymer, the addition of a cationization agent is possible, as depicted in Figure S8 in the Supporting Information. However, both with and without addition of a cationization agent, decent spectra were observed. The MALDI-ToF spectrum shown in Figure 4 was collected

Figure 4. MALDI-ToF spectrum of the copolymer resulted from the reaction with 50:50 ratio of ϵ -CL/ β -lactam.

without addition of a cationization agent. From this spectrum, the end group of the copolymer can be determined, and corresponds to $M = x + (n \times \text{mass of repeating unit}) + H^+$, where x is the mass of the end groups, n is the degree of polymerization, and the mass of the repeating unit of copolymer is 185.12. In Table S1 Supporting Information, the nine different end groups that were found are summarized. For example, the total mass for a copolymer with $n = 6$ and a hydroxyl-acid end group ($x = 18$) is 1129.72 Da, and in the spectrum of Figure 4 we found a signal at 1129.47 Da. Poly(ϵ -CL-*co*- β -lactam) had a $DP_{\max}$ of 12 and the most abundant degree of polymerization $\overline{DP}$ was 6.

Analysis of the MALDI-ToF spectra indicated that the ring-opening copolymerization of ϵ -CL and β -lactam resulted in the formation of a mixture of copolymers with alternating repeating

units and a random copolymer which is composed of alternating repeating units with oligo(ϵ -CL) and oligo(β -lactam) segments attached to the repeating units. Structure analysis of the MALDI-ToF spectra implicated cyclic and linear structures with nine different end groups, as displayed in Table S1 Supporting Information. Furthermore, it was shown that the alternating copolymer was the most abundant structure. These results are in good agreement with the 1H NMR analysis which shows a 1:1 ratio of amide and ester bonds. Nevertheless, the formation of random copolymers was observed as well. It has been suggested that the formation of a random chain structure is caused by CAL-B that catalyzes *trans*-acylation reactions.³⁸ Consequently, the reaction also occurred between the alternating repeating units already formed and the acyl-enzyme complex of the monomers (ϵ -CL or β -lactam), oligo(ϵ -CL), or oligo(β -lactam).

By combining the mechanisms of eROP of ϵ -CL^{27,28,38} and β -lactam,^{11,35} a proposed reaction mechanism of the copolymerization of ϵ -CL and β -lactam is summarized in Scheme S1 in the Supporting Information. The mechanism starts with the formation of acyl-enzyme complex and activation of monomers using water, continues with propagation, and subsequently leads to the formation of four probable species ((1), (2), (3), or (4)). The alternating structure will be formed when the propagation species (2) or (3) reacts further with the respective other monomers. For instance, when species (2) is the acyl-enzyme complex of β -lactam reacts with the activated monomer of ϵ -CL, as shown in Scheme S1, Supporting Information. However, other types of structures can also be formed when CAL-B activates the formation of acyl-enzyme complex from oligo(ϵ -CL) or oligo(β -lactam) and further reacted with species (1), (2), (3), or (4). As a result, poly(ϵ -CL-*co*- β -lactam) demonstrated at least nine different structures.

Feed ratios of 25:75 and 75:25 ϵ -CL/ β -lactam resulted in a different distribution of alternating and random copolymers. For the enzymatic ring-opening copolymerization with a feed ratio of 25:75 ϵ -CL/ β -lactam, a random copolymer structure composed of alternating repeating units with oligo(β -lactam) segments attached to the alternating repeating units was most abundant. For copolymerization with 75:25 ϵ -CL/ β -lactam, the most abundant species was a random copolymer consisting of alternating repeating units with oligo(ϵ -CL) segments attached to it, as derived from Figures S9 and S10 of the Supporting Information. This result was in good agreement with 1H NMR data. The ratio of ester/amide bonds in 1H NMR was determined to be 2:1 for the copolymer when an initial feed ratio of 75:25 ϵ -CL/ β -lactam was used (see Figure 3).

Molecular weight analysis of the product that dissolved in chloroform was performed by GPC. The results are summarized in Table 2. The highest molecular weight was

Table 2. Molecular Weight of Poly(ϵ -CL-*co*- β -lactam) and Poly(ϵ -CL)

feed ratio		M_n^a (g/mol)	M_w^a (g/mol)	PDI ^a (M_w/M_n)
ϵ -CL	β -lactam			
25	75	557	1773	3.18
50	50	1329	3659	2.75
75	25	1707	5242	3.04
100	0	7293	9565	1.31

^aMolecular weight determination was performed by GPC in chloroform. Therefore, no poly(β -lactam) is presented in the table.

observed for the poly(ϵ -CL) homopolymer. The copolymerization resulted in low molecular weight products and a high polydispersity index (PDI). The poor solubility of the copolymer during the reaction in toluene caused the low chain length of the final copolymer. The copolymer resulting from the reaction with feed ratio 75:25 of ϵ -CL/ β -lactam showed a slightly higher molecular weight compared to the copolymers from the reactions with 50:50 and 25:75 of ϵ -CL/ β -lactam. This is due to the increased solubility of the copolymer in toluene when it contains more ϵ -CL units. Considering the reaction performed with a high amount of β -lactam, the copolymer started to precipitate after a certain chain length, and the copolymer, poly(β -lactam), and CAL-B aggregated due to the sticky precipitate. After the aggregation of CAL-B enzyme with the product, the reaction was completely stopped. As a result, a low molecular weight product was observed together with a broad polydispersity.

The thermal stability of poly(ϵ -CL), poly(β -lactam), and poly(ϵ -CL-co- β -lactam) is investigated by TGA measurements, presented in Figure 5. The degradation temperature for the

Figure 5. TGA curves of poly(ϵ -CL), poly(β -lactam), and poly(ϵ -CL-co- β -lactam) resulted from the reaction with a feed ratio of 50:50 ϵ -CL/ β -lactam.

copolymer is the intermediate of its homopolymers, around 330 °C. Contrary to the parent homopolymers, the TGA curve of the copolymer clearly demonstrates one degradation step, which eliminates the probability of a block structure. When a block structure was present in the copolymer, we would have observed two or more degradation steps; but instead we only observed one degradation step. This result is in good agreement with ^1H NMR and MALDI-ToF data presented above, which indicated a 1:1 ratio of ester:amide bonds and an alternating structure.

The thermal properties of the homopolymers and copolymer were further analyzed by DSC measurements. DSC curves of poly(ϵ -CL) and poly(β -lactam) homopolymers are displayed in Figures S11 and S12 in the Supporting Information. Poly(ϵ -CL) presents a distinct melting temperature at 52 °C and a glass transition temperature (T_g) of -61 °C, which clearly indicates that poly(ϵ -CL) is a semicrystalline polymer. For poly(β -lactam), the melting temperature was observed to be very high around 320 °C. Above 300 °C, poly(β -lactam) not only starts to melt but also starts to decompose. Consequently,

in the second heating scan we cannot observe a melting temperature for poly(β -lactam) anymore.

DSC curves of the copolymer without quenching and after quenching are shown in Figure 6a,b. At least two melting temperatures can be observed in both quenched and non-quenched curves. In addition, the glass transition temperature (T_g) is clearly present in the samples treated without (at -8 °C) and with quenching (at -13 °C). The observed difference of 5 °C is caused by the difference in crystallinity. For the quenched sample, two cold crystallization exotherms were visibly in the first heating scan (at 29 and 74 °C). These two crystallization peaks indicate that complete crystallization can be suppressed using quenching. After quenching, the sample is more amorphous and during heating above its T_g the amorphous part can recrystallize.³⁹

In Figure 6a,b, at least two melting endotherms are observed. The double melting behavior is most probably due to partial recrystallization during heating. After a partial heating scan, in which the sample was heated up to in between the two melting peaks and subsequently cooled and reheated, only one larger melting peak together with a broad shoulder on the position of the original lower temperature melting peak was observed, as shown in Figure 6d. Moreover, a larger heat of fusion was determined after the partial scan (~ 37.65 J/g) compared to the total value of both peaks before the partial scan (32.40 J/g), as demonstrated in Figure 6a,d. The single melting temperature after the partial scan is equal to the temperature of the highest temperature peak before the partial scan.

By performing the partial heating scan, two different T_g values were observed, as shown in Figure 6c,d, around -15 and 50 °C. These two glass transitions are causing the two cold crystallization exotherms (at 29 and 74 °C), since cold crystallization occurs during heating above the T_g . The two T_g s are also an indication for strong phase separation, because the copolymer consisted of an alternating sequence and an oligomer (oligo(β -lactam) or oligo(ϵ -CL)) sequence attached to that alternating part, which may crystallize separately.

XRD spectra of poly(ϵ -CL), poly(β -lactam), and of poly(ϵ -CL-co- β -lactam) are depicted in Figure 7. From the XRD profiles, it was clearly demonstrated that the poly(ϵ -CL) and poly(β -lactam) homopolymers are both highly crystalline materials. Furthermore, the copolymer of ϵ -CL and β -lactam is found to be a semicrystalline polymer. These results are in good agreement with the previously discussed DSC results, since we observed a clear melting temperature for the homopolymers and copolymer and glass transition temperatures for both poly(ϵ -CL) and the copolymer.

CONCLUSION

Poly(ϵ -CL-co- β -lactam) was synthesized by enzymatic ring-opening copolymerization of ϵ -CL and β -lactam using CAL-B as catalyst. The homopolymers of poly(ϵ -CL) and poly(β -lactam) were obtained as side products during the copolymerization. Poly(ϵ -CL-co- β -lactam) was detected as alternating and random copolymer. The random copolymer contains alternating repeating units with short oligo(ϵ -CL) or oligo(β -lactam) segments. By varying the ϵ -CL/ β -lactam feed ratios of 75:25, 50:50, and 25:75, the alternating and random copolymer could be observed in different distributions. Alternating copolymer is observed as the most abundant structure in poly(ϵ -CL-co- β -lactam), resulting from copolymerization with an ϵ -CL/ β -lactam feed ratio of 50:50. The random copolymer contains short oligo(ϵ -CL) attached to alternating repeating

Figure 6. DSC curves of poly(ϵ -CL-co- β -lactam) resulting from the reaction with a feed ratio of 50:50 ϵ -CL/ β -lactam: (a) without quenching, (b) with quenching, (c) first partial heating scan, and (d) second heating scan.

Figure 7. XRD spectra of (a) poly(ϵ -CL-co- β -lactam) resulted from the reaction with feed ratio of 50:50 ϵ -CL/ β -lactam, (b) poly(β -lactam), and (c) poly(ϵ -CL).

units as the dominant structure, and is observed in the product of the copolymerization of 75:25 ϵ -CL/ β -lactam. Furthermore, the product resulting from the copolymerization with ϵ -CL/ β -lactam feed ratio of 25:75 is a random copolymer that contains short oligo(β -lactam) attached to alternating repeating units as the most dominant structure.

Yields up to 50% can be achieved for the copolymerization of 50:50 ϵ -CL/ β -lactam. Increasing amount of either ϵ -CL or β -lactam in the reaction mixture does not increase the yield. The

highest DP_{max} of 12 for poly(ϵ -CL-co- β -lactam) resulted from the copolymerization of 50:50 ϵ -CL/ β -lactam and showed nine different structures.

By comparing thermal properties of the copolymer with its homopolymers, it was demonstrated that the degradation temperature of poly(ϵ -CL) > poly(ϵ -CL-co- β -lactam) > poly(β -lactam). Considering the melting temperatures, poly(β -lactam) > poly(ϵ -CL-co- β -lactam) > poly(ϵ -CL). Poly(ϵ -CL-co- β -lactam) is a semicrystalline copolymer with T_g s at -15 and 50 °C and T_m at 124 °C. Cold crystallization could be observed at 29 and 74 °C.

■ ASSOCIATED CONTENT

Supporting Information

Percent yield calculation, 1H NMR spectra, MALDI-ToF MS spectra, and table information of structures of copolymer from different variation of feed ratios of ϵ -CL and β -lactam, 1H NMR spectra, MALDI-ToF MS spectra, and DSC curves of poly(ϵ -CL) and poly(β -lactam). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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