Enzyme-Catalyzed Ring-Opening Polymerization of 1,3-Dioxan-2-one to Poly(trimethylene carbonate)

Shuichi Matsumura,* Keisuke Tsukada, and Kazunobu Toshima

Faculty of Science and Technology, Keio University, 3-14-1, Hiyoshi, Kohoku-ku, Yokohama 223, Japan

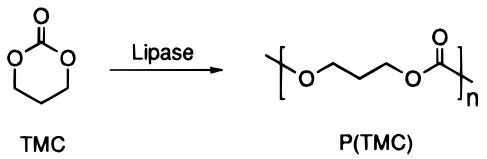
Received December 18, 1996
Revised Manuscript Received March 3, 1997

The introduction of carbonate linkages into a polymer chain will be one way of improving the mechanical properties of biodegradable polyesters, such as poly(hydroxyalkanoate), polycaprolactone, polylactide, and polyglycolide.[1−3] The carbonate linkage in the polymer chain may also be expected to be enzymatically hydrolyzable and more hydrolytically stable than an ester linkage. For the molecular design of biodegradable/bioabsorbable polymers, particularly for medical applications, the copolymerization method of cyclic carbonates with lactones or glycolides will be an effective way to attain a spectrum of properties such as degradation behavior and mechanical performances.

The polymerization of the six-membered cyclic carbonate 1,3-dioxan-2-one was first reported by Carothers et al.[4] They showed that it polymerized upon heating to produce an oligomer having a molecular weight of about 4000.[4] More recently, the ring-opening polymerization of cyclic carbonates has been studied and it was reported that aliphatic six-membered cyclic carbonates could polymerize by both anionic[5] and cationic polymerizations.[3,6] Although anionic polymerization of six-membered cyclic carbonates yielded corresponding linear polycarbonates,[5] the conventional cationic ring-opening polymerization of cyclic carbonates yielded a linear polycarbonate with a small amount of ether linkages formed by the partial elimination of carbon dioxide.[3,6] Also, the copolymerization of cyclic lactones and carbonates needs extremely pure monomers and anhydrous conditions as well as organometallic catalysts, which must be completely removed before use in medical applications. To avoid these difficult restrictions for the ring-opening polymerization of cyclic carbonates by chemical methods, enzyme-catalyzed polymerization may be one of the feasible methods to obtain polycarbonates. The enzymatic ring-opening polymerization of six- and seven-membered lactones was first conducted using lipase as a catalyst.[7] The enzyme-catalyzed ring-opening polymerization of various lactones has also been published.[8−11] However, the ring-opening polymerization of a cyclic carbonate using lipase, so far, has not been reported. In this report, the preparation of poly(trimethylene carbonate) [P(TMC)] by the enzymatic ring-opening polymerization of the six-membered 1,3-dioxan-2-one (TMC) was studied with respect to the reaction conditions and the origin of the enzyme.

Enzymatic ring-opening polymerization of TMC was carried out as shown in Scheme 1. TMC was synthesized from 1,3-propanediol and ethyl chloroformate in the presence of triethylamine according to the literature.[2] TMC was further purified by silica gel column chromatography using chloroform−ethyl acetate−diethyl ether (1:1:1) as an eluent. P(TMC)s are synthesized in bulk using commercially available lipases.[12] A typical polymerization of P(TMC) with an M_w of 84 700 (entry 5 in Table 1) was carried out as follows. A mixture of porcine pancreatic lipase (PPL: 0.5 mg) and TMC (100 mg) was stirred in an argon atmosphere in a sealed tube placed in a thermostated oil bath at 100 °C for 24 h. After the reaction, the reaction mixture was dissolved in chloroform (5 mL), and the insoluble enzyme was removed by filtration. The chloroform was then evaporated under reduced pressure to quantitatively obtain the polymer. The monomer conversion as determined by ^{1}H-NMR was 96%.[13] The polymer was further purified by reprecipitation (chloroform as a good solvent; methanol as a poor solvent) to yield P(TMC) in 95% yield. The molecular weight relative to polystyrene and the molecular weight dispersion as measured by GPC were M_w = 84 700 and M_w/M_n = 3.9, respectively.[14] The molecular structure was analyzed by FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopies and elemental analysis.[15]

Scheme 1



TMC P(TMC)

It was found that TMC was readily polymerized in bulk in the presence of PPL to yield P(TMC) with an M_w of up to 169 000 at 100 °C after 24 h. Under these conditions, no elimination reaction of carbon dioxide with subsequent formation of an ether linkage in the polycarbonate polymer was confirmed by ^{1}H-NMR spectroscopy, since the triplet at 3.4 ppm characteristic of an ether group (−CH_2−O−CH_2−) was not observed.[3,15] It was also confirmed that TMC remained unchanged without lipase at 60, 80, and 100 °C after 24 h, indicating that the lipase actually promoted the polymerization of TMC. Table 1 shows the typical ring-opening polymerization of TMC with/without enzyme. It was confirmed that polymerization occurred with all lipases tested except for Novozym 435. However, a significant difference between the enzymes was observed with respect to the monomer conversion and molecular weight of the resultant polymer. Among the lipases tested, PPL showed the highest activities for the polymerization. Both the conversion and M_w increased with increasing reaction temperature from 60 to 100 °C when PPL was used. At 120 °C, the M_w was decreased when compared with the M_w at 100 °C. That is, the most preferable temperature for the polymerization of TMC by PPL will be 100 °C with respect to the M_w. The M_w of the P(TMC) decreased with increasing lipase concentration from 0.25% to 10%. The maximum M_w of P(TMC) was obtained at a lipase concentration of around 0.25% at 100 °C. On the other hand, the conversion of TMC to P(TMC) remained over 90% with increasing lipase concentration from 0.25% to 5.0%. These tendencies are in agreement with the lipase-catalyzed ring-opening polymerization of β-propiolactone.[11] Similar TMC polymerization results for lipase PS were obtained at a lipase concentration between 0.5 and 10% at 100 °C. From the GPC and ^{1}H-NMR analyses, it was found that the TMC monomer was rapidly polymerized in the presence of PPL and lipase PS within the first 8 h; then the M_w and monomer conversion slightly increased to their maximum values

S0024-9297(96)01862-1 CCC: $14.00

Table 1. Typical Ring-Opening Polymerization of 1,3-Dioxane-2-one (TMC) with/without Lipase

entry[a]	lipase[b]	wt %[c]	temp (°C)	time (h)	conv (%)	$\bar{M}_w$[d]	$\bar{M}_w/\bar{M}_n$
1	PPL	1.0	60	96	5	1 200	1.2
2	PPL	1.0	80	96	33	5 400	1.9
3	PPL	0.1	100	24	51	156 000	3.8
4	PPL	0.25	100	24	96	169 000	3.5
5	PPL	0.5	100	24	96	84 700	3.9
6	PPL	1.0	100	24	99	76 500	4.1
7	PPL	5	100	24	91	25 100	5.2
8	PPL	10	100	24	86	17 800	5.0
9	PPL	0.5	120	24	97	29 900	3.0
10	PS	0.5	100	24	97	24 000	1.9
11	PS	1.0	100	24	94	25 400	2.4
12	PS	5	100	24	95	16 100	2.4
13	PS	10	100	24	93	14 000	3.1
14	CC	1.0	100	24	5	1 000	1.2
15	Lipo-IM	1.0	100	24	94	11 200	1.8
16	Novo	1.0	100	24	0		
17	PPL-IM	0.05	60	24	40	3 200	1.5
18	PPL-IM	0.05	80	24	88	35 200	2.1
19	PPL-IM	0.05	100	24	93	46 800	2.1
20	PPL-IM	0.5	80	24	90	11 000	2.3
21	PPL-IM[e]	0.5	80	24	81	13 500	1.5
22	PS-IM	0.05	100	24	85	27 000	2.0
23	blank		60	24	0		
24	blank		80	24	0		
25	blank		100	24	0		

[a] Entries 23−25: blank tests. [b] PPL: porcine pancreatic lipase. PS: lipase PS. CC: *Candida cylindracea* lipase. Lipo-IM: Lipozyme IM. Novo: Novozym 435. PPL-IM: immobilized PPL. PS-IM: immobilized PS. [c] The 1.0% concentration of immobilized lipase corresponds to approximately 0.05% lipase concentration based on the monomer. [d] Determined by GPC analysis, calibrated with polystyrene standards. [e] Recovered enzyme from entry 20 was used for the polymerization in entry 21.

up to 24−30 h. No significant formation of the TMC oligomer was detected by GPC.

It is reported that immobilization of enzymes on Celite showed higher activities in anhydrous organic solvents.[9,10,16] By using the immobilized lipase prepared according to the literature,[10] both the monomer conversion and M_w were significantly increased compared to those of naked lipase, particularly at the lower temperatures of 60 and 80 °C.[17] Also, TMC was efficiently polymerized with a low enzyme concentration of 0.05% by the immobilization on Celite. It was possible to recycle the immobilized enzyme without any decrease in its activity after the polymerization took place. That is, after the polymerization of TMC, the polymerization mixture was dissolved in chloroform, and the immobilized enzyme was recovered almost quantitatively by filtration. Thus recovered immobilized enzyme again worked actively for the polymerization of TMC as in the first use (Table 1, entries 20 and 21).

In conclusion, it was found that cyclic carbonate TMC was readily polymerized in the presence of lipase at a temperature between 60 and 100 °C for 24 h to yield the corresponding P(TMC). Among the lipases tested, PPL showed the best results with respect to the monomer conversion and the molecular weight of the resultant polymer. The most preferable concentration of lipase PPL was 0.25%. No elimination of carbon dioxide by the enzymatic polymerization of TMC was detected. Enzymatic polymerization of TMC was significantly enhanced by the immobilization of PPL on Celite. The recovered immobilized enzyme again worked actively for the polymerization of TMC as in the first use.

References and Notes

(1) (a) Albertsson, A.-C.; Eklund, M. *J. Appl. Polym. Sci.* **1995**, *57*, 87. (b) Albertsson, A.-C.; Sjoling, M. *J. Macromol. Sci., Pure Appl. Chem.* **1992**, *A29* (1), 43. (c) Albertsson, A.-C.; Eklund, M. *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 265. (d) Albertsson, A.-C.; Lofgren, A. *J. Macromol. Sci.-Pure Appl. Chem.* **1995**, *A32*, 41. (e) Lofgren, A.; Albertsson, A.-C.; Dubois, Ph.; Jerome, R.; Teyssie, Ph. *Macromolecules* **1994**, *27*, 5556. (f) Buchholz, B. *J. Mater. Sci., Mater. Med.* **1993**, *4*, 381. (g) Hori, Y.; Gonda, Y.; Takahashi, Y.; Hagiwara, T. *Macromolecules* **1996**, *29*, 804. (h) Katz, A. R.; Mukherjee, D.; Kaganov, A. L.; Gordon, S. *Surg. Gynecol. Obstet.* **1985**, *161*, 213. (i) Ariga, T.; Takata, T.; Endo, T. *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 1393. (j) Kricheldorf, H. R.; Jenssen, J.; Kreiser-Saunders, I. *Makromol. Chem.* **1991**, *192*, 2391. (k) Zhu, K. J.; Hendren, R. W.; Jensen, K.; Pitt, C. G. *Macromolecules* **1991**, *24*, 1736. (l) Keul, H.; Hocker, H.; Leitz, E.; Ott, K.-H.; Morbitzer, L. *Makromol. Chem.* **1988**, *189*, 2303. (m) Wurm, B.; Keul, H.; Hocker, H.; Sylvester, G.; Leitz, E.; Ott, K.-H. *Makromol. Chem., Rapid Commun.* **1992**, *13*, 9.

(2) Ariga, T.; Takata, T.; Endo, T. *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 581.

(3) Kricheldorf, H. R.; Jenssen, J. *J. Macromol. Sci., Pure Appl. Chem.* **1989**, *A26* (4), 631.

(4) (a) Carothers, W. H.; Van Natta, F. J. *J. Am. Chem. Soc.* **1930**, *52*, 314. (b) Carothers, W. H.; Dorough, G. L.; Van Natta, F. J. *J. Am. Chem. Soc.* **1932**, *54*, 761.

(5) (a) Sarel, S.; Pohoryles, L. A. *J. Am. Chem. Soc.* **1958**, *80*, 4596. (b) Kuhling, S.; Keul, H.; Hocker, H. *Makromol. Chem. Suppl.* **1989**, *15*, 9. (c) Kuhling, S.; Keul, H.; Hocker, H. *Makromol. Chem.* **1990**, *191*, 1611. (d) Keul, H.; Bacher, R.; Hocker, H. *Makromol. Chem.* **1990**, *191*, 2579.

(6) Kricheldorf, H. R.; Dunsing, R.; Serra i Albet, A. *Makromol. Chem.* **1987**, *188*, 2453.

(7) Uyama, H.; Kobayashi, S. *Chem. Lett.* **1993**, 1149.

(8) (a) Knani, D.; Gutman, A. L.; Kohn, D. H. *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 1221. (b) MacDonald, R. T.; Pulapura, S. K.; Svirkin, Y. Y.; Gross, R. J.; Kaplan, D. L.; Akkara, J.; Swift, G.; Wolk, S. *Macromolecules* **1995**, *28*, 73. (c) Uyama, H.; Takeya, K.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 56. (d) Kobayashi, S.; Shoda, S.; Uyama, H. *Adv. Polym. Sci.* **1995**, *121*, 1. (e) Matsumura, S.; Beppu, H.; Nakamura, K.; Osanai, S.; Toshima. K. *Chem. Lett.* **1996**, 795. (f) Namekawa, S.; Uyama, H.; Kobayashi, S. *Polym. J.* **1996**, *28*, 730. (g) Nobes, G. A. R.; Kazlauskas, R. J.; Marchessault, R. H. *Macromolecules* **1996**, *29*, 4829. (h) Zhang, L.; Deng, X.; Huang, Z. *Biotechnol. Lett.* **1996**, *18*, 1051.

(9) Uyama, H.; Takeya, K.; Hoshi, N.; Kobayashi, S. *Macromolecules* **1995**, *28*, 7046.

(10) Uyama, H.; Kikuchi, H.; Takeya, K.; Hoshi, N.; Kobayashi, S. *Chem. Lett.* **1996**, 107.

(11) Matsumura, S.; Beppu, H.; Tsukada, K.; Toshima, K. *Biotechnol. Lett.* **1996**, *18*, 1041.

(12) Enzymes: Porcine pancreatic lipase (PPL, 41 U/mg protein, according to the supplier) and lipase from *Candida cylindracea* (CC, 500 U/mg, according to the supplier) were purchased from Sigma Chemical Co. (St. Louis, MO). Lipase PS was kindly supplied by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). Novozym 435 (triacylglycerol hydrolase + carboxylesterase) having 7000 PLU/g (propyl laurate units) and Lipozyme IM were kindly supplied by Novo Nordisk A/S (Bagsvaerd, Denmark).

(13) The conversion of TMC to P(TMC) was determined by comparison of the ^{1}H NMR spectral integration intensities for the $\delta = 4.5$ ppm peak (OCH_2) corresponding to the two methylene groups adjacent to the carbonate group in TMC with the corresponding methylene protons of P(TMC) at $\delta = 4.2$ ppm (OCH_2).

(14) The number-average molecular weight (M_n), weight-averge molecular weight (M_w), and molecular weight dispersion (M_w/M_n) were measured by gel permeation chromatography (GPC) using GPC columns (Shodex K-803L + K-806L + K-800D, Showa Denko Co., Ltd., Tokyo, Japan) with a reflective index detector. Chloroform was used as the eluent. The GPC system was calibrated with polystyrene standards of narrow molecular weight distribution.

(15) The spectral data and elemental analysis of P(TMC) having an M_w of 84 700 are shown (entry 5 in Table 1) to be representative. IR (KBr): 2973, 1460 (CH_2), 1748 (carbonate C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$): $\delta = 2.0$ (quintet, 2H, 6.21), 4.17 (t, 4H, 6.21). ^{13}C-NMR ($CDCl_3$): $\delta = 28.0$ (CH_2CH_2-

CH_2), 64.2 (CH_2O), 154.8 (C═O). Anal. Calcd for $(C_4H_6O_3)_n$: C, 47.06; H, 5.92. Found: C, 46.79; H, 6.08.

(16) (a) Yamane, T.; Ichiryu, T.; Nagata, M.; Ueno, A.; Shimizu, S. *Biotechnol. Bioeng.* **1990**, *36*, 1063. (b) Reslow, M.; Adlercreutz, P.; Mattiasson, B. *Eur. J. Biochem.* **1988**, *172*, 573.

(17) Immobilized lipase was prepared as follows.[10] Lipase (50 mg) and D-sucrose (50 mg) were dissolved in 50 mL of phosphate buffer (pH 8), and then 1.0 g of Celite (Celite 545, Junsei Chemical Co., Ltd., Tokyo, Japan) was suspended in the enzyme solution for 10 min with stirring at room temperature, followed by lyophilization of the suspension for 10 h.

MA961862G