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Polyester and polycarbonate synthesis by in vitro enzyme catalysis

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Abstract Enzyme technology has significantly expanded in scope and impact over the past 10 years to include organic transformations in non-traditional environments. This is in part due to an increased understanding and capability of using enzyme catalysis in a wide variety of organic solvents, at interfaces, and at high temperatures and pressures. This review focuses on a relatively new but rapidly expanding research activity where in vitro enzyme catalysis is used for the synthesis of non-natural polyesters and polycarbonates. The inclination to use of enzymes for polymer synthesis has been fueled by a desire to carry out these reactions in the absence of heavy metals, at lower temperatures, and with increased selectivity. Aspects of this work that include enzyme-catalyzed step-growth condensation reactions, chain-growth ring-opening polymerizations, and corresponding transesterification of macromolecular substrates are discussed.

Introduction

Enzymes represent a diverse family of natural catalysts that carry out a wide range of organic transformations. They function under diverse conditions and have extraordinary ability to regulate the enantio- and regioselectivity of reactions. Increasingly, research has been carried out to explore the ability of enzymes to function in non-traditional media such as in organic solvents. Researchers have found that many enzymes can function with specificity on a surprisingly broad range of non-natural substrates. Prior to 1995, almost all of these activities were directed towards developing knowledge on organic transformations of small molecules. More recently,

the use of enzymes for polymer synthesis is gaining attention and is showing great promise. This review is focused on the use of in vitro enzyme catalysis for polyester and polycarbonate synthesis.

Enzyme-catalyzed polyester synthesis

Lipase-catalyzed condensation polymerizations

A pioneering study by Margolin et al. (1987) described the synthesis of optically active oligoesters by exploiting lipase enantioselectivity. These researchers prepared enantioenriched oligoesters by porcine pancreatic lipase (PPL)-catalyzed reactions between both a racemic diester and an achiral diol, or, a racemic diol and an achiral diester. In both cases, trimers and tetramers of type AA-BB-AA and AA-BB-AA-BB-AA and very low quantities of higher oligomers were formed. They observed formation of hydroxyl-capped oligomers since an excess of the diol was used. Wallace and Morrow (1989) were also early contributors to this new field. They recognized the importance of stoichiometry and studied polycondensation using equimolar quantities of trihaloalkyl diesters and primary diols. Halogenated alcohols such as 2,2,2-trichloroethyl activated the acyl donor and thereby improved the polymerization kinetics. They also removed byproducts periodically during the reactions to facilitate the growth of chains. Wallace and Morrow (1989; Morrow and Wallace 1992) investigated PPL-catalyzed copolymerization of *bis*(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate and 1,4-butanediol. They reported the synthesis of product with $M_w=7.9 \times 10^3$ g/mol after 120 h. In addition, the polyesters formed by Wallace and Morrow (1989) had high optical purity (>95%). Knani et al. (1993) studied the influence of enzyme type, solvent, concentration, reaction time, and other parameters on the self-condensation of methyl ϵ -hydroxyhexanoate. They observed no chain growth with aromatic monomers. Reactions conducted in bulk gave oligoesters with longer chain length than similar reactions conducted in

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solvents. In a separate study on the polymerization of lateral-substituted hydroxyesters (Knani and Kohn 1993), an increase in size of the lateral substituent from Me<Et<Ph gave slower polymerizations with higher enantioselectivity.

Chaudhary et al. (1997) summarized work carried out using lipase-catalysis for condensation-type polymerizations. They emphasized that moderate molecular weight polyesters required efficient methods to shift the thermodynamic equilibrium towards product formation. For example, Novozyme-435 was used to catalyze the solventless copolymerization of divinyl adipate and 1,4-butanediol to form a polyester with M_w 23.2×10^3 g/mol. From this work and that by others, it was concluded that the product molecular weight and end-group structure are a function of: (1) enzyme water content, (2) the enzyme/substrate ratio, (3) monomer-substrate stoichiometry, and (4) reaction temperature. Later, Rodney et al. (1999) described Novozyme-435-catalyzed AA-BB type condensation polymerizations to form aromatic polycarbonates and polyesters. Reactions of aromatic diols with 1,3-propanediol dicarbonate or divinyl adipate gave products of low molecular weight (up to 5,200 Daltons). The polymerizations were performed in tetrahydrofuran at 70, 90, and 110 °C for 24 h and at 50 °C for 72 h in bulk. In addition, they observed for reactions with benzenedimethanol that parahydroxyls react more rapidly than *meta*- and *ortho*-hydroxyl structural isomers. Lipase-catalyzed copolymerizations of aromatic dicarboxylic acid divinyl esters with glycols has also been studied by Uyama et al. (1999). Divinyl esters of dicarboxylic acids (isophthalic acid, terephthalic acid, *p*-phenylene diacetic acid, sebacic acid) were reacted with glycols at 60 °C for 48 h in heptane using various lipases including those from *Candida antarctica*, *Candida cylindracea*, *Mucor meihei*, *Pseudomonas cepacia*, *Pseudomonas fluorescens* and porcine pancreas. Of these lipases, that from *C. antarctica* gave the product polymer in the highest %-yield (74%) and molecular weight (5.5×10^3 g/mol). The polymer yield and molecular weight were a function of the glycol chain length. Comparing polymerizations of divinyl adipate with 1,4-butanediol, 1,6-hexanediol, and 1,10-decanediol showed that the product yield and molecular weight were highest when using 1,6-hexanediol and 1,10-decanediol, respectively. Self-condensation of cholic acid to its oligomers in the presence of lipase B of *Candida antarctica* has also been demonstrated (Pavel and Ritter 1996).

Enzyme-catalyzed ring-opening polymerizations

Ring-opening polymerizations of lactones and cyclic carbonates circumvents the generation of leaving groups that can limit propagation kinetics and product molecular weight. Publications have appeared on lipase-catalyzed ring-opening polymerizations of ϵ -caprolactone (ϵ -CL) (Uyama and Kobayashi 1993; Macdonald et al. 1995; Kumar and Gross 2000a), δ -valerolactone (δ -VL)

(Uyama and Kobayashi 1993), α -methyl- δ -valerolactone (Kullmer et al. 1998), α -methyl- ϵ -caprolactone (Kullmer et al. 1998), β -propiolactone (Nobes et al. 1996), β -methyl- β -propiolactone, (\pm)- α -methyl- β -propiolactone (Svikrin et al. 1996), γ -butyrolactone (Dong et al. 1998; Nobes et al. 1996), 8-octanolide (Kobayashi et al. 1998), and others. In all of these reports, the polymerizations proceeded with slow propagation kinetics and gave low molecular weight products. However, as will be seen in certain examples below, in the few years since these reports, significant improvements in polymerization efficiencies and product molecular weights have been made.

In contrast to 4-, 6- and 7-membered lactone polymerizations, the polymerization of macrolactones by traditional chemical methods proceeds slowly and gives low molecular weight polymers (Dubois et al. 1992). Lipase-catalyzed polymerizations of macrolactones have in some cases proved advantageous relative to chemical preparative routes. Kobayashi and coworkers were the first to investigate lipase-catalyzed polymerization of ω -undecanolide (UDL) (Uyama et al. 1996), ω -dodecanolide (DDL) (Uyama et al. 1995a), and ω -pentadecanolide (PDL) (Uyama et al. 1996) (12-, 13-, and 16-membered lactones). Screening of enzymes for the polymerization of UDL, DDL, and PDL using lipases including those from *Aspergillus niger*, *Candida cylindracea* (lipase B), *Candida rugosa*, *Rhizopus delmar*, *Rhizopus javanicus*, *Pseudomonas fluorescens* (lipase P, Cosmo Bio.) *Pseudomonas* sp. (lipase PS, Amano) as well as phospholipase and PPL was carried out (Uyama et al. 1995b). Quantitative conversions of UDL to poly(UDL) were achieved within 120 h using lipase P and PS. The highest number average molecular weight reported by these workers was for poly(DDL) synthesis ($M_n = 25.0 \times 10^3$ g/mol, 75 °C, 120 h) using the immobilized lipase PS from a *Pseudomonas* sp. (lipase PS, Toyobe Co.) (Uyama et al. 1995b). Bisht et al. (1997a) reported that, by using lipase PS-30 immobilized on celite, the solventless polymerization of PDL gave poly(PDL) having M_n 62.0×10^3 g/mol and PDI 1.9. Kumar et al. (2000b) reported poly(PDL) having M_n of 86.4×10^3 g/mol using Novozyme-435 as catalyst in low levels of toluene. This result demonstrated that the low molecular weights of products reported in previous work were not a consequence of an intrinsic limitation of lipase-catalyst systems.

The choice of a lipase as the polymerization catalyst creates opportunities for structural control by regioselective transformations. This characteristic of lipases was exploited to initiate polymerizations selectively from specific sites of multifunctional initiators. For example, α -, β -ethyl glucoside was used as an initiator for ϵ -caprolactone ring-opening polymerization. PPL allowed initiation and subsequent propagation to occur exclusively at and from the primary six-hydroxyl group (Bisht et al. 1998). A similar result was also reported by Cordova et al. (1998a). In addition, Ihre et al. (1996) used a hexahydroxy-functional dendrimer from 2,2-bis(hydroxy-

methyl)propionic acid as a multifunctional initiator for ϵ -caprolactone ring-opening polymerization. They reported that the polymerization proceeded from only one of the possible six-hydroxyl sites (Ihre et al. 1996; Cordova et al. 1998b).

Copolymerization of two or more monomers is an important strategy for the "tailoring" of polymeric materials. Since in vitro enzyme-catalyzed polymerization is a relatively new area of study, copolymerizations have thus far received little attention. Namekawa et al. (1996) reported the lipase-catalyzed copolymerization of β -propiolactone and ϵ -CL. Uyama et al. (1996) studied *Pseudomonas fluorescens* lipase-catalyzed copolymerizations of PDL with DDL, UDL, δ -VL, and ϵ -CL. The rates of these reactions were slow and yielded low molecular weight copolymers ($M_n < 6000$ g/mol). Later, Kobayashi et al. (1998) reported the formation of copolymers from δ -VL and ϵ -CL using the lipase from *P. fluorescens*. In addition, the lipase from *Candida antarctica* was used for the copolymerization of 8-OL with ϵ -CL and DDL in isoctane. The latter copolymerization gave %-monomer conversions of about 80% in 48 h at 60 °C (lipase 0.10 g/1.0 mmol monomer) and products with $M_n < 9.0 \times 10^3$ g/mol (Kobayashi et al. 1998). Matsumura et al. (1999) reported the preparation of poly(ester/carbonate) copolymers. Lactide and trimethylene carbonate were copolymerized in bulk using PPL (2.5 mg/1 per mmol of monomer) at 100 °C for 7 days. These conditions were reported to give poly(lactide-co-trimethylene carbonate) with M_n values between 8.0×10^3 and 21.0×10^3 g/mol. It is noteworthy that, at 100 °C, trimethylene carbonate will polymerize thermally (Bisht et al. 1997b). Therefore, it may be that this polymerization was strongly influenced by non-enzyme-mediated reactions that did not occur at the lipase box or the catalytic triad. Dong et al. (1998) reported the copolymerization of ϵ -caprolactone with cyclic and linear monomers using the lipase from *Pseudomonas* sp. Copolymerizations were carried out at 45 °C for 20 days in bulk (lipase 40 mg/0.1 mmol of monomer). The highest M_n reported by these workers was 8.4×10^3 Da for the copolymerization of ϵ -caprolactone with PDL.

Since the first reported studies of lipase-catalyzed oligoester formation by Margolin et al. (1987), it has been evident that dramatic increases in polymerization rates and molecular weights would be needed for this work to be considered of practical importance. In an effort to improve propagation kinetics and increase product molecular weights, parameters such as solvent, reaction temperature, monomer to solvent ratio, enzyme concentration, enzyme source, and reaction water content were studied (Kumar and Gross 2000a; Deng and Gross 1999). The focus of these efforts has been using an immobilized form of *Candida antarctica* lipase B (Novozyme-435). This catalyst has proven to be the most versatile and efficient of the lipases studied thus far for lactone ring-opening polymerizations. Our laboratory (Kumar and Gross 2000a; Kumar et al. 2000b) studied the effects of solvent polarity and concentration for Novozyme-435 catalyzed

ϵ -CL and PDL polymerizations. We found that the addition of toluene to the polymerizations (toluene to monomer 2:1 vol/wt) resulted in large improvements in product molecular weights and propagation kinetics. Under these conditions, at 70 °C for 45 min, copolymerization of ϵ -caprolactone and pentadecalactone (monomer feed ratio 1:1) gave a copolymer in 88% yield with M_n 20.0×10^3 g/mol. The reactivity ratios of the two monomers showed that PDL polymerization was 13 times faster than that of ϵ -caprolactone. However, irrespective of the large differences in comonomer reactivity, the copolymers had repeat-unit sequence distributions that fit closely random or Bernoullian propagation statistics. These seemingly contradictory results are explained by lipase-catalyzed polymer transacylation or transesterification reactions that occur between chains (see discussion below). Furthermore, toluene appears to enhance the thermal stability of Novozyme-435. This permits enzyme-mediated polymerizations to be efficiently run at temperatures between 90 and 100 °C.

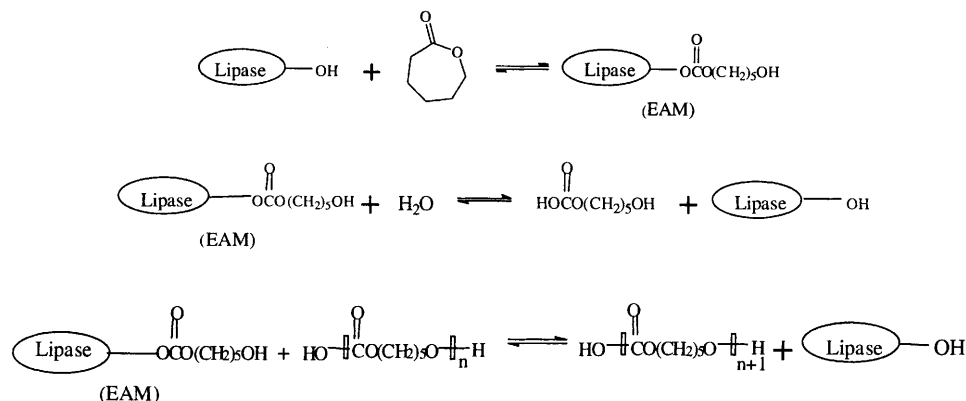
Kinetic and mechanistic investigations of lipase-catalyzed ring-opening polymerization of lactones

Thus far, the kinetics and mechanism of lipase-catalyzed ring-opening polymerizations have been investigated with only a limited set of monomers and enzyme-catalyst systems. However, for these systems, work has been conducted to begin developing knowledge of how reaction parameters such as solvent, temperature, enzyme concentration, and water and monomer concentration influence the rate of propagation, molecular weight and polydispersity. In addition, the potential to achieve controlled or living polymerizations has been explored. Summaries of studies that have addressed these questions are given below.

Henderson and Gross (2000) and Bisht et al. (1998) believe that lipase-catalyzed lactone ring-opening polymerization proceeds by the nucleophilic attack of lipase serine residues at lactone carbonyl groups. This results in the formation of an enzyme-activated monomer (EAM) complex. For initiation, a nucleophile such as water, normally present in small quantities, can react with the EAM complex to form the mono-adduct. If other nucleophiles such as alcohols or amines are added to the polymerization, they can replace water as the initiating species. Polymer chain growth or propagation takes place when the hydroxyl terminal group of a chain acts as the nucleophile that reacts with the EAM complex to give a product that is elongated by one repeat unit (see Scheme 1).

For the ϵ -CL/PPL monomer enzyme system, three nucleophiles (butanol, water, and butylamine) were used by us to study their effects on chain initiation and propagation (Henderson et al. 1996). An experimental rate equation was derived and the living/immortal nature of the polymerization was tested by a classical polymer chem-

Scheme 1 Mechanism of lipase-catalyzed polymerization



istry approach. The results indicated that monomer consumption followed a first-order rate law and was independent of the type and concentration of the nucleophile. Lipase-catalyzed polymerization of ϵ -CL was found to share many features with that of an immortal polymerization. Subsequently, this work was extended to the ϵ -CL/Novozyme-435 monomer enzyme system. Deng et al. (1999) observed that the rate of chain initiation (k_i) was slow relative to chain propagation (k_p). As above, the diagnostic tools for living polymerizations were used to assess these reactions. The plots of $\text{Ln}\{([M]_0 - [M]_t)/([M]_t - [M]_i)\}$ vs time were linear (r^2 ranged from 0.992–0.996), indicating that there was no chain termination and monomer consumption followed a first-order rate law. Furthermore, plots of M_n vs conversion showed linear relationships, suggesting that chain transfer does not occur (Deng et al. 1999). The absence of chain transfer and/or chain termination in these reaction systems implies that the product molecular weight may be controlled by reactant stoichiometry.

Bisht et al. (1997a) studied the effect of water content on the %-monomer conversion for immobilized and non-immobilized PS-30-catalyzed PDL polymerization. With an increase in water content, enhanced polymerization rates were observed. For example, for non-immobilized PS-30, %-monomer conversion was 67 and 10% at 3 h reaction times for 5.60 and 0.20% wt/wt water, respectively. Further, for immobilized PS-30-catalyzed reactions conducted for 1 h, increasing the water content from 0.20 to 0.50% wt/wt resulted in an increase in %-monomer conversion from 35 to 95%. The increase in reaction water content caused an increase in the number of propagation chain ends in the reaction and, thus, the polymerization rate. Water content in reactions was also found to regulate product molecular weight (Bisht et al. 1997a). The M_n increased from 9.1×10^3 to 35.0×10^3 g/mol as a result of a decrease in reaction water content from 5.60 to 0.20% for PS-30-catalyzed PDL polymerization.

The reader is advised that the available research on kinetics and mechanism is limited and may be specific to the monomer-catalyst systems. Thus, at this stage, the above information on the kinetics and mechanism of lipase-catalyzed polymerizations should not be generalized to other lipases and monomer systems.

Transesterification or transacylation reactions

Transacylation reactions catalyzed by lipases in organic media are well known to occur between small molecules. For example, lipase-catalyzed reactions between an achiral alcohol and a racemic carboxylic acid ester have been used to resolve the latter (Drauz and Waldmann 1995). However, the activity of lipases for the catalysis of transacylation reactions between macromolecule intrachain esters may be very different. Our laboratory has explored lipase-catalyzed transacylation reactions with the following substrate combinations: (1) monomer and polymer, (2) two homopolyesters that differ in main chain structure (Kumar and Gross 2000c). These transacylation reactions were studied using Novozyme-435 in toluene or by dispersing the catalyst in a solventless reaction medium. In bulk, the Novozyme-435-catalyzed transacylation reaction between polypentadecalactone (4.3×10^3 g/mol) and polycaprolactone (9.2×10^3 g/mol) resulted in random copolymers in only 1 h. An increase in the molecular weight of the homopolymers (polycaprolactone, M_n 44.0×10^3 , PDI 1.65; polypentadecalactone, M_n 40.0×10^3 , PDI 1.71) for Novozyme-435 catalyzed transacylation slowed the reaction so that, in 1 h, a multiblock copolymer was formed (M_n 18.2×10^3 , PDI 1.92). Thus, the rate of transacylation between preformed polyesters is molecular-weight-dependent such that chains of relatively lower molecular weight react more rapidly. Considering the requirement that two macromolecules must be in close proximity at the lipase active site for these reactions, it is surprising that they occur so rapidly.

Apparently, lipase catalysis results in the cleavage of internal ester linkages along chains. We postulated that the resulting enzyme-activated-polymer complex (EAP) then reacts with terminal hydroxyl groups at chain ends to form ester linkages. These reactions can be used to reshuffle the repeat unit sequence distribution between different aliphatic polyester chains (Kumar and Gross 2000c).

Polycarbonates

Lipases have also been used to catalyze the ring-opening polymerization of trimethylene carbonate (TMC) (Bisht

et al. 1997b) and substituted cyclic carbonate monomers (e.g., 5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one) (Al-Azemi and Bisht 1999). Novozyme-435 gave almost quantitative monomer conversion (97%) in 120 h at 70 °C to form poly(TMC) (M_n 15.0×10³ g/mol). Studies by proton and carbon NMR showed that propagation occurred without decarboxylation (Bisht et al. 1997b). Novozyme-435-catalyzed TMC polymerization occurs by slow initiation and relatively faster propagation (Bisht et al. 1997b; Deng and Gross 1999). No chain termination was observed during the polymerization.

Conclusions

Recent advances in in vitro enzyme-catalyzed polymerizations has led to: (1) a new and potentially better way to achieve unprecedented levels of control over polymer structure by exploiting enzyme enantio- and regioselectivity, (2) the ability of substituting heavy metal catalysts with environmentally benign enzymes, (3) opportunities to harness the extraordinary selectivity of enzymes to meet current needs for complex but well-defined architectural polymers that create ordered nanoscale assemblies, and (4) newly discovered enzyme activities that occur under mild conditions and result in a wide array of polymer transformations such as the transesterification of macromolecular substrates. Since research activities in the application of in vitro enzyme-catalysis to non-natural polyester and polycarbonate synthesis was almost non-existent until about 1995, many exciting findings are rapidly being uncovered and many more challenges lie ahead. Based on the work carried out thus far, it appears that some of the first applications of enzyme technology that may make their way into industrial polymer processes will likely involve the synthesis of specialty monomers, macromers, and polymer building blocks such as diols for polyurethane chemistry. Already, enzyme catalysis by lipases has been used to carry out polymerizations that would otherwise be difficult or non-achievable if conventional chemical catalysts were only available. Some examples that illustrate the special characteristics of in vitro enzyme transformations in polymer chemistry are: (1) their high activity for macrolactone polymerizations (e.g., ω-pentadecalactone), (2) regioselectivity that allows the multifunctional initiator α, β-ethyl glucose to initiate ring-opening polymerizations from only the C-6 hydroxyl group, and (3) enantioselectivity that should be transferable to a wide array of stereo-elective polymerizations (e.g., α-methyl-β-propiolactone ring-opening polymerization).

At present, a number of problems still exist in transferring these methods from the laboratory to industrial processes. There remains a need to further improve the catalytic activities displayed by enzymes when used in non-aqueous media. To circumvent this problem, larger amounts of enzymes than desired are currently needed to ensure that the process proceeds within a practical time scale. The development of highly stable catalysts, suit-

able supports, and processes that facilitate the recycling of the enzymes will surely help to alleviate difficulties associated with lower than desired enzyme activity. Ultimately, the amount and cost of an enzyme necessary for carrying out a polymer-related transformation will play a decisive role in determining whether the process can possibly be considered for practice by industry. Although the environmental benefits of the process and products may be clear, current market trends indicate that this will add little market value. Thus, it will be critical to find ways to use enzyme selectivity to improve the quality and performance of products giving them a clear advantage in the market place.

Future developments in enzyme technology will focus on addressing many of the aforementioned challenges that currently limit the use of enzymes for in vitro polymer synthesis. With that in mind, it is evident that we must explore the genetic diversity that can now be rapidly generated in the test-tube and followed by rapid screening to identify new versions of starting genes. Integration of these powerful new tools in enzyme engineering with the current challenges in enzyme-catalyzed polymerizations will surely become a prominent activity in future years that will empower current activities.

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