Polymersome Nanoreactors for Enzymatic Ring-Opening Polymerization

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Polystyrene-polyisocyanopeptide (PS-PIAT) polymersomes containing CALB in two different locations, one in the aqueous inner compartment and one in the bilayer, were investigated for enzymatic ring-opening polymerization of lactones in water. It is shown that the monomers 8-octanolactone and dodecalactone yield oligomers with this polymersome system. It is also observed that the polymerization activity is dependent on the position of the enzyme in the polymersome. SEM investigations show that the polymersome structures were destabilized during the polymerization. Further investigations show that the vesicular morphology of the polymersomes was destabilized only in the case of polymer product formation.

Introduction

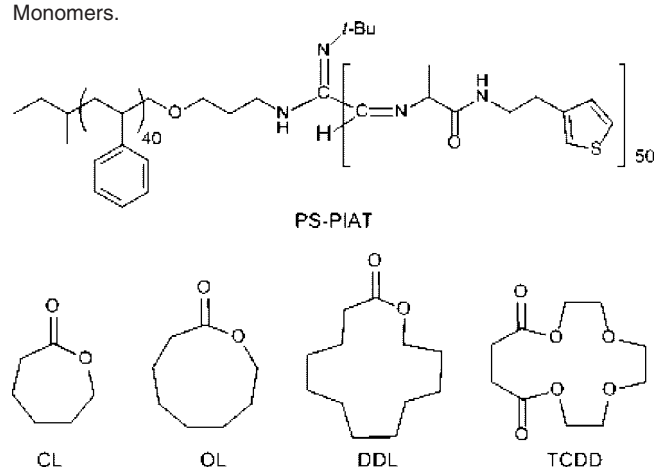
In recent years, in vitro enzymatic polymerization has received much attention as a selective and mild alternative route for polymer synthesis.[1] In particular, ring-opening polymerization (ROP) of lactones catalyzed by lipases to give high-molecular-weight polyesters has been extensively studied by several groups.[2–7] In most of the cases, the Lipase B of *Candida antarctica* (CALB) immobilized on an acrylic resin, commercially available as Novozym 435, has been used. Lipase-catalyzed esterification, transesterification, and polymerization have been performed in organic solvents, because hydrolysis will be the preferential reaction in the presence of water.[8] To our knowledge, there are very few reports in the literature that describe the lipase-catalyzed ring-opening polymerization in aqueous medium.[9,10]

In nature, living organisms are constantly producing different macromolecules in an aqueous environment. These macromolecules, such as polynucleotides and proteins, are essential for their survival.[1] Living organisms are capable of performing these complex biocatalytic polymerizations in a very efficient and selective manner. To achieve this, nature applies the concept of compartmentalization. With this concept, multistep reactions can be performed in specific environments within the cell.

Lately, the self-assembly of block copolymers into vesicular compartments (also known as polymersomes) has attracted increased attention.[11,12] In our laboratories, we have developed polystyrene-polyisocyanopeptide (PS-PIAT) block copolymers (Chart 1) that form stable polymersomes.[13] Both in the aqueous core and in the bilayer of these aggregates, enzymes were incorporated.[14] The encapsulated enzymes were shown to be still catalytically active, and due to the porous structure of the PS-PIAT polymersome, substrate and product could freely diffuse in and out of the inner aqueous compartment.

To mimic naturally occurring enzymatic polymerization using the idea of compartmentalization, we decided to study the ability of these enzyme-filled polymersomes to synthesize polymers and oligomers. In this paper, we present for the first time the enzymatic ring-opening polymerization of lactones in polymersomes in aqueous medium.[9,10] Although these conditions normally would favor hydrolysis of the lactone into the hydroxy acid, we anticipated that the compartmentalization of CALB can change the polymerization characteristics and possibly result in a shift of the equilibrium toward polymers or oligomers. To test this, we have incorporated CALB in the bilayer and in the aqueous core of PS-PIAT polymersomes and have investigated the effect of the position of the enzyme in either the hydrophobic or aqueous environment on lactone ring-opening polymerization (Figure 1).

Chart 1. Chemical Structures of PS-PIAT and the Lactone Monomers.



The importance of the lactone ring size, and hence its polarity, was investigated by studying polar lactones such as ϵ-caprolactone (CL) and the polar cyclic diester 4,7,10-tetraoxacyclotetradecane-11,14-dione (TCDD), medium polar 8-octanolactone (OL), and apolar 12-dodecanolactone (DDL) (Chart 1).

Materials and Methods

Candida antarctica. Lipase (CALB) was purchased from Fluka. Polystyrene-polyisocyanopeptide (PS-PIAT) diblock copolymer was

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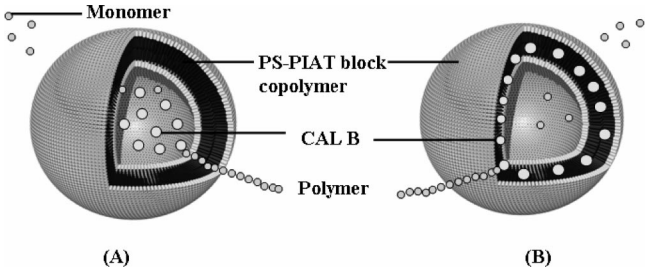

Figure 1. Schematic representation of enzymatic polymerization in polymersomes. (A) CALB in the aqueous compartment (B) CALB embedded in the bilayer.

synthesized as described earlier.[13] 6,8-Difluoro-4-methylumbelliferyl (DiFMU) octanoate was purchased from Molecular Probes. The monomers 1,4,7,10-tetraoxacyclotetradecane-11,14-dione (TCDD) and 8-octanolactone (OL) were synthesized according to previously published methods.[15,16] ϵ-Caprolactone (CL) and 12-dodecanolactone (DDL) were purchased from Aldrich.

Analytical Methods. Analysis of the polymer samples was carried out by gel permeation chromatography (GPC) using a Shimadzu apparatus with a refractive index (RI) detector. The samples were dissolved in chloroform and injected onto a RESIPORE column with a flow rate of 1 mL/min. The calibration was performed with polystyrene standards.

MALDI-ToF mass spectra were recorded with an Applied Biosystems Voyager System 6020 both in linear and reflector modes. To remove the monomer present in the samples, a preparative GPC purification was carried out using the same setup as described above. The GPC system was flushed thoroughly with fresh chloroform before injecting the samples. For fractionating of the samples, an eluent flow rate of 1 mL/min was maintained. The samples were collected after the RI detector. The elution volume ($\sim$2.5 mL) was collected in a vial from the appearance of polymer peak until the beginning of monomer peak. The fractionated samples were further concentrated to $\sim$0.2 mL by drying with helium. α-Cyano-4-hydroxycinnamic acid (CHCA) was used as a matrix.[17]

Fluorescent product formation from DiFMU octanoate was measured using a Wallac Victor 1420 multilabel counter.

For TEM and SEM imaging, JEOL JEM-1010 and JEOL JSM-6630F were used respectively. Samples for TEM were prepared by drying a drop of the polymersome suspension on a carbon-coated copper grid and blotting away the excess liquid with a filter paper. SEM samples were prepared in the same manner, but the sample was coated by sputtering a 1.5 nm layer of Pd/Au with a Cressington 208 HR sputter coater fitted with a Cressington thickness controller.

Encapsulation of CALB in PS-PIAT Polymersomes. *(a) Encapsulation in the Aqueous Core of Polymersomes.* For encapsulating enzymes inside the polymersomes, the following procedure was followed: 0.5 mL of a solution of 1 mg/ml PS-PIAT in THF was added dropwise to a solution of 2.5 mL of CALB (final concentration 0.16 mg/ml) in MilliQ H_2O. After 28 h of equilibration, the suspension was filtered 8 times to remove the nonencapsulated enzymes using Amicon Ultra Free-MC centrifugal filters (0.1 μm filter). After filtration, the suspension of PS-PIAT polymersomes was adjusted back to 3 mL with MilliQ H_2O.

b) Encapsulation in the Bilayer of Polymersomes. For encapsulating enzymes in the bilayer of the polymersomes, the following procedure was followed: 1 mL of a solution of 1 mg/ml PS-PIAT in THF was added to a solution of 0.2 mL of 2.5 mg/ml CALB in MilliQ H_2O. The above mixture was lyophilized in a vacuum lyophilizer. The lyophilized product was resuspended in 0.5 mL of THF and added dropwise to 2.5 mL of MilliQ H_2O. After equilibrating the mixture for 5 h, the nonencapsulated enzymes were removed as described above, and the suspension of PS-PIAT polymersomes was adjusted back to 3 mL with MilliQ H_2O.

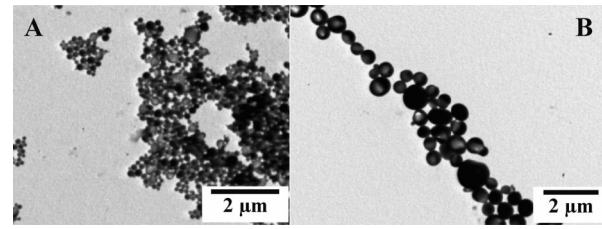

Figure 2. TEM image of PS-PIAT polymersomes: (A) CALB in the aqueous core of polymersomes, (B) CALB in the bilayer of polymersomes.

The structure of the polymersomes with CALB encapsulated in the inner aqueous compartment and in the bilayer was characterized by SEM and TEM.

Characterization of Enzyme-Encapsulated PS-PIAT Polymersomes. *Functionality Test of CALB in Polymersomes.* DiFMU octanoate was used as a substrate to check the activity of CALB inside polymersomes.[13] In a typical reaction assay, 100 μL of PS-PIAT polymersome suspension with and without CALB was taken and 2 μL of a 1 mM DiFMU octanoate stock solution in DMSO was added. The reaction assays were performed in a 96 well plate. Fluorescence product formation after hydrolysis was monitored (λ_{ex}: 355 nm/λ_{em}: 460 nm). The assay was performed for both CALB encapsulated in the aqueous core and in the bilayer of polymersomes.

Ring-Opening Polymerization of Lactones with CALB Polymersomes. 8-Octanolactone (48 mg, 0.365 mmol) and 0.5 mL of polymersome solution were added into a glass tube that was closed with a stopper. Similar reaction mixtures were made with 52 mg of 12-dodecanolactone (0.242 mmol), 55.3 mg of ϵ-caprolactone (0.464 mmol), and 42 mg of 1,4,7,10-tetraoxacyclotetradecane-11,14-dione (TCDD) (0.181 mmol). Control reactions were performed using CALB suspended in MilliQ H_2O. The reaction mixtures were kept at room temperature, and the reactions were allowed to proceed for 1, 3, and 6 days. For extracting the polymer, 1 mL of chloroform with 0.1 mL of anisole (as an internal standard) was added to the reaction mixture, which was shaken well, after which 0.5 mL of chloroform phase was collected. The polymerization product was analyzed by GPC, MALDI ToF mass spectrometry, and GC-MS.

Results and Discussion

Characterization of PS-PIAT Polymersomes Containing CALB. The procedures for encapsulation of enzymes in PS-PIAT polymersomes is well established in our laboratories.[14,18] Following our previously described protocols (see Materials and Methods Section).[14,18] CALB was encapsulated in PS-PIAT polymersomes at two different locations: one in the aqueous core of the polymersomes and one in their bilayers. For encapsulating CALB in the aqueous core of the polymersomes, the PS-PIAT in THF was injected into the enzymatic solution. To immobilize the CALB in the bilayer of the polymersomes, the solution of PS-PIAT in THF was mixed with the enzymatic solution and lyophilized. The lyophilized mixture was then redissolved in THF and injected into MilliQ H_2O. In the aqueous core, the enzymes remain more or less in their natural aqueous environment, whereas in the bilayer, they are immobilized in a more hydrophobic surrounding. The enzyme-containing polymersomes were visualized using transmission (TEM) and scanning (SEM) electron microscopy. Representative SEM and TEM images for CALB in the water pool, or lumen (Figures 2A and 3A), of the polymersomes and CALB in the bilayer (Figures 2B and 3B) are given in Figures 2 and 3, respectively. The particle size distribution of the former aggregates was more monodisperse (Figures 2A and 3A) than the particle size distribution of the aggregates in which CALB was embedded

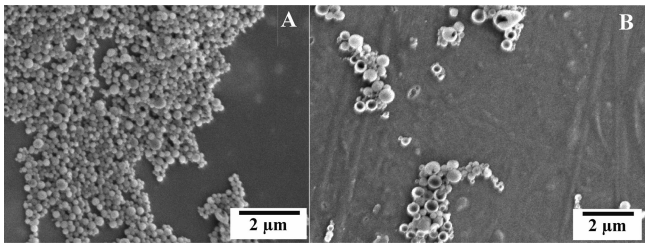

Figure 3. SEM image of PS-PIAT polymersomes: (A) CALB in the aqueous core of polymersomes, (B) CALB in the bilayer of polymersomes.

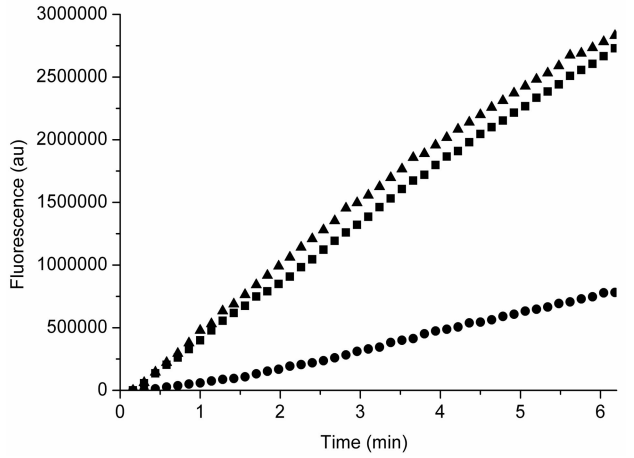

Figure 4. DiFMU conversion by CALB entrapped within PS-PIAT polymersomes, (■) CALB in the inner aqueous compartment of the polymersomes, (●) CALB in the bilayer of the polymersomes, and by free CALB (▲).

in the bilayer. The latter polymersomes were also slightly larger in diameter (Figures 2B and 3B).[19]

These differences in size are a result of the presence of the enzyme in the bilayer. It is known that there are several factors that influence the self-assembly behavior and morphology of these polymersomes,[20] and the effect of the inclusion of proteins on the polymersome morphology has been observed before.[18]

Activity of CALB in the Polymersomes. To evaluate the activity of CALB after encapsulation in the polymersomes, the hydrolysis of DiFMU octanoate into a fluorescent DiFMU product (λ_{ex} 355 nm/ λ_{em} 460 nm) was investigated (Figure 4).[13] From Figure 4, it is evident that the enzymes inside the polymersomes are active. To exclude the possibility that this activity originated from nonencapsulated enzyme, as a control, filtrates after washing 8 times were also measured for DiFMU conversion activities. It was observed that in this case no hydrolysis of DiFMU octanoate took place. As reported earlier, we may conclude therefore that DiFMU is only converted due to the presence of CALB encapsulated either inside or in the membrane of the polymersomes.[18] To compare the DiFMU conversion activities of free enzyme with the enzyme encapsulated inside polymersomes, a 10 times diluted solution of CALB (initial concentration; 0.16 mg/ml) was prepared based on estimations previously reported[14] and its activity was studied.

Figure 4 shows that there is a clear difference in activity when CALB is located at the two different positions in the polymersomes, with CALB in the water pool being more active than CALB in the bilayer, although the same initial concentrations of CALB were used for encapsulation. This difference may have several reasons. It could be that the amount of enzyme trapped in the water pool is higher than the amount encapsulated in the bilayer. This seems to be unlikely because the procedure for encapsulating CALB in the bilayer should even result in a higher efficiency of encapsulation.

A second explanation might be that the resuspension of the lyophilized product in THF compromises the stability of the enzyme and therefore its activity.[21] To investigate the effect of THF and lyophilization, 1 mL of THF was added to 0.2 mL of enzymatic solution, followed by lyophilization. After lyophilization, this mixture was resuspended in water and the activity of the enzyme was compared with the enzyme before lyophilizing. No difference was observed between the kinetics of DiFMU conversion (see Supporting Information). A third plausible explanation for the slow rate of product formation in the case of CALB entrapped in the bilayer of the polymersomes may be the restricted access of substrate molecules due to the thickness of the hydrophobic polystyrene layer.[22]

Enzymatic Polymerization in CALB-Containing Polymersomes. The screening of four different monomers, ϵ-caprolactone (CL), 1,4,7,10-tetraoxacyclotetradecane-11,14-dione (TCDD), 8-octanolactone (OL), and 12-dodecanolactone (DDL) for ring-opening polymerization by CALB in the polymersomes was performed at room temperature in a glass tube closed with a stopper (see Materials and Methods Section). CL and TCDD did not yield any polymer, and these monomers, therefore, were not investigated further. The inactivity of these relatively hydrophilic monomers in our reactions suggests that lipase-catalyzed polymerization of lactone monomers is only favorable for monomers that possess certain hydrophobicity, as reported earlier.[10] In the case of OL and DDL, polymerization was observed. Monomers were added to three different samples: (a) polymersomes with CALB in their inner aqueous compartment, (b) polymersomes with CALB in their bilayers, and (c) CALB in MilliQ water. Experiments were performed in such a way that equal amounts of monomers were weighed into different glass tubes together with 0.5 mL aliquots of polymersome suspensions containing CALB at different positions or 0.5 mL of CALB (0.16 mg/ml) in water.

The reaction was stopped by adding chloroform to the mixture after fixed periods of time, and the polymer products were extracted into this solvent. The obtained solution was dried under nitrogen. The dried samples were dissolved in 1 mL of chloroform and filtered using a 0.2 μm filter, after which 20 μL was injected into a GPC to analyze the polymer product formation. The molecular weights of the obtained polymers were calculated based on calibration with polystyrene standards and are shown in Table 1 and Figure 5. To follow the rate of polymer product formation, experiments were performed for 6 days and samples were taken after day 1 and after day 3. In all cases, formation of oligomers was observed. However, there was a difference between the molecular weights of the poly (OL) obtained from the different experiments (Figure 5 A–C, Table 1, entries 1, 2, and 3). Interestingly, the GPC profiles and the molecular weight distribution of the polymer formed after 6 days by the enzyme present in the inner aqueous compartment of the polymersomes and in bulk water was similar for monomer OL (Figure 5B,C, Table 1, entries 1 and 3). For the polymerization using the enzyme in the bilayer, lower molecular weight products of poly (OL) were obtained (Figure 5A, Table 1, entry 2). For DDL, all polymerization experiments gave products with similar molecular weight distributions after day 1, day 3 (Supporting Information), and after 6 days (Figure 5D, Table 1, entries 4, 5, and 6). It should be noted however that precipitation of the oligomeric product occurred during polymerization of DDL.

Table 1. Molecular Weight Distribution of the Polymer Formed by Enzymatic Polymerization of Lactones with Polymersomes Containing CALB in Water[a]

entry	monomers	type of samples	molecular weight		
			day 1	day 3	day 6
1	OL	CALB in the aqueous core of the polymersomes	242–897	261–1212	266–2701
2	OL	CALB in the bilayer of the polymersomes	*b*	245–917	256–917
3	OL	CALB in water	261–1226	264–3303	247–3303
4	DDL	CALB in the aqueous core of the polymersomes	465–1143	475–1143	495–1143
5	DDL	CALB in the bilayer of the polymersomes	460–1143	475–1156	495–1156
6	DDL	CALB in water	460–1143	475–1156	490–1143

[a] All the molecular weights were calculated from the GPC peaks based on polystyrene standards. [b] Not determined.

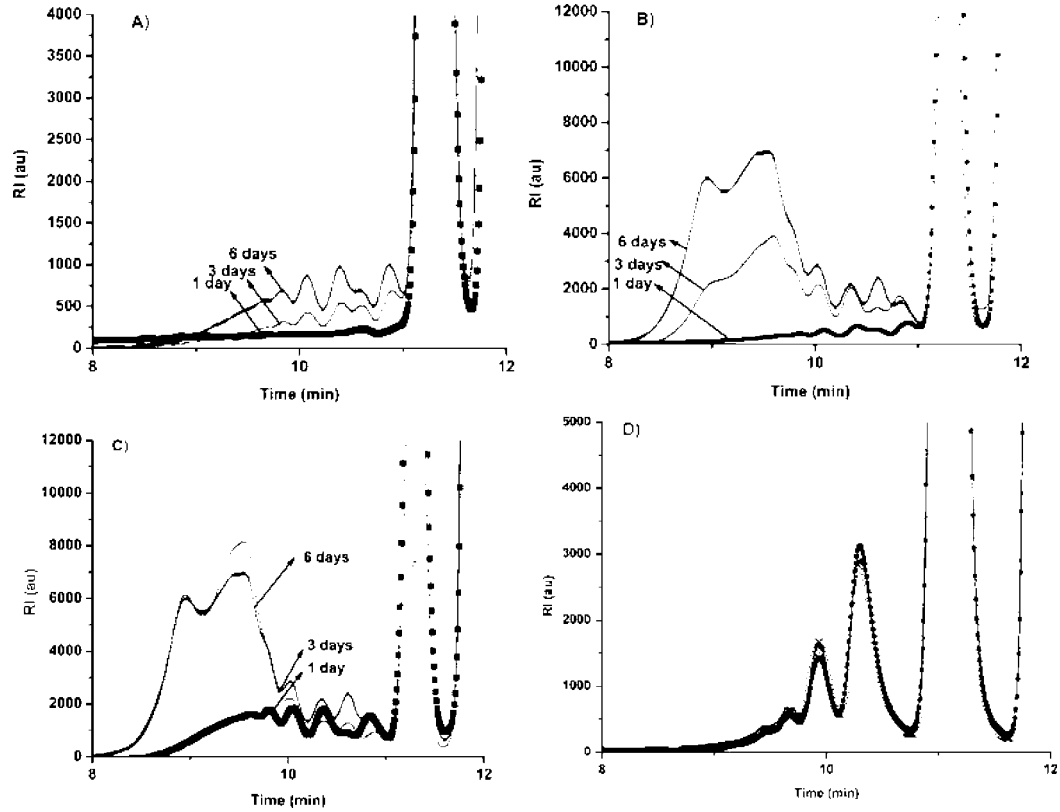

Figure 5. GPC profiles of the polymers formed by enzymatic polymerization of lactones with CALB-containing polymersomes in water. (A) Poly (OL) formed by CALB present in the bilayer of polymersomes. (B) Poly (OL) formed by CALB entrapped in the aqueous compartment of the polymersomes. (C) Poly (OL) formed by CALB in water. (D) Poly (DDL) formed after 6 days by polymersomes containing CALB entrapped in the aqueous compartment (■); CALB present in the bilayer of the polymersomes (—); CALB in water (×). All the peaks are calibrated against polystyrene standards.

To assess the exact molecular weight distribution, fractionated samples of poly (OL) and poly (DDL) isolated after 6 days by preparative GPC were analyzed by MALDI-ToF mass spectrometry (Figure 6). From Figure 6, it is clear that the mass spectrometry results confirm the previous observations made by GPC: the molecular weight distributions of poly (OL) obtained by ring-opening polymerization with CALB in the inner aqueous compartment of the polymersomes and in water are similar. For CALB embedded in the bilayer, the polymerization of OL yields polymer with a lower molecular weight. In the case of poly (DDL), MALDI-ToF mass spectra showed similar molecular weight distributions for polymers prepared by CALB polymersomes or present in water (Supporting Information).

In contrast to a previously published report,[10] our results show that OL can be polymerized by a lipase present in an aqueous medium. Notably, both in our studies and those by Kobayashi and co-workers,[10] the DDL monomer yielded polymeric products. This discrepancy may be explained by the fact that the polymerization behavior of OL is greatly dependent on the origin of the lipase.[23] Our polymerization results furthermore indicate that CALB inside the water pool of a polymersome behaves similarly to CALB in water, which means that the encapsulated enzyme is freely accessible and is not hindered by the polymeric shell. In the case of CALB encapsulated in the bilayer, only lower molecular weight oligoesters can be made, which may be caused by a decreased accessibility of the enzyme active site of the enzyme for longer oligomeric species.

The reason that this effect is not observed for the polymerization of DDL may be due to the fact that precipitation of the polymer occurred. In this case, the molecular weight is determined by the lack of solubility of the polymer and hence the inability of the growing species to react beyond a certain molecular weight. Formation of poly (DDL) with a constant molecular weight are in agreement with earlier reported polymerizations of DDL carried out in bulk at 45 °C.[24]

Morphology of Polymersomes during Enzymatic Polymerization. Enzymatic polymerization in aqueous solutions has been performed at temperatures of 45 °C or above to increase the solubility of the components of the reaction and

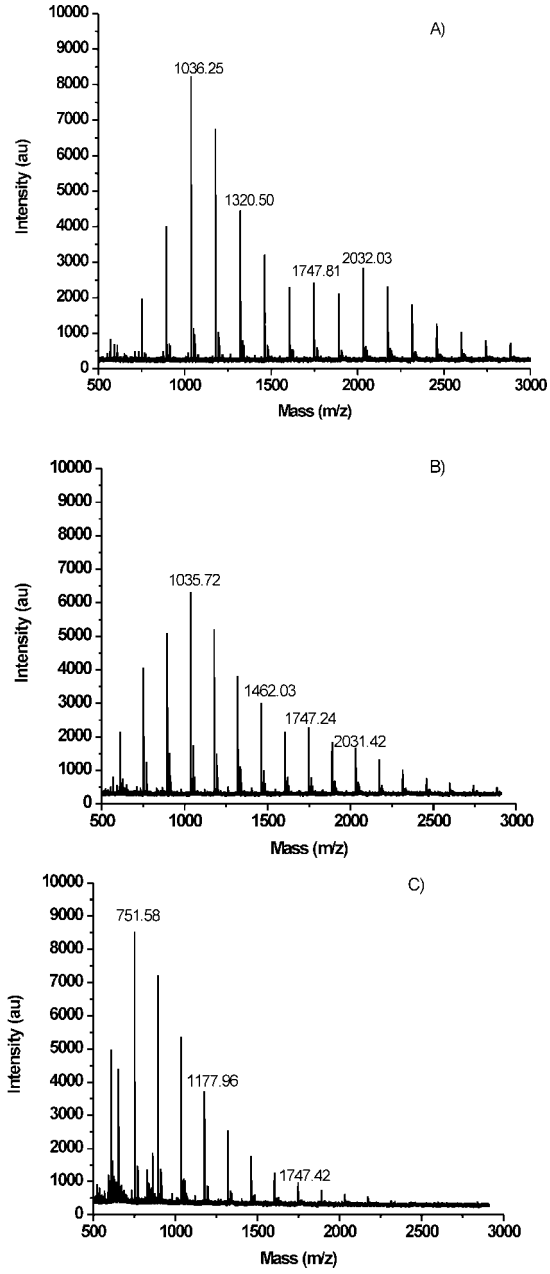

Figure 6. MALDI-ToF mass spectra of poly (OL) catalyzed by CALB in MilliQ water (A), CALB present in the inner aqueous compartment of the polymersomes (B), CALB in the bilayer of polymersomes (C).

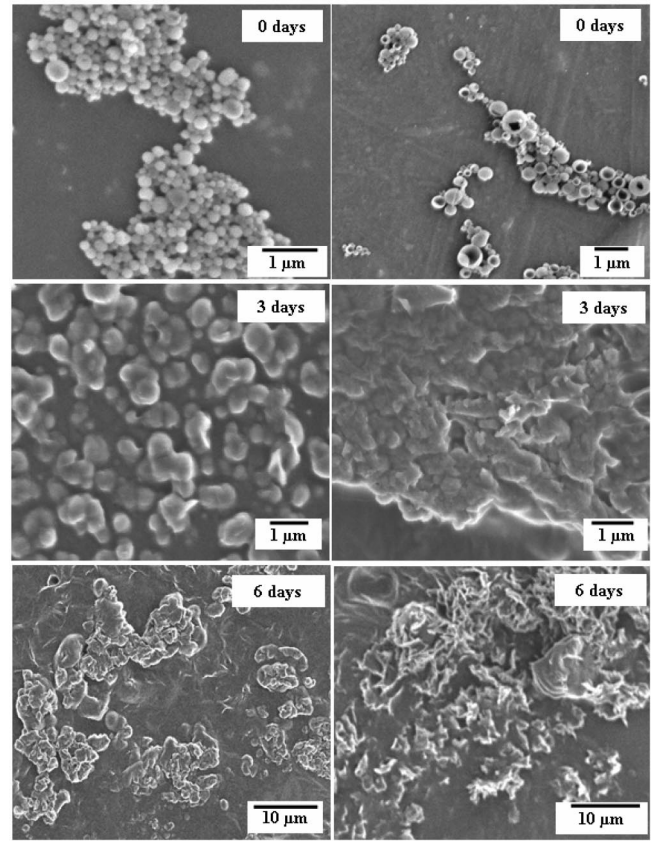

Figure 7. SEM image of PS-PIAT polymersomes with CALB inside and in the membrane before and during enzymatic polymerization. Polymersomes during polymerization of OL by CALB in the inner aqueous compartment of the polymersomes (left), polymersomes during enzymatic polymerization of OL by CALB in the bilayer of the polymersomes (right).

maintain low viscosity.[9,10] We performed the enzymatic polymerizations at room temperature in order to avoid any deformation of the polymersomes as a result of high temperature. From the GPC profile of poly (OL) (Figure 5B), it is evident that a significant increase in product formation was observed for CALB present in the inner aqueous compartment of the polymersomes after three days. This increase indicates that the monomer has easy access to the enzyme, which could be due to the leakage of enzymes from the polymersomes. This observation prompted us to investigate the morphology of the polymersomes during enzymatic ring-opening polymerization in more detail.

The morphology of PS-PIAT polymersomes was studied by SEM. The samples were taken from the crude reaction mixture (see Materials and Methods Section for details). Figure 7 shows SEM images of PS-PIAT polymersomes with CALB in the inner aqueous compartment and in the membrane before and during enzymatic polymerization. We observed that the polymersomes containing CALB were destabilized during the enzymatic polymerization of OL and DDL. In case of OL, the morphological changes were observed after 3 days and the polymersomes were completely deformed after day 6. In the case of DDL, the polymersomes were destabilized completely after 3 days of enzymatic polymerization (Supporting Information). Interestingly, in the case of OL, after deformation of the polymersomes, no significant increase in product formation (Figure 5A) was observed for CALB in the bilayer of the polymersomes, suggesting that CALB was still trapped in the polymer membrane. One possible explanation for the destabilization of these generally regarded tough block copolymer vesicles[25] is that the oligoester formed during the enzymatic polymerization plasticizes the bilayer of polymersomes. It has been reported before that short oligoesters condense in the presence of surfactants to form polyester particles.[9] To investigate this possibility, the polymerization experiments were performed in such a way that the monomers (CL, OL, and DDL) were added to an aqueous CALB solution also containing empty PS-PIAT polymersomes. The polymerization time was kept the same as for the enzymatic polymerization inside the polymersomes. Figure 8 shows SEM images of the empty polymersomes after 6 days of enzymatic polymerization in solution. For the nonreactive monomer (CL), the vesicular morphology of the polymersomes was still present, but for the reactive monomers (OL, DDL), the vesicular morphology of polymersomes changed. To distinguish between polymersome destabilization by the

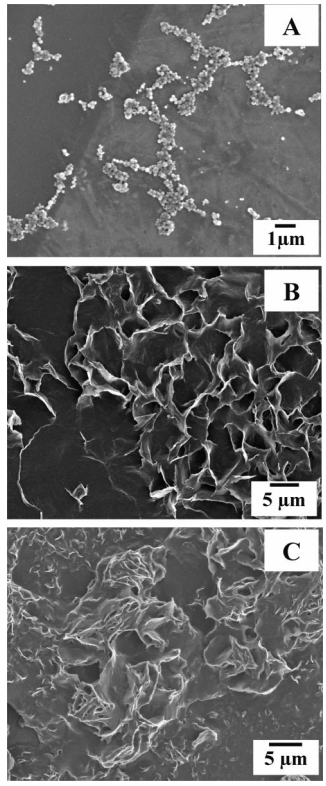

Figure 8. Morphology changes of polymersomes after 6 days of enzymatic polymerization of nonreactive monomer CL (A) with monomer OL (B) and monomer DDL (C).

formed oligomers and destabilization by the added monomers (OL, DDL), the latter compounds were added to polymersomes loaded with a nonreactive protein (BSA). SEM investigations indicated that, in the presence of OL, no polymersome destabilization takes place. In the case of DDL, some fusion could be observed, however, to a much reduced extent compared to experiment with CALB-loaded polymersomes (see Supporting Information). These results suggest that the destabilization of polymersomes during the enzymatic polymerization is much more pronounced than the addition of monomer itself.

Conclusion

In this paper, the enzymatic ring-opening polymerization of a series of lactones catalyzed by CALB encapsulated in either the water pool or the bilayer of PS-PIAT polymersomes was studied. It was demonstrated that oligomer formation was possible for the monomers 8-octanolactone and dodecalactone. Depending on the position of the enzyme, differences in polymerization activity were observed. Whereas the enzyme entrapped in the water pool showed similar reactivity as the free enzyme in aqueous solution, the enzyme located in the bilayer was sterically less accessible and produced shorter oligoester fragments. SEM investigation of the polymersomes before and during the course of enzymatic polymerization showed that the formed oligoesters caused destabilization of the vesicular structure of PS-PIAT. We are currently investigating whether deformation of the polymersomes can be avoided by slightly cross-linking the surface of the polymersomes.

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Supporting Information Available. Activity of CALB was evaluated by DiFMU hydrolysis before and after lyophilizing in the presence of THF. Morphological changes of polymersomes during enzymatic polymerization. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Gross, R. A.; Kumar, A.; Kalra, B. *Chem. Rev.* **2001**, *101*, 2097–2124.
(2) Uyama, H.; Kobayashi, S. *Chem. Lett.* **1993**, 1149–1150.
(3) Kumar, A.; Kalra, B.; Dekhterman, A.; Gross, R. A. *Macromolecules* **2000**, *33*, 6303–6309.
(4) Peeters, J. W.; van Leeuwen, O.; Palmans, A. R. A.; Meijer, E. W. *Macromolecules* **2005**, *38*, 5587–5592.
(5) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793–3818.
(6) Mei, Y.; Kumar, A.; Gross, R. A. *Macromolecules* **2002**, *35*, 5444–5448.
(7) Kumar, A.; Gross, R. A. *Biomacromolecules* **2000**, *1*, 133–138.
(8) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351–3403.
(9) Taden, A.; Antonietti, M.; Landfester, K. *Macromol. Rapid Commun.* **2003**, *24*, 512–516.
(10) Namekawa, S.; Uyama, H.; Kobayashi, S. *Polym. J.* **1998**, *30*, 269–271.
(11) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967–973.
(12) Vriezema, D. M.; Aragones, M. C.; Elemans, J. A. A. W.; Cornelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M. *Chem. Rev.* **2005**, *105*, 1445–1489.
(13) Vriezema, D. M.; Hoogboom, J.; Velonia, K.; Takazawa, K.; Christianen, P. C. M.; Maan, J. C.; Rowan, A. E.; Nolte, R. J. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 772–776.
(14) Vriezema, D. M.; Garcia, P. M. L.; Sancho Oltra, N.; Hatzakis, N. S.; Kuiper, S. M.; Nolte, R. J. M.; Rowan, A. E.; van Hest, J.C.M. *Angew. Chem., Int. Ed.* **2007**, *46*, 7378–7382.
(15) Bachrach, A.; Zilkha, A. *Eur. Polym. J.* **1982**, *18*, 421–425.
(16) van der Mee, L.; Helmich, F.; de Bruijn, R.; Vekemans, J. A. J. M.; Palmans, A. R. A.; Meijer, E. W. *Macromolecules* **2006**, *39*, 5021–5027.
(17) Lou, X. W.; van Buijtenen, J.; Bastiaansen, J. J. A. M.; de Waal, B. F. M.; Langeveld, B. M. W.; van Dongen, J. L. J. *J. Mass Spectrom.* **2005**, *40*, 654–660.
(18) Vriezema, D. M. Polyisocyanide Polymersomes. Ph.D. Thesis, 2005.
(19) van Hest, J. C. M.; Vriezema, D. M.; Garcia, P. M. L.; Cornelissen, J. J. L. M.; Rowan, A. E.; Nolte, R. J. M. *Polym. Prepr.* **2006**, *47*, 238.
(20) Kita-Tokarczyk, K.; Grumelard, J.; Haefele, T.; Meier, W. *Polymer* **2005**, *46*, 3540–3563.
(21) Zaks, A.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 3194–3201.
(22) Opsteen, J. A.; Cornelissen, J. J. L. M.; van Hest, J. C. M. *Pure Appl. Chem.* **2004**, *76*, 1309–1319.
(23) Kobayashi, S.; Uyama, H.; Namekawa, S.; Hayakawa, H. *Macromolecules* **1998**, *31*, 5655–5659.
(24) Uyama, H.; Takeya, K.; Hoshi, N.; Kobayashi, S. *Macromolecules* **1995**, *28*, 7046–7050.
(25) Discher, B. M.; Won, Y. Y.; Ege, D. S.; Lee, J. C. M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143–1146.

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