Lipase-Catalyzed Ring-Opening Polymerization of Trimethylene Carbonate†

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ABSTRACT: This work was directed at extending the use of lipase-catalyzed ring-opening polymerizations to cyclic carbonate monomers. Of the seven lipases screened for bulk trimethylene carbonate (TMC) polymerization (70 °C, 120 h), Novozym-435 from *Candida antarctica* gave almost quantitative monomer conversion (97%) and poly(TMC) with a M_n = 15 000 (M_w/M_n = 2.2) with no apparent decarboxylation during propagation. The lipases from *Pseudomonas* species (AK and PS-30) and porcine pancreas (PPL) also exhibited high monomer conversions (>80%, 120 h) but gave lower molecular weight polymers with broad polydispersity. Analyses by ^{1}H-NMR spectroscopy suggested that poly(TMC) prepared by Novozym-435-catalyzed polymerization had terminal $-CH_2OH$ functionalities at both chain ends. A monotonic increase in monomer conversion with time and the rapid increase in M_n as a function of monomer conversion for Novozym-435-catalyzed TMC bulk polymerization at 70 °C suggest that the polymerization has chain-type propagation kinetics. An increase in conversion above 66% did not substantially change M_n. The percent conversion was larger when the reaction temperature was increased from 45 to 55 °C. Further increase in the reaction temperature from 55 to 85 °C did not give higher percent conversion values. The molecular weight decreased substantially as the reaction temperature was increased from 55 to 85 °C (M_n from 24 400 to 5 900). The highest poly(TMC) molecular weight (M_n = 24 400) was obtained by conducting the polymerization at 55 °C. Monomer conversion and molecular weight as a function of the percent reaction water content (w/w) were investigated. Increasing the water content resulted in enhanced polymerization rates and decreased molecular weights. Separation of the oligomeric products from polymerizations of TMC in dried dioxane and toluene catalyzed by porcine pancreatic lipase led to the isolation of di- and triadducts of trimethylene carbonate. Based on the symmetrical structure of these products and the end-group structure of high molecular weight chains, a mechanism for chain initiation and propagation for lipase-catalyzed TMC polymerization was proposed.

Introduction

The use of enzyme catalysis in organic media for polymer-forming reactions is gaining increasing attention. The hallmark of enzymes is their ability to achieve high enantio- and regioselectivity for various chemical transformations.[1] Furthermore, enzymes represent a family of "environmentally friendly" catalysts. Advantages of using enzymes in organic as opposed to aqueous media are as follows: (i) increased enzyme thermal stability, (ii) solubility of a wide range of substrate types in the reaction media, (iii) no requirement for pH adjustment as the reaction proceeds, and (iv) readily recyclable.[2,3] The application of these reactions to lipase-catalyzed lactone ring-opening polymerizations is described below.

Studies of enzyme-catalyzed lactone ring-opening polymerizations in organic media have been conducted for the polymerizations of ϵ-caprolactone (ϵ-CL),[4−6] δ-valerolactone (δ-VL),[4] β-propiolactone,[7] (±)-β-methyl-β-propiolactone,[7] (±)-α-methyl-β-propiolactone,[8] and γ-butyrolactone.[7] The enzyme-catalyzed copolymerization of β-propiolactone with ϵ-CL was also investigated.[9] Enantio-enriched polymers were obtained by the stereoelective enzyme-catalyzed polymerization of (±)-α-methyl-β-propiolactone.[8] Mechanistic investigations into lipase-catalyzed reactions are further exploiting enzymes as catalysts. For example, porcine pancreatic lipase (PPL) catalyzed ϵ-CL polymerizations were shown to provide "controlled" polymerizations where the molecular weight was a function of the reaction monomer to initiator stoichiometry.[10] In general, polymerizations of 4−7-membered ring lactones resulted in low molecular weight products after long reaction times. Additional mechanistic studies will be needed to improve propagation kinetics and product molecular weights.

In contrast to the studies described above with small-ring-size lactone monomers, enzyme-catalyzed polymerizations of macrolactones have already resulted in improved propagation kinetics and/or molecular weights when compared to chemical preparative routes. The enzyme-catalyzed polymerizations of ω-undecanolide (UDL), ω-dodecanolide (DDL), and ω-pentadecanolide (PDL) (12-, 13-, and 16-membered lactones) were first investigated by Kobayashi and co-workers.[11−13] Recently, we reported the ring-opening polymerization of ω-pentadecalactone catalyzed by immobilized and non-immobilized forms of the lipase PS-30.[14] When immobilized PS-30 was used for bulk polymerizations at 70 °C, we obtained poly(PDL) with a high number-

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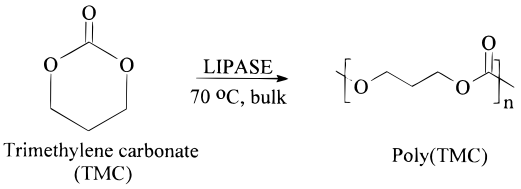
Scheme 1



average molecular weight (M_n = 62 000) and moderate dispersity (M_w/M_n = 1.9).[14] Further study of this enzyme−monomer system showed that reaction water content and temperature were important factors that controlled not only the rate of monomer conversion but also the polymer molecular weight.[14] Specifically, we showed that, by decreasing the water content in reactions, slower polymerization rates but higher poly(PDL) molecular weights resulted. Furthermore, poly(PDL) molecular weights were highest at reaction temperatures between 80 and 90 °C. One objective of this paper was to determine how the above reaction parameters would effect lipase-catalyzed ring-opening polymerizations carried out with a different enzyme−monomer pair.

A number of aliphatic polycarbonates and their copolymers were reported to be environmentally biodegradable and are of interest as bioresorbable biomedical materials.[15,16] Also, cyclic carbonates have been recognized as a novel class of monomers showing expansion in volume on polymerization.[17] Ring-opening polymerization of 1,3-dioxan-2-one (TMC) was first studied by Carothers and Van-natta.[18] More recent investigations have reported the polymerization of TMC to linear high molecular weight chains by using organometallic catalysts.[19,20] Early work by Abramowicz and Keese[21] reported the enzymatic transesterification of diphenyl carbonate (DPC) with a wide variety of alcohols and phenols. The reaction products included the substitution of one or both of the phenoxide groups on DPC. Transesterification of DPC with bifunctional alcohols catalyzed by the *Candida* lipase resulted in the synthesis of oligocarbonates having molecular weights of about 900.[21] Based on the work of Abramowicz and Keese,[21] a preliminary report of lipase-catalyzed carbonate polymerization by us,[22] and other studies described above using lactones, it appeared possible that cyclic carbonate enzyme-catalyzed polymerization might provide a route to linear polymers of substantial molecular weight. Furthermore, within a time period similar to that of our report,[22] Matsumura *et al.*[23] and Kobayashi *et al.*[24] also communicated results on the lipase-catalyzed polymerization of TMC. Matsumura *et al.*[23] claimed that extraordinarily high molecular weight poly(TMC) (M_w = 156 000, M_w/M_n = 3.8) was obtained by using only low quantities of PPL (∼0.1 wt %) as the catalyst at very high reaction temperature (100 °C).[23] In contrast, Kobayashi *et al.*[24] reported the formation of low molecular weight poly(TMC) (M_n = 800, M_w/M_n = 1.4) by PPL (50.0 wt %) catalyzed polymerization at 75 °C. Indeed, comparison of the papers in refs 22−24 showed considerable points of variation, which, in part, were further explored in this work.

In this paper, we explored the effects of reaction parameters on the lipase-catalyzed ring-opening polymerization of cyclic carbonates. Commercially available lipases from different sources were screened for their ability to catalyze the bulk polymerization of TMC at 70 °C (Scheme 1). Based upon monomer conversion and molecular weight measurements, Novozym-435 (lipase from *Candida antarctica*) was identified as being most suitable for TMC bulk polymerization. Monomer conversion and product molecular weight during Novozym-435-catalyzed polymerization of TMC were evaluated as a function of (i) reaction time, (ii) reaction temperature (45−85 °C), and (iii) reaction water content. High molecular weight poly(TMC) was prepared without decarboxylation during propagation.

Experimental Section

Materials. Solvents were dried, distilled, and stored over 4 Å molecular sieves prior to their use. All other chemicals were of analytical grade and were used as received. Porcine pancreatic lipase (PPL) Type II crude (activity = 61 units/mg of protein) and *Candida cylindracea* lipase (CCL) Type VII (activity = 4570 units/mg of protein) were obtained from Sigma Chemical Co. The lipases PS-30, AK, and MAP-10 from *Pseudomonas cepacia*, *Pseudomonas fluorescens*, and *Mucor javanicus*, respectively, were obtained from Amano Enzymes Co., Ltd., Lombard, IL (specified activities at pH 7.0 were 30 000, 20 000, and 10 000 units/g, respectively. Enzyme activities reported herein are the corresponding manufacturer's specification based on the olive oil method, with a unit defined as the amount of enzyme which releases 1 μmol of fatty acid from olive oil/min. Immobilized lipases from *Candida antarctica* (Novozym-435) and *Mucor miehei* (Lipozyme IM) were gifts from Novo Nordisk Bioindustrials, Inc., Danbury, CT.

Synthesis of Trimethylene Carbonate. Trimethylene carbonate (TMC) was synthesized following a procedure described elsewhere.[25] The product was recrystallized twice from diethyl ether. White crystals were obtained in 55% yield: mp 45 °C (lit.[25] mp 45 °C). The ^{1}H NMR spectrum (4H, 4.50 ppm; 2H, 2.21 ppm) was consistent with that reported previously.[25]

Preparation of Thermally Deactivated Novozym-435. Novozym-435 (2.0 g) was suspended in 25 mL of nanopure water in a 100 mL round-bottomed flask fitted with a water-cooled condenser. The lipase suspension was refluxed for 14 h. The suspension was then allowed to cool to room temperature, and water was removed by freeze drying. The lipolytic activity of the lipase was assessed according to a Sigma titrometric method (Procedure No. 800 for lipases). The reagents for this assay (Kit 800-A) were also purchased from Sigma Chemical Co. (St. Louis, MO). Typically, in 20 mL reaction vials, nanopure water (2.5 mL), Sigma lipase substrate (stabilized olive oil emulsion, 50% v/v, with 0.1% sodium azide; 10.0 mL), Trizma buffer (0.2 mol/L, pH 7.7 at 37 °C; 1.0 mL), and the lipase (50 mg) were added. The reaction vials were capped tightly and shaken vigorously for a few minutes. The reaction vials were then placed in a constant-temperature water bath maintained at 37 °C for 3 h with magnetic stirring. The contents of the vials was then transferred to 50 mL Erlenmeyer flasks, and the vials were rinsed with 3.0 mL of 95% ethanol. Thymolphthalein indicator (6 drops) was added, and the reaction mixture was titrated against a standard 0.05 N NaOH solution to a slight but permanent blue color. Blank assay vials were also set up as described above, only lipase was not added. Novozym-435 and the deactivated lipase had activity values of 42.0 and 0.45 units/mg of the solid, respectively.

Enzymatic Polymerization of TMC. TMC was polymerized with and without solvent. The following are representative procedures for enzymatic polymerization reactions. For bulk polymerizations, the monomer and lipase were dried separately in 6 mL reaction vials. In all cases, the monomer was dried over P_2O_5 in a desiccator (0.1 mmHg, 38 h, room temperature). Four levels of reaction water content were obtained by drying the enzyme by one of the following methods: (1) used without drying, (2) dried over P_2O_5 in a desiccator (0.1 mmHg, 38 h, room temperature), (3) dried as in method 2 for 5 min, and (4) dried over P_2O_5 using a diffusion pump in a drying pistol (65 μmHg, 38 h, 56 °C). For all of the lipases evaluated in the screening experiment, drying was by method 2. The lipase (50 mg) was then transferred, under an argon atmosphere, into oven-dried 6 mL reaction vials containing 200 mg of dried TMC. The vials were immediately

stoppered, further sealed with Teflon tape, and placed in a constant-temperature oil bath for predetermined times. For solution polymerizations, lipase PPL (750 mg) and TMC (550 mg) were first dried in a vacuum desiccator (0.1 mmHg, 25 °C, 16 h) and then transferred under an argon atmosphere into oven-dried 20 mL reaction vials. Subsequently, 5 mL of solvent (toluene or dioxane) dried by distilling over CaH_2 was added via syringe under argon. The reaction vials were placed into a shaker incubator (65 °C, 200 rpm) for predetermined times. Control reactions with thermally deactivated Novozym-435 were also set up as described. Both bulk and solution reactions were terminated by adding 5 mL of chloroform, cooling to room temperature, stirring for 15 min, and removing of enzyme by filtration (glass-fritted filter, medium-pore porosity). The filtered substances were washed three times with 5 mL portions of chloroform. The filtrates were combined, the solvents were removed by rotoevaporation, and the residues (crude product which may consist of unreacted trimethylene carbonate and oligomeric and polymeric products) were analyzed by proton (^{1}H) NMR and gel permeation chromatography (GPC). When specified, monomer was removed from products to obtain purified poly(TMC). This was carried out by addition of a chloroform solution of crude poly(TMC) to methanol which precipitated the polymer.

Isolation of Low Molecular Weight Intermediates. The separation of dimer and trimer from crude product was accomplished by column chromatography using silica gel (Merck, 230−400 mesh, 60 Å), a column with a length and diameter of 25 and 2 cm, respectively, and a 70:1 ratio of silica gel to crude product. Chromatography was performed by using chloroform/methanol (20:1) as the eluent and a flow rate of 2 mL/min and by collecting 20 mL fractions. After solvent removal by rotoevaporation, the fractions were analyzed by GPC and ^{1}H NMR. The product which eluted from 80 to 100 mL was identified as the α,ω-dihydroxy trimer of trimethylene carbonate (TTMC). ^{1}H NMR of TTMC ($CDCl_3$, 250 MHz) was as follows: 4.3 (t, 8H), 3.8 (t, 4H), 2.1 (m, 2H), 1.9 (m, 4H) ppm. The product that eluted from 120 to 140 mL was identified as the α,ω-dihydroxy dimer of trimethylene carbonate (DTMC). ^{1}H NMR of DTMC ($CDCl_3$, 250 MHz) was as follows: 4.3 (t, 4H), 3.8 (t, 4H), 1.9 (m, 4H), 1.8 (m, 2H) ppm. GPC elution times for TTMC and DTMC were 42.20 and 44.31 min, respectively (see chromatograms in Figure 9).

Instrumental Methods. ^{1}H NMR spectra were recorded on a Bruker ARX-250 spectrometer at 250 MHz. Chemical shifts (ppm) were reported downfield from 0.00 ppm using tetramethylsilane (TMS) as the internal standard. The concentrations used were ∼4% w/v in deuterated chloroform ($CDCl_3$). The instrument parameters were as follows: temperature, 300 K; pulse width, 4.9 μs (30°); data points, 32K; acquisition time, 3.17 s; relaxation delay, 1 s; 32 transients. For ^{1}H−^{1}H COSY experiments the data were collected in a 1024 × 128 matrix and zero-filled to 512 × 512 using 8 scans/increment, a 2173 sweep width, and 1.96 s delay between transients. The data were processed using sine-bell weighting. ^{13}C NMR spectra were recorded at 62.9 MHz on a Bruker ARX-250 spectrometer. Chemical shifts were referenced relative to $CDCl_3$ at 77.00 ppm. The instrument parameters were as follows: temperature, 300 K; pulse width, 30°; data points, 64K; acquisition time, 1.638 s; delay time, 1 s; 15 000−20 000 transients.

Monomer conversions were determined from the relative peak areas of signals corresponding to methylene (−CH_2-OCOO) protons of the polymer and monomer at 4.28 and 4.51 ppm, respectively. In cases where 1,3-propanediol was found in products, signal intensities at 1.90 (CCH_2C) and 3.90 (HOCH_2CH$_2$CH$_2$OH) ppm were used for quantitation. The values of M_n reported for low (M_n < 6000) molecular weight products were calculated by ^{1}H-NMR from the relative areas of signals at 3.78 (−CH_2OH) and 4.28 ppm.

Molecular weights, M_n, >6000 were measured by GPC using a Waters HPLC system equipped with a Model 510 HPLC pump, a Model 717 autosampler, a Model 410 refractive index detector (RI), a Viscotek Model T-60 viscosity detector, and 500, 10^3, 10^4, and 10^5 Å ultrastyragel columns in series. Chloroform (HPLC grade) was used as eluent at a flow rate of 1.0 mL/min. The sample concentration and injection volumes were 1.0% (w/v) and 100 μL, respectively. Viscotek GPC-viscometry module software was used to calculate molecular weights based on a universal calibration curve generated by narrow molecular weight distribution polystyrene standards (3.00×10^2, 1.00×10^3, 2.50×10^3, 4.00×10^3, 1.40×10^4, 9.00×10^4, and 2.07×10^5; Polysciences). The refractive index increment ($dn/dc = 0.0409 + 0.0004$) was also measured using Viscotek Trisec GPC software.

Total reaction water contents (wt % water; g of water/g of reaction mixture) were measured by using a Mettler DL 18 Karl Fischer titrator with Hydranal-Titrant 5 (Fisher Scientific) and Hydranal Solvent (Fisher Scientific). The water contents in the enzyme and the monomer were determined separately by stirring either 50 mg of enzyme or 100 mg of monomer in 2.5 mL of anhydrous methanol for 8 h in a closed septum vial under an argon atmosphere. The water content was calculated by subtracting the value for a methanol control.

Results and Discussion

Screening of Enzymes. To compare the enzyme−substrate specificity of lipases for bulk TMC ring-opening polymerizations (70 °C, 120 h), seven commercial lipases (see the Experimental Section) were evaluated. Based on this study, Novozym-435 appeared most promising. Novozym-435 gave almost quantitative monomer conversion (97%) and poly(TMC) with M_n = 15 000 and a polydispersity (M_w/M_n) of 2.2. The lipases AK, PS-30, and PPL also exhibited high monomer conversion (>80%) over the 120 h polymerization time. However, lower molecular weight polymers (M_n values 3300, 4500, and 3500, respectively) with broad polydispersities (>3.0) were obtained. Lipases CCL, MAP-10, and lipozyme-IM gave poor TMC conversions (∼10%). Therefore, Novozym-435 (lipase from *Candida antarctica*) was selected to investigate the effects of various reaction parameters on bulk TMC polymerizations (see below). In a control reaction (70 °C, 26 h) using thermally deactivated Novozym-435 in place of the active lipase preparation (see the Experimental Section), no TMC polymerization was observed.[26] This was similarly observed by Kobayashi *et al.*[24] Hence, Novozym-435 polymerization of TMC occurs due to specific enzyme catalysis and not by nonspecific reactions at nucleophilic functionalities of the protein preparation.[26]

The above results are generally consistent with those reported by Kobayashi *et al.*[24] except that the highest M_n noted by those authors using the lipase from *Candida antarctica* was 2500. The higher M_n poly(TMC) obtained in this paper may be due to differences in the water content of reactions (see below). However, the above results are substantially different from those reported by Matsumura *et al.*[23] The latter authors found that TMC was not polymerized using Novozym-435 (1.0 wt %) as the catalyst at 100 °C. This is likely explained by the thermal deactivation of Novozym-435 at 100 °C.[24] It is also noteworthy to mention that, thus far, we have no evidence to confirm the claim by Matsumura *et al.*[23] that PPL can catalyze TMC polymerization at 100 °C to obtain high molecular weight poly(TMC) (unpublished results).

^{1}H-NMR End-Group Analysis of Poly(TMC). Poly(TMC) prepared in bulk (70 °C, 120 h, % water content = 0.52 w/w) using Novozym-435 and purified by precipitation had M_n = 15 000. The 250 MHz ^{1}H NMR spectrum of this product is shown in Figure 1. The signals at 4.28 and 2.08 ppm were due to intrachain a and b methylene protons, respectively.[19] The proton resonances at 4.32, 3.78, and 1.95 ppm were assigned to the end-group protons a_1, c_1, and b_1, respectively, based on a ^{1}H−^{1}H COSY spectrum of oligo(TMC) (submitted as Supporting Information). The upfield

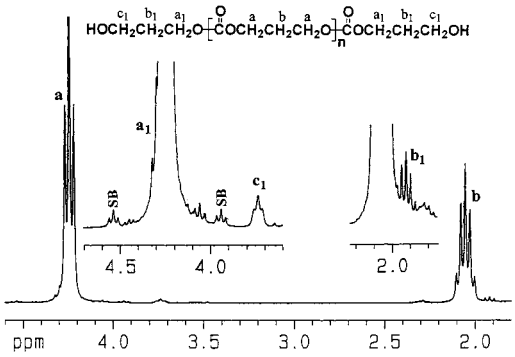


Figure 1. ^{1}H NMR (250 MHz, $CDCl_3$) of poly(TMC) purified by precipitation. SB stands for side bands observed as a consequence of the signal due to a protons.

position of protons c_1 relative to a was expected based on model compounds.[27] Furthermore, the signal corresponding to c_1 was no longer observed at 3.78 ppm after derivatization with trifluoroacetic anhydride. When the product of M_n = 10 000 was analyzed, M_n values determined by GPC (see the Experimental Section) and by ^{1}H NMR end-group analysis (see the Experimental Section) calculated assuming that both chain terminal groups were $-CH_2OH$ were in very good agreement (deviation < 7%). The ^{13}C NMR spectrum (not shown) of this product had prominent signals at 155.10, 64.51, and 28.88 ppm, which were due to intrachain repeat unit carbonyl ($-COO(CH_2)_3O-$), methylene ($-COOCH_2(CH_2)_2O-$), and methylene ($-COOCH_2CH_2CH_2O-$) carbons, respectively.[28] The low-intensity signals in the spectrum at 155.48, 65.27, 59.20, and 31.91 ppm were assigned to the end-group carbonyl ($-COO(CH_2)_3OH$), methylene ($-COOCH_2(CH_2)_2OH$), $-CH_2OH$, and $-CH_2CH_2OH$ carbons, respectively. These assignments were based on empirical calculations and comparisons with model compounds.[27] Also, after trifluoroacetylation of end groups, upfield shifts of 0.40, 0.45, and 4.00 ppm of the resonances at 155.48, 65.27, and 31.91 ppm, respectively, in conjunction with a downfield shift of 5.00 ppm for the signal at 59.20 ppm substantiated these assignments. From the above we conclude that poly(TMC) prepared by Novozym-435-catalyzed TMC ring-opening polymerization had terminal $-CH_2OH$ functionalities at both chain ends. The proposed structure of poly(TMC) is different from the one suggested by Kobayashi *et al.*[24] These workers described one of the chain ends as being a monocarbonic acid group. This conclusion was based on the assignment of the triplet at 4.32 ppm to the α-methylene of the terminal monocarbonic acid group.[24] However, based on the work described above, we believe that the signal at 4.32 ppm should be assigned to a_1 (see Figure 1). Furthermore, we would not expect that a monocarbonic acid end group would be stable under the polymerization conditions used.

In some cases during chemical-catalyzed ring-opening polymerizations of cyclic carbonates, loss of CO_2 during propagation resulted in ether linkages along the chain.[19,20] If ether linkages ($-CH_2OCH_2-$) occurred along poly(TMC) chains, ^{1}H NMR spectra would show a triplet at 3.45 ppm ($-CH_2OCH_2-$).[19,20] However, ^{1}H NMR spectra of poly(TMC) products from lipase-catalyzed TMC polymerizations carried out in this work showed no evidence of decarboxylation during propagation (Figure 1). Under similar polymerization conditions, Kobayashi *et al.*[24] also concluded that no decarboxylation occurred.

Bulk Polymerizations. TMC polymerization was conducted at 70 °C, with a reaction water content of

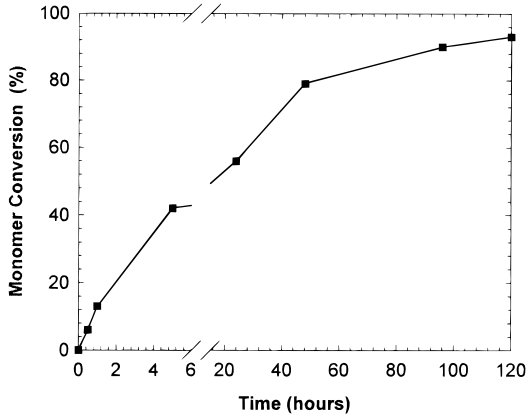


Figure 2. Variation in percent monomer conversion as a function of reaction time for the bulk polymerization at 70 °C. The reaction water content was 0.52% w/w.

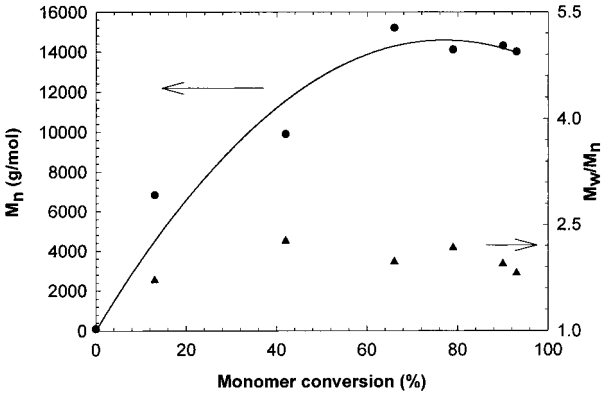


Figure 3. Number-average molecular weight and molecular weight distribution as a function of percent monomer conversion for the bulk polymerization at 70 °C. The reaction water content was 0.52% w/w.

0.52% by weight (drying method 2; see the Experimental Section) and Novozym-435 as the catalyst. Figure 2 shows percent monomer conversion versus time. The monotonic increase in monomer conversion implies that consumption is not zero order with respect to monomer concentration. Initially, monomer conversion reached 42% within only a 5 h reaction time. However, 48 and 120 h were required to reach 80 and 93% monomer conversions, respectively. The decrease in polymerization rate as the polymerization proceeds is consistent with slowed diffusion of monomer and propagating chain ends with increased reaction viscosity. A similar behavior was observed for the lipase-catalyzed bulk polymerization of ω-pentadecalactone (ω-PDL).[14]

The variation in poly(TMC) molecular weight and polydispersity versus percent conversion shown in Figure 3 was studied for the same set of experiments described above for Figure 2. Poly(TMC) M_n increased rapidly with conversion (6800 by 13%) so that, by 66% conversion, M_n was 15 200. However, further increase in conversion did not substantially change M_n. The polydispersity varied irregularly between 1.8 and 2.2 for percent conversions above 40%. The formation of high molecular weight chains by about 66% conversion is consistent with polymerizations having chain-type kinetics[29] where the rate of propagation is much faster than initiation. That poly(TMC) M_n showed little or no change with increases in percent conversion above about 66% may result from an increase in the ratio of chain degradation to chain propagation reaction rates. Indeed, it is expected that chain propagation will decrease for bulk reactions as conversion increases due to increased reaction viscosity and lower monomer concen-

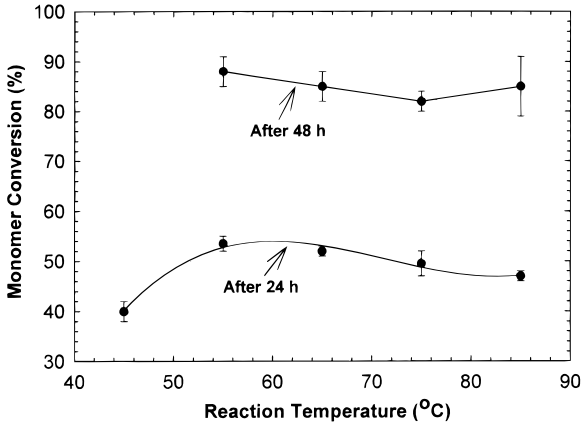


Figure 4. Effect of reaction temperature on percent monomer conversion for bulk TMC polymerizations with 0.48% w/w reaction water content. Error bars give the deviation from the mean for $n = 2$.

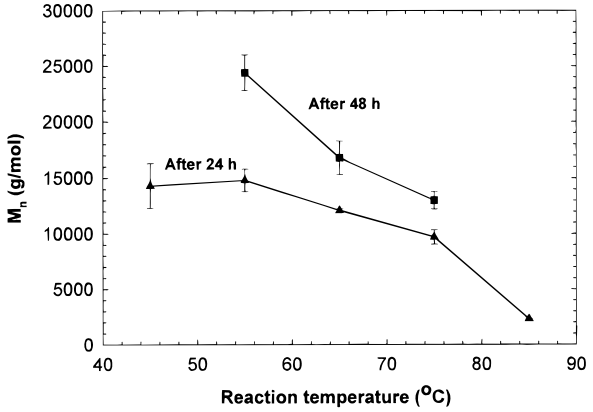


Figure 5. Effect of reaction temperature on poly(TMC) number-average molecular weight for the reaction with water content 0.48% w/w. Error bars give the deviation from the mean for $n = 2$.

tration. The effects of reaction conditions such as temperature and water concentration on chain degradation, percent monomer conversion, and product molecular weight are described below.

Effect of Reaction Temperature. Ring-opening bulk polymerizations of TMC catalyzed by Novozym-435 were performed at 45, 55, 65, 75, and 85 °C for 24 and 48 h (Figure 4). For all of these polymerizations, the reaction water content was 0.48% by weight (enzyme dried by method 4; see the Experimental Section). For a 24 h reaction time, the percent conversion increased by increasing the reaction temperature from 45 to 55 °C (Figure 4). Since the melting temperature of TMC is 45 °C, the increase in monomer conversion at 55 °C may result from an increase in monomer and propagating chain end diffusivity. However, reactions conducted for 24 h at reaction temperatures from 55 to 85 °C gave similar percent conversion values (~50%). Furthermore, 48 h reactions also gave percent monomer conversions that appeared invariable (~85%) for reaction temperatures from 55 to 85 °C. Therefore, further increase in reaction temperature above 55 °C was not useful for achieving increased percent monomer conversion. In contrast, for immobilized PS-30-catalyzed ω-PDL polymerizations, an increase in the reaction temperature to 70 °C resulted in higher monomer conversion.[14] This was explained by the increased diffusivity of propagating chain ends and ω-PDL at elevated reaction temperatures.[14] Since the behavior of the Novozym-435-catalyzed TMC polymerization differs in response to increasing reaction temperature, it may be that the Novozym-435−TMC system is relatively less constrained by diffusion. Furthermore, increasing the reaction temperature may result in partial denaturation of the Novozym-435 enzyme that catalyzes TMC polymerization. Thus, increasing catalytic conversion may be offset by lower catalyst concentration.

The relationship between poly(TMC) M_n and reaction temperature was studied (Figure 5). Inspection of Figure 5 shows that increasing the reaction temperature above 55 °C resulted in substantially lower product M_n. For example, 48 h polymerizations conducted at 55 and 75 °C had M_n values of 24 400 and 13 000, respectively. These results are in contrast to those reported by our laboratory[14] and by Uyama *et al.*[11−13] for the lipase-catalyzed polymerization of macrolactones. For example, immobilized PS-30-catalyzed ω-PDL bulk polymerizations gave poly(PDL) of higher M_n by increasing the reaction temperature from 60 to 80 °C.[14] This was explained by the fact that elevated reaction temperatures permit poly(PDL) of relatively higher molecular weight to continue growing prior to reaching an upper limit where diffusion constrains propagation.[14] For Novozym-435-catalyzed bulk TMC polymerizations, it appears that the increase in chain diffusivity at elevated reaction temperatures (above 55 °C) does not dominate product molecular weight behavior. Thus, to improve both percent conversion and poly(TMC) M_n, polymerizations at 55 °C are preferred. Decreases in poly(TMC) M_n as the reaction temperature was increased may result from one or more of the following: (i) increased reactions that cause chain scission, (ii) increased chain initiation, and (iii) increased chain depolymerization due to backbiting reactions. It also may be that the Novozym-435 enzyme(s) that catalyze TMC polymerization is partially denatured at elevated reaction temperatures, which could result in enhanced rates for the above reactions relative to chain propagation.

Interestingly, Figure 5 also shows that M_n values were higher at constant reaction temperature by increasing the reaction time from 24 to 48 h. This increase in M_n was largest at 55 °C (14 500−24 400) but was still substantial at 75 °C (9 700−13 000). In contrast, Figure 3 describes TMC polymerizations conducted at 70 °C which did not result in increased poly(TMC) M_n with increased percent monomer conversion from 66 to 93%. The key difference between experiments described in Figures 3 and 5 is that, for the latter, the conditions (method 4) used to dry Novozym-435 was more rigorous (see earlier and the Experimental Section). Thus, we believe that one outcome of restricting water in TMC polymerizations is to decrease the rate of chain cleavage and/or initiation reactions so that chain M_n can increase in bulk reactions to high (~90%) TMC conversion.

Figure 6 shows GPC traces recorded for reactions conducted for 48 h at 55, 65, 75, and 85 °C. The polymerizations were performed using method 4 to dry Novozym-435 (see the Experimental Section). For reactions conducted between 55 and 75 °C, M_w/M_n values show only small variations (between 2.4 and 2.6) and GPC traces appear unimodal. However, reactions conducted at 85 °C show a bimodal distribution. It was estimated, relative to the polystyrene standards, by assuming Bernoullian shape curves and using a curve fit program ($r^2 = 0.997$) that the higher molecular weight component (~75% by weight of the product) had $M_n = 20\,300$ ($M_w/M_n = 2.5$). Similarly, the lower molecular weight component (~25% by weight of the product) had $M_n = 3000$ ($M_w/M_n = 2.2$). The reaction

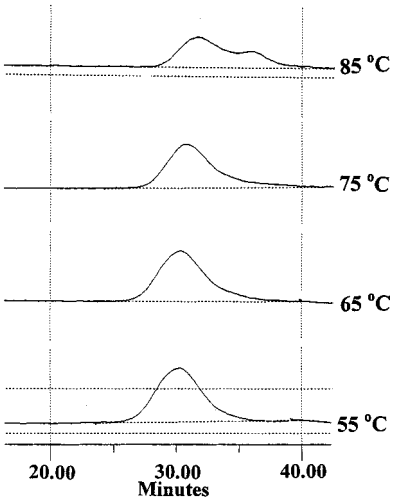


Figure 6. GPC traces of poly(TMC) products for polymerizations conducted at 55, 65, 75, and 85 °C after 48 h.

conducted for 24 h at 85 °C did not show a bimodal molecular weight distribution. In a separate experiment, purified poly(TMC) $M_n = 23\,000$ (from the reaction conducted at 55 °C, see above) was incubated in bulk at 85 °C for 24 and 48 h with and without Novozym-435. The reactions were conducted following the procedure described above for TMC polymerization using method 2 to dry Novozym-435 (see the Experimental Section). GPC analysis of the products indicated significantly lower poly(TMC) molecular weight (M_n about 8 000) for 24 and 48 h reactions carried out with the lipase. In contrast, for reactions conducted for 24 and 48 h without the lipase, no change in poly(TMC) molecular weight was observed. Also, a bimodal molecular weight distribution similar to that observed for TMC polymerizations conducted at 85 °C (Figure 6) was observed after incubation of poly(TMC) with the lipase at 85 °C. These results are consistent with the hypothesis presented above that enzyme-catalyzed reactions leading to chain degradation are accelerated relative to propagation as the temperature is increased above 55 °C. Moreover, it may be that lower chain length products are more easily hydrolyzed by the lipase, leading to the evolution of a bimodal distribution.

From the results of increasing temperature, there are apparent differences in the experimental activation energies, E_a, for the rate of polymerization and molecular weight. By using the Arrhenius and Mayo equations[30] in conjunction with mechanistic studies,[6,10,14] the following expressions for activation energies were defined:

$$E_R = E_i + E_p \quad (1)$$

$$E_x = E_p - E_{tr} \quad (2)$$

where E_R and E_x are the overall activation energies for the rate of polymerization and degree of polymerization, respectively. Equations 1 and 2 were simplified by assuming the absence of termination and initiation by the activated monomer mechanism {see the proposed mechanism below and refs 6 and 10}. Since no changes in the rate of monomer conversion were noted as a function of temperature, a low positive value for E_R is proposed. This is not surprising since the existence of an activated monomer complex is believed to be a low-energy pathway. In eq 2, a negative overall activation energy for the degree of polymerization is consistent

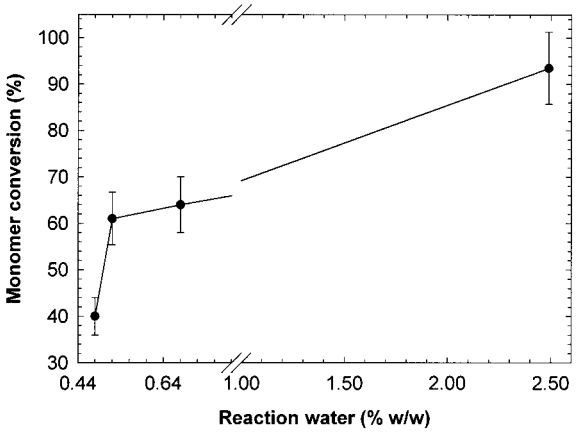


Figure 7. Percent monomer conversion as a function of reaction water content (% by weight) for bulk polymerizations at 70 °C after 24 h. Error bars give the standard deviation for $n = 3$.

with our observations. Contrary to the above, the negative value implies that other chain-breaking reactions occur and is given the term E_{tr}. As temperature increases, these rates are enhanced relative to propagation and thus are favored over propagation. This is consistent with the above experimental observations where increasing the polymerization temperature contributes to chain hydrolysis or depolymerization.

Effect of Reaction Water Content. The water content for Novozym-435-catalyzed TMC polymerization reactions conducted at 70 °C for 24 h was varied by using four different methods to dry the enzyme (see the Experimental Section). Monomer conversion as a function of the total reaction water content (% by weight) is plotted in Figure 7. Increasing the water content resulted in enhanced polymerization rates. For example, by increasing the reaction water content from 0.68 to 2.49%, the monomer conversion increased from 62 to 93%. Interestingly, a small increase in the water content from 0.48 to 0.52% resulted in a rather large increase in the percent monomer conversion, i.e., from 40 to 61%. These observations are similar to those made for PS-30-catalyzed ω-PDL polymerizations and can be explained in a similar fashion.[14] In summary, we proposed that, by increasing the total water in reactions, polymerization rates may be increased due to an increase in the number of propagating chain ends (see Proposed Mechanism section).[14] Also, increased water content in the system may result in enhanced enzyme activity.[14,31]

For the same set of experiments discussed above, the water content in reactions was also found to be important in regulating product molecular weight (Figure 8). Poly(TMC) M_n increased from 3330 to 13 500 by decreasing the reaction water content from 2.49 to 0.68%. A further decrease in the reaction water content from 0.68 to 0.48% did not result in any additional apparent poly(TMC) M_n change. Taking into account the variation in M_n as a function of percent conversion (Figures 3−5), it is expected that, at equivalent percent conversion values, M_n would be greater for poly(TMC) formed at 0.48 as opposed to 0.52% water. Thus, it is concluded that, by choosing the appropriate incremental change in reaction water content, one can alter poly(TMC) M_n. This was similarly observed for PDL polymerizations[14] and might be a consequence of a decrease in the total chain number at lower reaction water contents[6,10] (see Proposed Mechanism section).

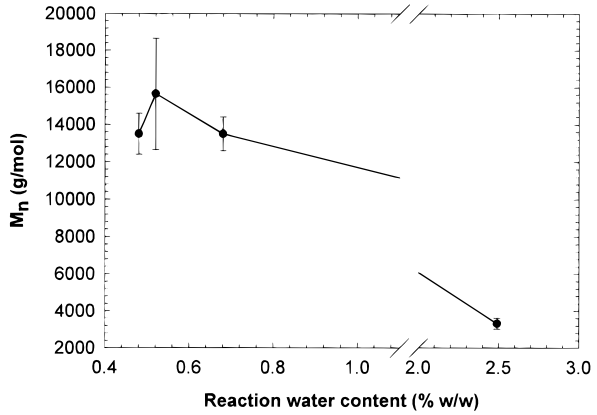


Figure 8. Variation in poly(TMC) number-average molecular weight as a function of the reaction water content (% by weight) for bulk polymerizations at 70 °C after 24 h. Error bars give the standard deviation for $n = 3$.

Proposed Mechanism

Isolation of oligo(TMC) products was used to elucidate a general mechanism for lipase-catalyzed TMC polymerization. Earlier, it was described that PPL-catalyzed TMC polymerization in bulk at 70 °C gave products of relatively low M_n (3500) for >80% conversion. Furthermore, preliminary studies showed that PPL-catalyzed solution polymerizations at 65 °C were useful in obtaining low molecular weight.[22] Therefore, PPL, an important catalyst studied by us and others for lactone polymerizations,[6,7,12,13] was used for the oligomerization of TMC in dried toluene and dioxane solutions (0.15 and 0.21 weight % of reaction water content, respectively). These reactions in toluene and dioxane were conducted for 144 h at 65 °C. This resulted in oligo(TMC) with percent monomer conversions of 95 and 97%, respectively, and degree of polymerization (dp) values from ^{1}H NMR analyses of 3 and 9, respectively. Control reactions, maintained under the same conditions but without the addition of enzyme, did not result in TMC ring opening. Low molecular weight products formed by polymerizations conducted in toluene and dioxane were carefully isolated and characterized. The product isolation was accomplished by column chromatography using silica gel as the stationary phase (see the Experimental Section). Products isolated were identified as α,ω-dihydroxy trimer of trimethylene carbonate (TTMC), α,ω-dihydroxy dimer of trimethylene carbonate (DTMC), and 1,3-propanediol. NMR analyses of these products which include ^{1}H NMR spectra of DTMC and TTMC as well as a ^{1}H−^{1}H COSY NMR spectrum of oligo(TMC) are given as Supporting Information. GPC traces of the oligo(TMC) products prepared by PPL catalysis in toluene and dioxane are shown in Figure 9. The GPC peaks corresponding to DTMC and TTMC at 44.31 and 42.20 min, respectively, were identified from GPC traces of these purified compounds as well as from GPC traces of oligo(TMC) mixed separately with DTMC and TTMC. ^{1}H NMR spectra of 1,3-propanediol showed resonances at 3.90 and 1.90 ppm. The reactions conducted in toluene and dioxane had 1 and 29% of ring-opened product as 1,3-propanediol. The relatively higher quantities of 1,3-propanediol isolated from the polymerization in dioxane is consistent with the relatively lower oligo(TMC) dp.

The following mechanism for lipase-catalyzed TMC ring-opening polymerization shown in Scheme 2 was proposed based on the following: (1) identification of 1,3-propandiol, DTMC, and TTMC; (2) the symmetrical primary hydroxyl end-group structure of low and high molecular weight TMC polymerization products; (3) the effects of water on poly(TMC) M_n; and (4) previous mechanisms proposed for lipase-catalyzed lactone ring-opening polymerizations.[6,8] Initiation involves: (i) the reaction of TMC with the lipase to form the lipase−TMC enzyme-activated-monomer (EAM) complex and (ii) reaction of the EAM with water followed by rapid decarboxylation to form 1,3-propanediol. Propagation as defined by the presence of a carbonate functionality involves the formation of DTMC by reaction of the EAM with 1,3-propanediol, TTMC synthesis by reaction of DTMC with the EAM, and subsequent propagation reactions to form high molecular weight chains. It is important to note that, in the proposed model, it is assumed that the serine residue is the catalytically essential region, where the enzyme reacts with the TMC carbonate functionality by attacking the carbonyl group. This would result in the formation of an EAM complex which contains a highly reactive carbonate functionality. The high reactivity of serine residues is generally accepted for lipases which catalyze the hydrolysis of triglycerides.[32]

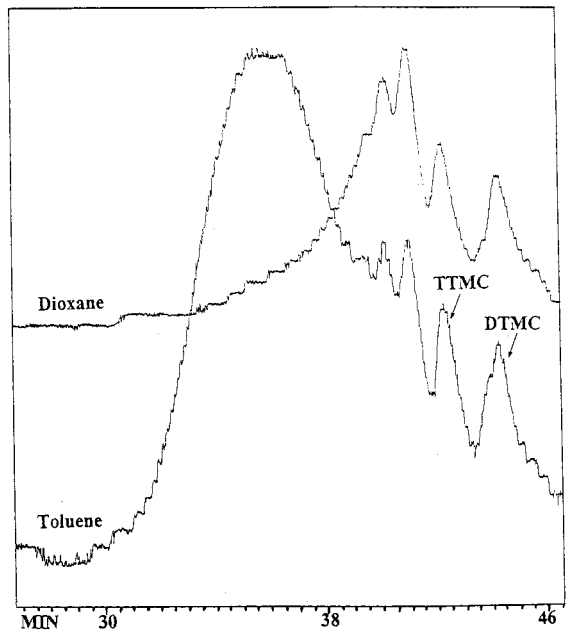
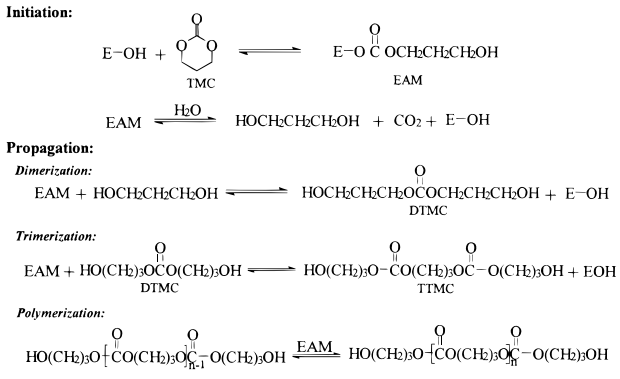


Figure 9. GPC traces of the oligo(TMC) products obtained by PPL-catalyzed solution polymerizations.

Scheme 2



Comparison of Lipase- and Chemical-Catalyzed TMC Polymerizations

Research has been conducted with a goal of identifying suitable chemical catalysts for cyclic carbonate ring-opening polymerizations that result in high molecular weight chains without monomer decarboxylation during

propagation.[19,20,25,33] Recently, TMC polymerization using organometallic initiators gave linear high molecular weight chains with low ether linkage content.[19,20] For example, poly(TMC) having an M_n of >50 000 with ~2% intrachain ether linkages was formed by using $(C_4H_9)_2SnO$ and $BF_3O(C_2H_5)_2$ as catalysts (60−100 °C, 5−8 h).[19,28] In another study involving tin, bismuth, and zinc catalyst systems, poly(TMC) was prepared without decarboxylation.[34] However, M_w values reported were only in the range of 17 000−20 000.[34] Much higher molecular weight poly(TMC) (M_w = 250 000) was reported using $BuSnCl_3$ as catalyst in bulk at 90 °C for 8 h.[20] In this paper, Novozym-435 was used to give percent monomer conversions of 42% (M_n = 10 000) within a 5 h reaction time. Furthermore, a 48 h polymerization of TMC catalyzed by Novozym-435 at 55 °C gave poly(TMC) with M_n = 25 000 without detectable decarboxylation. At this early stage in the use of enzymes to catalyze polymer-forming reactions, these comparative results are surely encouraging. It is important to consider that lipases did not evolve to carry out cyclic carbonate polymerizations. Future work to redesign and optimize enzyme catalysts and critical reaction parameters may very well bring new tailored environmentally friendly enzyme systems that are the catalyst of choice for various polymer-forming reactions.

Summary of Results

The ability of lipases to catalyze bulk polymerization of cyclic carbonates was demonstrated. In polymerizations of TMC carried out at 70 °C, of the seven lipases studied, the lipase from *Candida antarctica* (Novozym-435) gave the most rapid polymerization reaction. Quantitative monomer conversion (97%) and poly(TMC) M_n = 15 000 (M_w/M_n = 2.2) were achieved in 120 h. Analyses by NMR showed that poly(TMC) has a symmetrical structure where both chain termini had CH_2-OH groups. Consistent with our previous findings, reaction temperature and water content controlled not only the rate of monomer conversion but also the polymer molecular weight. The highest molecular weight poly(TMC), M_n = 24 400 (M_w/M_n = 2.4), resulted from a polymerization conducted at 55 °C. Increasing the reaction temperature from 55 to 85 °C led to similar monomer conversion and decreased molecular weight. Reactions including hydrolysis, increased chain initiation, and/or increased chain depolymerization such as backbiting reactions might be responsible for this. A bimodal molecular weight distribution in reactions conducted at 85 °C suggested that enzyme-catalyzed reactions leading to chain degradation are accelerated relative to propagation as the temperature is increased. By decreasing the water content, higher molecular weight and lower polymerization rates resulted. A mechanism for lipase-catalyzed ring-opening polymerization of TMC was proposed based on the analysis of low molecular weight products. Also, we showed that decarboxylation did not occur during Novozym-435-catalyzed poly(TMC) chain growth.

Acknowledgment. We are grateful for the financial support received from Rohm & Haas Co.

Supporting Information Available: ^1H-NMR spectra of DTMC and TTMC; $^1H-^1H$ COSY and ^{13}C-NMR spectra (before and after trifluoroacetylation) of the oligo(TMC) (11 pages). This material is contained in many libraries on microfiche, immediately follows this paper in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the internet; see any current masthead page for ordering information and Internet access instruction.

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