Polymer Synthesis by In Vitro Enzyme Catalysis

Richard A. Gross,* Ajay Kumar, and Bhanu Kalra

NSF IUCRC for Biocatalysis and BioProcessing of Macromolecules, Department of Chemistry and Chemical Engineering, Polytechnic University, Six Metrotech Center, Brooklyn, New York 11201

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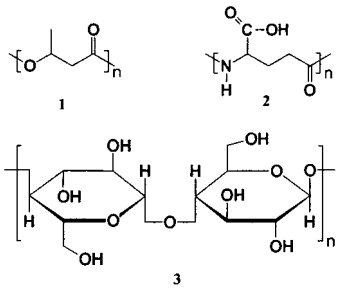
I. Introduction

In nature, living organisms are constantly producing different macromolecules for their metabolic needs. These macromolecules, such as polysaccharides, polynucleotides, proteins, or polyesters, are essential to organism survival. Their synthesis generally involves in vivo enzyme-catalyzed chain-growth polymerization reactions of activated monomers, which are generally formed within the cells by complex metabolic processes. Because of their diversity and renewability, microbial polymers such as polysaccharides, bacterial polyhydroxyalkanoates (**1**) and polyanions such as poly(γ-glutamic acid) (**2**) have received increasing attention as candidates for industrial applications. In many cases, microorganisms carry out polymer syntheses that are impractical or impossible to accomplish with conventional chemistry. Thus, microbial catalysts enable the production of materials that might otherwise be unavailable. Microbial polymers are synthesized from renewable low-cost feedstocks, and the polymerizations operate under mild process conditions with minimal environmental impact. In addition, microbial polymers provide products that, when disposed, can degrade to nontoxic products.



Certainly, there are many technical challenges to the development of commercial processes that involve microbial polymerizations. The identification of a microorganism with the desired synthetic capabilities can require an extensive search. Microbes are often effective only in a restricted physiological set of conditions (temperature, pH, in water) that are not compatible with reactant stability or reaction thermodynamics. Living catalysts are susceptible to chemical toxins and strain instability. Compared to chemical catalysis, microbial syntheses often occur with relatively slow reaction kinetics. In addition, the isolation and purification of polymer products by low-cost processes often proves challenging. Furthermore, to better control in vivo processes where reactions occur within complex assemblies of natural substances, fundamental studies are desperately needed to better define the structure of participating cata-

* Corresponding author e-mail: rgross@poly.edu; telephone: (718)-260-3024; fax: (718)875-9646.

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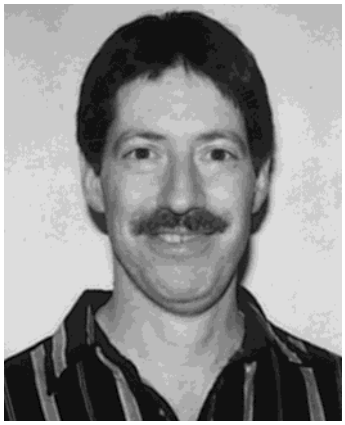
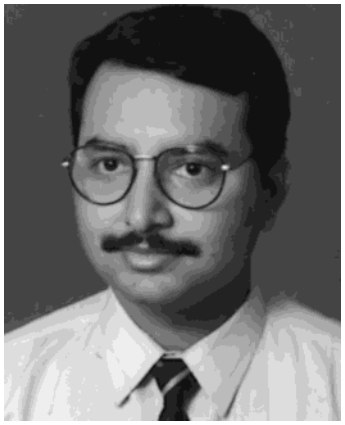


Richard A Gross received his B.S. in Chemistry from SUNY at Albany in 1979. In 1986, he received his Ph.D. in Organic-Polymer Chemistry under the supervision of Professor Mark M. Green at the Polytechnic University (Brooklyn, NY). His doctoral work focused on macromolecular stereochemistry, and his dissertation title was "The Effect of Pendant Group Structure on Polymer Conformation in Polyisocyanates and Polyisonitriles". From 1986 to 1988, he worked as a postdoctoral fellow with Prof. Robert W. Lenz at the University of Massachusetts Amherst, specializing in the synthesis of microbial polyhydroxyalkanoates (PHAs) by microbial and chemical routes as well as studies of structure–property relationships. In 1988, he joined the University of Massachusetts Lowell as an assistant professor of chemistry. In 1990, he received a NSF Presidential Young Investigator Award. Dr. Gross was promoted to full professor in 1995. Dr. Gross has served as the President of the U.S. Society on Biodegradable Polymers (1999), Founder and Co-Editor of the *Journal of Environmental Polymer Degradation* (1993–1998), and Co-Director of the NSF I/UCRC Center Biodegradable Polymer Research Center (1993–1998). In 1998 he joined the Polytechnic University (Brooklyn, NY) as the Herman F. Mark Chaired Professor. He is the Director of the NSF I/UCRC Center for Biocatalysis and Bioprocessing of Macromolecules (NSF–BBM). His current interests include integrated chemical and biocatalytic strategies for the synthesis of monomers, polymers, and their selective modification; design/synthesis of bioresorbable polymers and study of their interactions with biological systems; synthesis and selective modification of microbial glycolipids and studies of their biological properties; and polymer therapeutic systems for drug delivery.



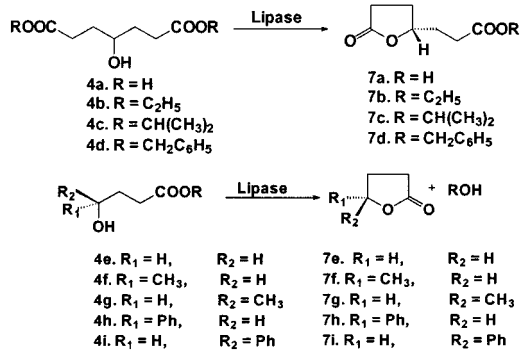
Ajay Kumar received his B.Sc. and M.Sc. in Chemistry at the University of Delhi, India. He received his Ph.D. in 1997 under the supervision of Professor V. S. Parmar at the University of Delhi, India. His doctoral work was on biotransformations of esters and amides of polyacetoxy aromatic carboxylic acids and synthetic studies on nitrogen containing hetrocyclic compounds. He joined Professor Richard A. Gross in 1997 as a postdoctoral research associate and worked on enzyme-assisted ring-opening polymerizations. He is now a Senior Research Fellow in the National Science Foundation I/UCRC Center for Biocatalysis and Bioprocessing of Macromolecules at Polytechnic University, Brooklyn, NY. His current interest includes biotransformations on drug molecules, efficient and environmentally friendly synthetic routes to monomers, and polymers preparations (biodegradable polymers of importance in medicine that are difficult to synthesize using pure chemical means).

Bhanu Kalra received her B.Sc. and M.Sc. in Chemistry at the University of Delhi, India. She received her Ph.D. in 1997 under the supervision of Professor S. M. S. Chauhan at the University of Delhi, India. Her doctoral work was on biomimetic oxidation reactions of selected phenolics. She joined Professor Richard A. Gross in 1998 as a postdoctoral research associate and worked on enzyme-mediated vinyl polymerizations, chemical modification of polysuccinimide with different phenolic amines to prepare polyaspartamides copolymers, and oxidative cross-linking of phenol-decorated water-soluble polymers with oxidases to form gels and to study the gel properties. She is now a Senior Research Fellow in the National Science Foundation I/UCRC Center for Biocatalysis and Bioprocessing of Macromolecules at the Polytechnic University, Brooklyn, NY. Her current interest includes enzyme-catalyzed synthesis and polymerization of vinyl monomers from sugars, nucleoside bases, and other functional moieties.

lysts, their organization, pathway regulation, and mechanisms at a molecular level.

A separate activity that has taken on increased importance in recent years is the use of isolated enzymes. These enzymes, harvested from living organisms, are finding increasing use as catalysts for polymer synthesis in vitro. The hallmark of enzyme catalysis is its superior catalytic power and high selectivity under mild reaction conditions. In addition, enzyme catalysis in polymer synthesis offers several advantages relative to chemical preparative-routes. Enzymes, derived from renewable resources, (i) offer promising substrate conversion efficiency due to their high selectivity for a given organic transformation, (ii) have high enantio- and regioselectivity, (iii) offer catalyst recyclability, (iv) have the ability to be used in bulk reaction media avoiding organic solvents, and (v) can circumvent the use of potentially toxic catalysts. Polymers with well-defined structures can be prepared by enzyme-catalyzed process. In contrast, attempts to attain similar levels of polymer structural control by conventional methods may require undesirable protection−deprotection steps. Examples of well-defined polymeric structures that have been prepared by using in vitro enzyme catalysis are as follows: (i) enantioenriched polyesters synthesized by lipase-catalyzed stereoelective ring-opening polymerization,[1] (ii) cellulase-catalyzed[2] cellulose synthesis (**3**), (iii) horseradish peroxidase (HRP)-catalyzed polymerization of aromatic substrates (e.g., phenols, aniline, and their derivatives).[3] Recent advances in nonaqueous enzymology has significantly expanded the potential conditions that these reactions can be performed. Examples of alternative reaction environments include various organic

Scheme 1. Lipase-Catalyzed Stereoelective Lactonization of γ-Hyroxy Esters



solvents, biphasic organic solvent−aqueous mixtures, reversed micelle systems, and supercritical fluids. Nontraditional reaction media have allowed new options for the control of polymer molecular weight as well as the morphology and architecture of polymer products. From a processing viewpoint, diversity in reaction environments facilitates the engineering of systems to facilitate downstream product separation and enzyme reuse.

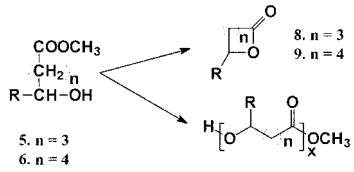
In addition to providing important elements of structural control, in vitro enzyme-catalyzed polymer synthesis has many related environmental benefits. Historically, one of the major missions in polymer science was to develop synthetic plastics to replace natural materials such as plant fibers and animal proteins. Materials such as wool, cotton, silk, and wood have been targets for these developments since they were perceived as fluctuating in both quality and availability. However, environmental pollution caused by the production and the disposal of petrochemical-derived plastics has raised increasing concerns within the chemical industry. Alternative approaches using environmentally benign processes to synthesize plastics that are engineered to degrade-on-demand are actively being pursued. Enzymes, in addition to the aforementioned remarkable features, are part of a sustainable environment. They come from natural systems, and when they are degraded, the amino acids of which they are made can be readily recycled back into natural substances. Their extended use in aqueous or solvent-less systems has made them particularly attractive to replace potentially toxic heavy metal catalysts for polymer synthesis. Furthermore, the products derived from enzyme catalysis, whether polyesters, polyphenols, polysaccharides, proteins, or other polymers, are in most cases biodegradable. Therefore, it is not surprising that the magnitude of research activity in this field has thrived in recent years.[4]

II. Lipase-Catalyzed Enantioenriched Monomer, Macromer, and Polymer Preparations

A. Lactones

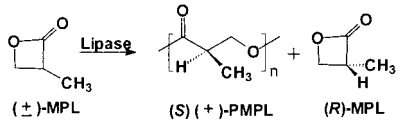
Lipases in organic media have been used successfully for lactonization of racemic γ-hydroxy esters (**4**), ω-hydroxy esters (**5**), and δ-hydroxy esters (**6**) into enantioenriched γ-butyrolactones (**7**), ω-lactones (**8**),

Scheme 2. Lipase-Catalyzed Lactonization of ω-Hyroxy Esters



and δ-lactones (**9**), respectively (Schemes 1 and 2).[5−7] Enzyme catalysis has generated stereochemical regularity and enantiopurity along chains. For example, the lipase-catalyzed polymerization of racemic α-methyl-β-propiolactone (MPL, **10**) occurred with substantial enantioselectivity[1] (Scheme 3). The polymeriza-

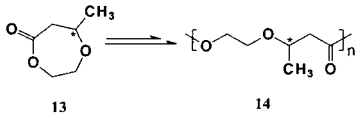
Scheme 3. Lipase-Catalyzed Stereoelective Ring-Opening Polymerization of α-Methyl-β-propiolactone



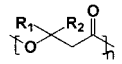
tion was catalyzed by lipase PS-30 (from *Pseudomonas cepacia)* in different organic solvents. With respect to reaction rates and enantioselectivity, toluene was the preferred solvent for preparing [*S*]-enriched PMPL (**11**) and [*R*]-enriched α-methyl-β-propiolactone (**12**). On the basis of the analysis of chain stereosequence distribution by ^{13}C NMR, stereoselectivity during propagation was said to result from catalyst enantiomorphic site control.

Similarly, Kobayashi and co-workers[8] have performed ring-opening polymerization of (*R*)- and (*S*)-3-methyl-4-oxa-6-hexanolides (MOHELs, **13**) using lipase PC as catalyst in bulk at 60 °C (Scheme 4). A

Scheme 4. Lipase-Catalyzed Ring-Opening Polymerization of MOHEL



comparison of the initial rate of polyMOHEL (**14**) formation from the (*R*) and (*S*) antipodes showed that the (*S*) enantiomer had an initial rate that was seven times larger. Lipase PA and PF catalyzed the polymerization of (*S*)-MOHEL but not (*R)*-MOHEL.



15.R_1=H, R_2=C_6H_5
16.R_1=C_2H_5, R_2=C_6H_5
17.R_1=CH_3, R_2=C_2H_5

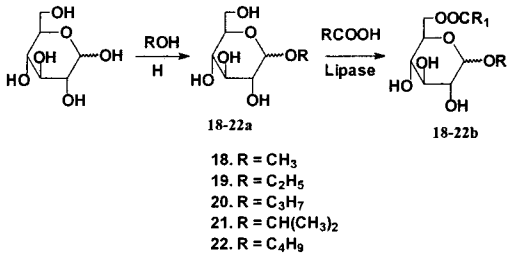
Chemical routes have shown variable levels of success for the preparation of enantioenriched lactones and their corresponding polymers. Examples of chemically prepared optically active polyesters include poly(α-phenyl-β-propiolactone) (**15**),[9] poly(α-ethyl-α-phenyl-β-propiolactone) (**16**),[10,11] poly(α-meth-

yl-α-ethyl-β-propiolactone) (**17**),[12] and poly(lactic acid).[13,14] Often, the synthetic methods used to prepare enantioenriched monomers either are tedious (requiring multistep reactions) or do not provide the monomer in sufficient enantiopurity. Attempts at carrying out stereoelective polymerizations of racemic lactones using enantiomerically pure polymerization catalysts have often failed to produce useful results. For example, an organometallic product from Zn-$(C_2H_5)_2$ and [*R*]-(−)-3,3-dimethyl-1,2-butanediol was evaluated for its ability to catalyze the stereoelective polymerization of [*R,S*]-β-methyl-β-propiolactone (**10**).[15] The results of this work showed only a small enantiomeric enrichment in the final polymer. Recently, Coates and co-workers[13] reported stereoelective copolymerizations of lactides (LL/DD monomers) using a zinc alkoxide complex. It acts as a single-site living initiator for the polymerization of *rac*-lactide that gives heterotactic PLA.[13] Heterotactic macromolecules are a rare type of polymer that have alternating pairs of stereogenic centers in the main chain (i.e., predominantly *mr*/*rm* diads are present).

B. Vinyl Monomers

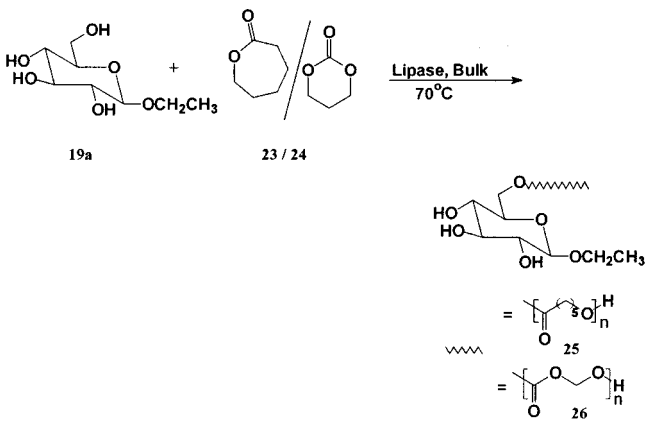
Work has been carried out to prepare vinyl monomers from carbohydrates by selective enzyme-catalyzed transformations. Motivations for such work have been to (i) selectively place vinyl functional groups at one site of multiple possible positions, thus circumventing tedious protection−deprotection steps; (ii) synthesize polymers from renewable carbohydrate feedstocks; and (iii) develop a new family of functional water-soluble monomers and polymers. Since Klibanov and co-workers[16,17] first demonstrated selective monosaccharide acylation catalyzed by lipases, numerous reports have appeared in the literature on enzyme-catalyzed selective carbohydrate acylations and deacylations.[18,19] However, the intrinsic polarity of carbohydrate compounds causes them to be soluble in only polar solvents (e.g., dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and pyridine).[20] In these media, many enzymes lose their activity due to the stripping away of critical or essential water. Consequently, in polar solvents only a small number of enzymes retain some activity that is manyfold decreased relative to their use in nonpolar solvents.[18,19] Alternative strategies to improve the miscibility between carbohydrates and hydrophobic organic substances have been pursued. Selected examples are as follows: (i) the use of solvent mixtures to achieve both satisfactory lipase activities and good solubility of the substrates,[21] (ii) the use of organoboronic acids to solubilize carbohydrates by complexation,[22] (iii) the preadsorption of carbohydrates on silica gel,[23] (iv) the use of *tert*-butyl alcohol that functions as a bulky polar solvent,[24] and (v) the prior modification of sugars by alkylation[25] and acetalization.[26] For example, Adelhorst and co-workers[26] performed the regioselective, solvent-free esterification of simple 1-*O*-alkylglycosides (**18**−**22**) using a slight molar excess of melted fatty acids (Scheme 5). A range of 1-*O*-alkyl-6-*O*-acylglucopyranosides (**18**−**22b**) were prepared in up to 90% yield, and the process has

Scheme 5. Lipase-Catalyzed Regioselective Acylation of Glucose



recently undergone pilot-scale trials by Novo Nordisk.[27] As an extension of the work by Adelhorst and co-workers,[26] our laboratory synthesized macromers by lipase-catalyzed ring-opening polymerizations of lactones from the hydroxyl moieties[28,29] of carbohydrates.[30] In summary, ethylglucopyranoside (EGP) (**19**) was used as the multifunctional initiator, and ϵ-caprolactone (**23**)/TMC (**24**) ring-opening polymerization was catalyzed by lipases (19 + 23 = 25, 19 + 24 = 26, Scheme 6).[30] Selective ring-opening from the

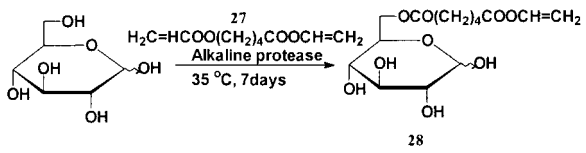
Scheme 6. EGP-Initiated Polymerization of TMC and ϵ-CL



6-hydroxyl position was achieved. This work is discussed in greater detail below in section IX.

Shibatani et al.[31] demonstrated the synthesis of vinyl sugar esters (e.g., 6-*O*-vinyladipoylglucose) (**28**) using glucose and activated fatty acids (**27**) in DMF (Scheme 7). Of the 34 different lipases and proteases

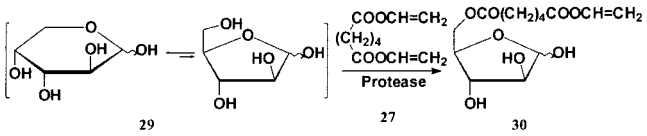
Scheme 7. Protease-Catalyzed Regioselective Synthesis of 6-*O*-Vinyladipoylglucose



screened, an alkaline protease from *Streptomyces* sp. had the highest transesterification activity giving 56% yield at 35 °C in 7 days. This protease was more effective than subtillisin from *Bacillus subtilis*.[22,32] Tokiwa and co-workers[33] investigated the protease-catalyzed synthesis of vinyl pentose esters such as that from arabinose and divinyl adipate. The transesterification catalyzed by the alkaline protease from

Streptomyces sp. of 0.5 M divinyl adipate (**27**) with 0.25 M arabinose (**29**) in DMF (30 °C, 7 days) gave 5-*O*-vinyladipoyl-D-arabinofuranose (**30**) in 50% yield (Scheme 8).[33] The low productivity and the reduced

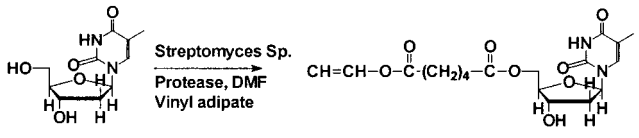
Scheme 8. Protease-Catalyzed Regioselective Synthesis of 5-*O*-Vinyladipoyl-D-arabinofuranose



29 27 30

operational stability of enzymes in these polar aprotic solvents (e.g., DMF and DMSO) are some of the factors that limit their use for these methods. Tokiwa and co-workers[34] also studied the transesterification reaction of 0.25 M thymidine (**31**) with 1 M divinyl adipate (**27**) in DMF using the alkaline protease (5 mg mL^{-1}) from *Streptomyces* sp. (20 units mg^{-1} min^{-1}). After 7 days at 30 °C, this reaction gave 5′-*O*-vinyladipoylthymidine (**32**) in 77% yield (Scheme 9).[34]

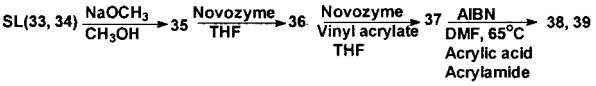
Scheme 9. Protease-Catalyzed Regioselective Synthesis of 5′-*O*-Vinyladipoylthymidine



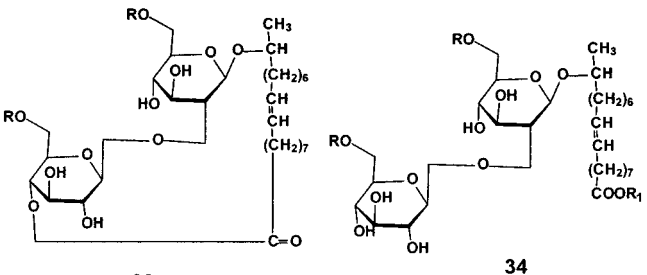
31 32

Our laboratory developed a chemoenzymatic route to a vinyl-decorated glycolipid monomer (6-*O*-acryl-sophorolactone, Scheme 10).[35] Sophorolipids (SLs)

Scheme 10. Chemoenzymatic Approach toward Synthesis of Poly(6-*O*-acryl sophorolactone) and Copolymers with Acrylic Acid or Acryamide

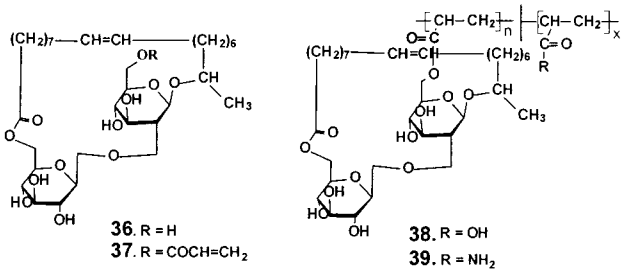


were prepared in our laboratory by fermentation of the yeast *Candida bombicola* on a glucose/oleic acid mixture.[36] The microbial synthesis of SLs results in a mixture of at least eight different compounds. SLs exist mainly in the lactonic (**33**) and acidic forms (**34**) and are acetylated to various extents at the 6′ and 6″ positions. Treatment of SL with sodium methoxide resulted in complete deacetylation and formation of the SL methyl ester (**35**). Novozyme-435 (immobilized catalyst containing the *C. antarctica* lipase B) was used to lactonize the SL-methyl ester forming a macrolactone (**36**). This same catalyst was then used to perform a highly selective vinyl esterification on the macrolactone (**37**). The resulting vinyl-functionalized glycolipid (**37**) was successfully homopolymerized and subsequently copolymerized with acrylic acid (**38**) and acrylamide (**39**) using traditional free radical initiation.[35] We are not aware of an alternative chemical route that could be used to prepare this complex SL monomer.



33 SL= R = H, $COCH_3$ 34

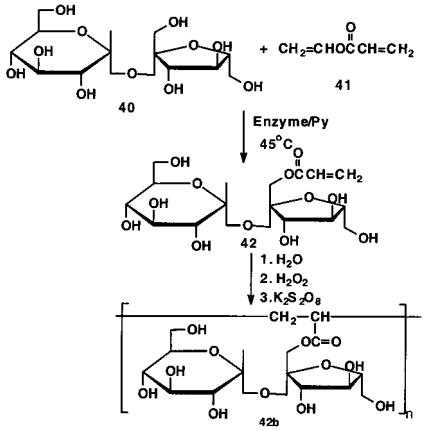
35 R = H, $COCH_3$, R_1 = CH_3



36. R = H
37. R = $COCH=CH_2$
38. R = OH
39. R = NH_2

Patil et al.[37] used Proleather (alkaline protease from *Bacillus* sp.) as a selective catalyst in pyridine for the reaction of vinyl acrylate (**41**) with one of three sucrose (**40**) primary hydroxyl groups. The resulting mono acryl derivative (**42**) was polymerized by conventional free radical methods to give a novel poly(sucrose acrylate) (**42b**, Scheme 11). Other examples

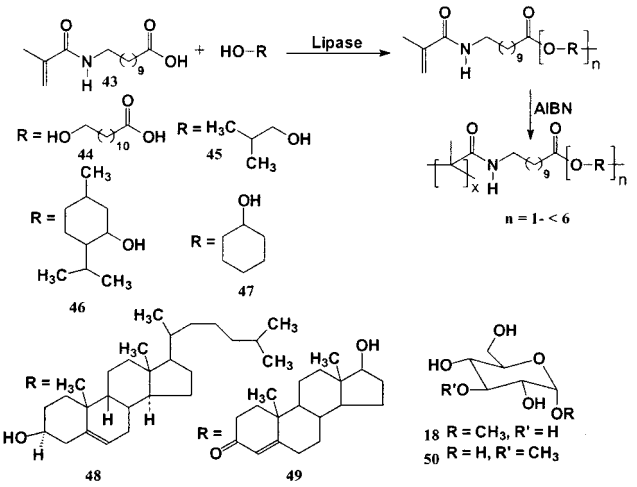
Scheme 11. Chemoenzymatic Synthesis of a Sucrose Polyester



42b

of chemoenzymatic methods that were used to prepare chiral monomers and functional polymers follow. Ritter and co-workers[38,39] used lipases from *C. cylindracea* and *C. antarctica* to catalyze the esterification of 11-methacryloylaminoundecanoic (**43**) acid with 12-hydroxyundecanoic acid (**44**), isobutyl alcohol (**45**), menthol (**46**), cyclohexanol (**47**), cholesterol (**48**), and testosterone (**49**). They also used *C. antartica* lipase to catalyze the regioselective esterifications of methyl α-D-glucopyranoside (**18**) and 3-*O*-methyl α-D-glucopyranose (**50**) with 11-methacryloylaminoundecanoic acid (**43**).[40] Subsequently, these monomers were polymerized by conventional free radical techniques (Scheme 12).

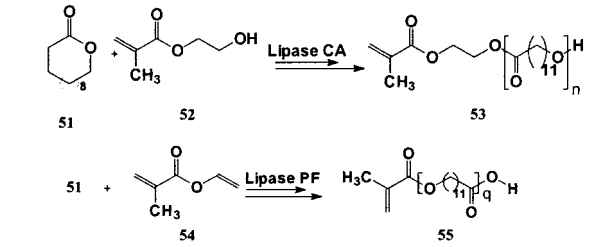
Scheme 12. Enzymatic Synthesis of a Polymerizable Oligoester



C. Macromers

Macromers are functional oligomers or low molecular weight polymers that are polymerizable. Kobayashi and co-workers[41] prepared polyesters with methacryloyl end groups (**53**, **55**). This was accomplished by the polymerization of DDL (**51**) in the presence of ethylene glycol methacrylate (**52**) and vinyl methacrylate (**54**) (Scheme 13). The acyl−

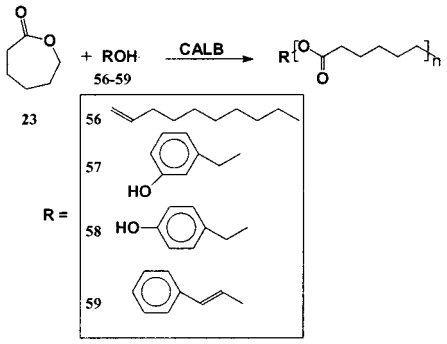
Scheme 13. Enzymatic Synthesis of a Functional Macromer



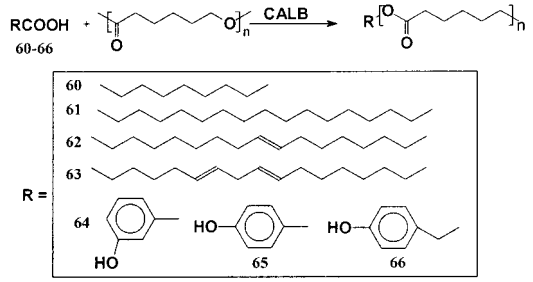
enzyme intermediate, formed by reaction of the lipase and the vinyl ester, reacted to terminate propagating chains.

Cordova and co-workers[42] prepared end-functionalized polycaprolactone (PCL) macromers using *C. antarctica* lipase B. ϵ-Caprolactone (CL) (**23**) was polymerized in the presence potential chain iniators that included 9-decenol (**56**), 2-(3-hydroxyphenyl)-ethanol (**57**), 2-(4-hydroxyphenyl)ethanol (**58**), and cinnamyl alcohol (**59**) (Scheme 14). Alternatively, CL polymerizations were conducted in the presence of potential chain-terminating carboxylic acids that included *n*-decanoic acid (**60**), octadecanoic acid (**61**), oleic acid (**62**), linoleic acid (**63**), 2-(3-hydroxyphenyl)-acetic acid (**64**), 2-(4-hydroxyphenyl)acetic acid (**65**), and 3-(4-hydroxyphenyl)propanoic) acid (**66**) (Scheme 15). In an effort to simultaneously control both the hydroxyl and the carboxyl end groups of macromers, esters [e.g., 9-decenyl oleate (**67**), 2-(4-hydroxyphenyl)ethyl acrylate (**68**), 9-decenyl-2-(4-hydroxyphenyl)acetate) (**69**), methyl linolenate (**70**)] or a sequence of an alcohol and an acid were added at various times during the course of *C. antarctica* lipase B-catalyzed CL (**23**) polymerizations (Scheme

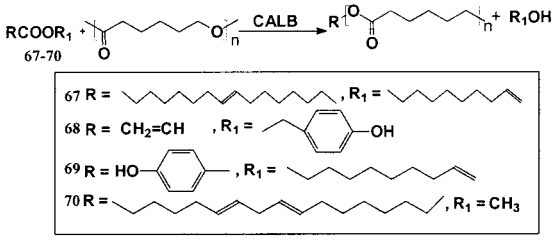
Scheme 14. Enzymatic Synthesis of End-Functionalized Polycaprolactone Monomers Using Alcohol as Initiating Group



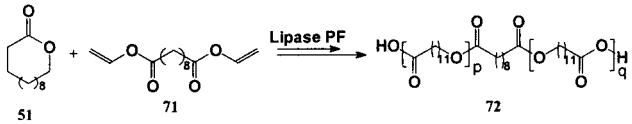
Scheme 15. Enzymatic Synthesis of End-Functionalized Polycaprolactone Monomers Using Carboxylic Acid as Chain-Terminating Group



Scheme 16. Enzymatic Synthesis of End-Functionalized Polycaprolactone Monomers Using Ester Functionality in the Reaction



Scheme 17. Enzymatic Synthesis of a Functional Macromer Using Dodecanolactone and Divinyl Sebacate



16). Similarly, a telechelic polymer (**72**) bearing carboxylic acid groups at both chain ends was formed by carrying out the lipase-catalyzed polymerization of DDL in the presence of divinyl sebacate (**71**) (Scheme 17).[41] In this case, divinyl sebacate acts as a coupling agent so that the hydroxyl propagating ends of poly(DDL) chains react with the lipase-activated acyl intermediate of divinyl sebacate.

III. Enzyme-Catalyzed Condensation Polymerizations

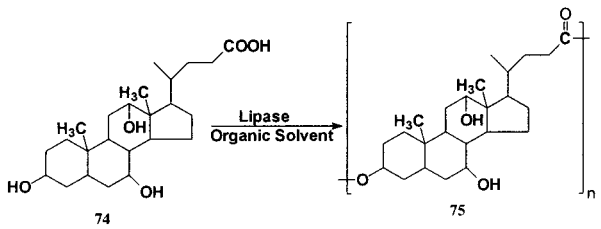
A. Self-Condensation Reactions

Polymerizations may be conducted with A−B type monomers, where the groups A and B can react with

other B and A groups, respectively. When this occurs with the evolution of small molecule leaving groups, these reactions are referred to as A−B type condensation polymerizations. An advantage of A−B type monomers relative to copolymerizations of A−A/B−B type monomers is that the latter requires equimolar quantities of A−A and B−B monomers to obtain high molecular weight polymers.

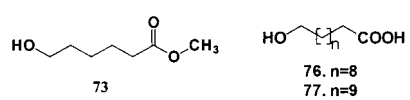
Gutman and co-workers[6] studied PPL-catalyzed A−B type polymerization of β-, δ-, and ϵ-hydroxyesters (**4**−**6**). Unsubstituted β−, δ-, and ϵ-hydroxyesters undergo exclusively intermolecular transesterification to afford the corresponding oligomers whereas substituted δ-methyl δ-hydroxyesters undergo lactonization (see section II). Knani and co-workers[43] were the first to consider the influence of enzyme origin, solvent, concentration, reaction time, and other parameters on self-condensation reactions. For this, they chose methyl ϵ-hydroxyhexanoate as a model monomer (**73**). The degree of polymerization (DP) of the polyester formed followed a S-shaped behavior with solvent log P ($-0.5 < \log P < 5$) with an increase in DP around log $P \sim 2.5$. Decreasing values of DP in good solvents for polyesters were attributed to the rapid removal of product oligomers from the enzyme surface, resulting in reduced substrate concentration near the enzyme. In a separate study by Knani et al.,[44] it was found that an increase in the size of hydroxyester lateral substituents from methyl to ethyl to phenyl gave slower polymerizations but higher enantioselectivity. Also Ritter and co-workers[38] reported the formation of oligomers from cholic acid (**74**, **75**) by self-condensation reactions catalyzed by the lipase from *C. antartica* (Scheme 18).

Scheme 18. *Candida antarctica* Catalyzed Self-Condensation Polymerization of Cholic Acid



74 75

Hagan and Zaidi[45,46] studied the *C. cylindracea* lipase-catalyzed condensation polymerization of 10-hydroxydecanoic acid (**76**) and 11-hydroxydecanoic acid (**77**). The polymerization of 10-hydroxydecanoic acid, carried out in hexane for 48 h at 55 °C with 3-Å molecular sieves, gave a polymer with $M_n = 9.3 \times 10^3$.[45,46] A time course study of 11-hydroxydecanoic acid polymerizations showed that oligomers are formed relatively rapidly and then later condense to generate higher molecular weight polyesters. By this method, within 7 days, they reported the formation of product with molecular weights up to 35×10^3. A compilation of lipase-catalyzed self-condensation polymerizations that have thus far appeared in the literature is given in Table 1.



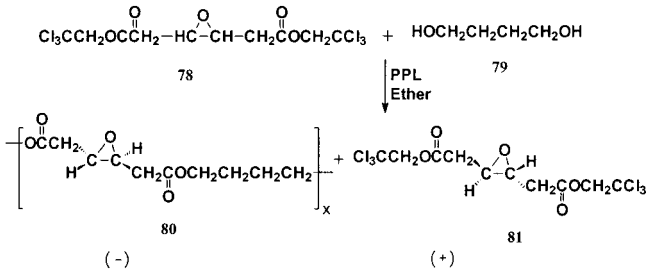
73 76. n=8 77. n=9

B. AA–BB Type Enzymatic Polytransesterifications

Okumara and co-workers[47] were the first to attempt the lipase-catalyzed synthesis of oligoesters from reactions between dicarboxylic acids (BB) and diols (AA). They showed that "trimer", "pentamer", and "heptamer" consisting of AA−BB−AA, AA−BB−AA−BB−AA, and AA−BB−AA−BB−AA−BB−AA, respectively, were formed. Klibanov and co-workers[48] used the stereoselectivity of lipases to prepare enantioenriched oligoesters. The reactions were conducted using racemic diester and an achiral diol or, conversely, a racemic diol and an achiral diester as monomers.

Wallace and Morrow[49,50] used halogenated alcohols such as 2,2,2-trichloroethyl to activate the acyl donor and thereby improve the polymerization kinetics. They also removed byproducts periodically during the reactions to further shift the equilibrium toward the growth of chains instead of chain degradation. Thus, these workers copolymerized bis(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate (**78**) and 1,4-butanediol (**79**) using porcine pancreatic lipase (PPL) as the catalyst. After 5 days, an enantioenriched polyester (**80**, **81**) with $M_w = 7900$ g/mol and an optical purity in excess of 95% was formed (Scheme 19).[49]

Scheme 19. Porcine Pancreatic Lipase-Catalyzed Synthesis of Enantioenriched Polyester with Epoxy Groups in the Main Chain



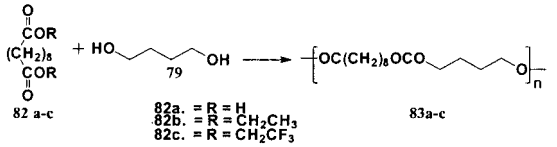
78 79 80 81 (−) (+)

Linko et al.[51] studied lipase-catalyzed polyesterification reactions where the degree of activation of the diacid was systematically varied. Thus, the synthesis of poly(1,4-butyl sebacate) (**83a**−**c**) from reactions of 1,4-butanediol (**79**) with sebacic acid (**82a**), diethyl sebacate (**82b**), or bis(2,2,2-trifluoroethyl) sebacate (**82c**) were performed in veratrole or diphenyl ether using the lipase from *Mucor miehei* (36.5 wt %). The effects of the removal of water or alcohol, the solvent character, and the substrate structure on the average molar mass of the products were determined. When vacuum was used to remove water formed during the polymerization, sebacic acid (**82a**) was directly polymerized with 1,4-butandiol in diphenyl ether to give a product with $M_w = 42 \times 10^3$ g/mol in 7 days at 37 °C (Scheme 20). The polymerization of bis(2,2,2-trifluoroethyl) sebacate (**82c**) with 1,4-butanediol in diphenyl ether gave the corresponding polyester with $M_w = 46\,400$ g/mol in 72 h at 37 °C with periodic removal of trifluoroethanol under vaccum (5 mmHg followed by 0.15 mmHg).[52] In addition, Linko et al.[52] systematically varied the chain length of the monomeric dicarboxylic acid [C-4 (**84**), C-6 (**85**), C-8 (**61**), C-10 (**82a**), and C-12 (**86**)]

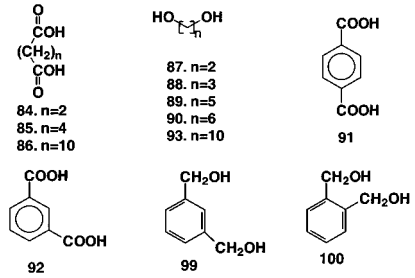
Table 1. Lipase-Catalyzed Condensation Polymerizations

enzyme	monomer	refs
Alcaligenes sp.	divinyl sebacate with glucose	67
Aspergillus niger (lipase A)	sebacic acid with 1,8-OL	8
	1,13-tridecandedoic acid with 1,3-propanediol	52
	bis(2-chloroethyl)(+ −)2,5-bromoadipate with 1,6-hexanediol	47
Candida antartica lipase B (Novozyme-435)	1,3-propanediol divinyl carbonate with 1,3-propanediol	61
	1,6-hexanediol divinyl carbonate with 1,2,4-butanetriol	61
	1,4-butanediol divinyl carbonate with glycerol	61
	divinyl carbonate with 1,2,4-butanetriol	61
	divinyl carbonate with 1,3-propanediol	61
	divinyl carbonate with 1,10-decanediol	61
	divinyl carbonate with 1,12-dodecanediol	61
	divinyl carbonate with 1,9-nonanediol	61
	divinyl carbonate with 1,3- benzenedimethanol	61
	divinyl carbonate with 1,4-benzenedimethanol	61
	divinyl carbonate with 2,6-pyridinedimethanol	61
	1,3-propanediol divinyl adipate with 1,3-benzenedimethanol	61
	1,3-propanediol divinyl adipate with 1,4-benzenedimethanol	61
	1,3-propanediol divinyl adipate with 2,6-pyridinedimethanol	61
	1,2-benzenedimethanol 4,4-isopropylidenebis[2-(2,6-dibromophenoxy)ethanol] bisphenol A	59
	divinyl isophthalate with 1,6-hexanediol divinyl terephthalate divinyl *p*-phenylene diacetate	59
	divinyl sebacate with *p*-xylene glycol	59
	1,3-propanediol divinyl dicarbonate with 1,3-propanediol	68
	1,3-propanediol divinyl dicarbonate with glycerol	68
	1,3-propanediol divinyl dicarbonate with 1,2,4-butanetriol	68
	1,3-propanediol divinyl dicarbonate with 1,2,6-trihydroxyhexane	68
	1,4-butanediol divinyl dicarbonate with glycerol	68
	1,4-butanediol divinyl dicarbonate with 1,2,4-butanetriol	68
	1,4-butanediol divinyl dicarbonate with 1,2,6-trihydroxyhexane	68
	1,6-hexanediol divinyl dicarbonate with glycerol	68
	1,6-hexanediol divinyl dicarbonate with 1,2,4-butanetriol	68
	1,6-hexanediol divinyl dicarbonate with 1,2,6-trihydroxyhexane	68
	divinyl adipate with glycerol	69
	divinyl adipate with 1,2,4-butanetriol	69
	divinyl adipate with 1,6-trihydroxyhexane	69
	adipic acid with 1,4-butanediol	55, 61
	divinyl adipate with 2,2,3,3-tetrafluoro-1,4-butanediol	62
	divinyl adipate with 2,2,3,3,4,4-hexafluoro-1,5-pentanediol	62
	divinyl adipate with 3,3,4,4,5,5,6,6-octafluorooctan-1,8-diol	62
	divinyl sebacate with glucose	67
	cholic acid	38
Candida cylinderacea (lipase CCL)	11-hydroxyundecanoic acid	46
	10-hydroxyundecanoic acid	45
	sebacic acid with 1,8-OL	8
Candida rugosa (lipase AYS)	bis(2,2,2-trifluoroethyl) sebacate with 1,4-butanediol	51
Klebsiella oxytota (lipase K)	sebacic acid with 1,8-OL	8
Mucor meihei (lipozyme)	sebacic acid with 1,4-butanediol	51
	diethyl sebacate with 1,4-butanediol	51
	bis(2,2,2-trifluoroethyl) sebacate with 1,4-butanediol	51
	bis(2,2,2-trifluoroethyl) sebacate with 1,4- butanediol	51, 52
	bis(2,2,2-trifluoroethyl) sebacate with 1,2-ethanediol	51
	bis(2,2,2-trifluoroethyl) sebacate with 1,3-propanediol	51
	bis(2,2,2-trifluoroethyl) sebacate with 1,5-pentanediol	51
	bis(2,2,2-trifluoroethyl) sebacate with 1,6-hexanediol	51
	divinyl adipate with glycerol	69
	divinyl adipate with 1,2,4-butanetriol	9
	divinyl adipate with 1,6-trihydroxyhexane	69
	bis(2,3-butanedionemonooxime) glutarate with 1,6-hexanediol	65
	dichloroethyl fumarate with 4,4′-isopropylidenebis[2-(2,6-dibromophenoxy)ethanol]	70
	adipic acid with 1,4-butanediol	71
porcine pancreatic lipase (PPL)	divinyl sebacate with glucose	67
	sebacic acid + 1,8-OL	8
	bis(2,2,2-trifluoroethyl) sebacate with 1,4-butanediol	58, 71
	bis(2,2,2-trifluoroethyl) glutarate with 1,4-butanediol	72
	bis(2,2,2-trichloroethyl) adipate with 1,4-butanediol	73
	bis(2,2,2-trichloroethyl) *trans*-3-hexanedioate (racemic mixture) with 1,4-butanediol	49
	methyl-5-hydroxypentanoate methyl-6-hydroxyhexanoate	43
Pseudomonas aeruginosa (lipase PA)	sebacic acid with 1,8-OL	8
Pseudomonas cepacia (lipase PS, PS-30)	sebacic acid + 1,8-OL	8
Pseudomonas fluoresenes (lipase PF)	sebacic acid with 1,8-OL	8
	divinyl adipate with 1,4-butanediol	57
	bis(2,2,2-trifluoroethyl) sebacate with 1,4-butanediol	51
Rhizopus delemer (lipase RD)	divinyl sebacate with glucose	67
Bacillus sp. Proleather	sucrose with bis(2,2,2-trifluoroethyladipate)	37
Pseudomonas sp. lipase (PSL)	divinyl sebacate with glucose	67
Steptomyces sp. alkaline protease	divinyl adipate with arabinose	33
	divinyl sebacate with glucose	67

Scheme 20. Lipase-Catalyzed Condensation Polymerization of Sebacic Acid Ester with Butanediol



and diol [C-2 (**87**), C-3 (**88**), C-4 (**79**), C-5 (**89**), and C-6 (**90**)] used for the polycondensation polymerization. Of the lipases and solvents screened, the *M. miehei* lipase and diphenyl ether, respectively, were found to be preferred. It was also observed that the molecular weight of the polymer increased with an increase in the substrate concentration of up to about 0.83 M. The reaction of adipic acid (**85**) with different diols showed the following trend with respect to polymer DP: 1,6-hexanediol > 1,4-butanediol > 1,5-decanediol > 1,3-decanediol >1,2-butanediol.[52] Similarly, the reaction of 1,6-hexanediol with different acids showed the following trend toward polymer DP: adipic acid > sebacic acid > octanedioic acid > dodecanoic acid > succinic acid.[52] *M. miehei* catalyzed the condensation polymerization of adipic acid (**85**) and hexanediol (**90**) in diphenyl ether at 37 °C for 7 days under reduced pressure (0.15 mmHg) to give the corresponding product with $M_w = 77.4 \times 10^3$ and PDI = 4.4.[52] Subsequently, using the preferred *M. miehei* lipase from above, Linko et al.[53]studied copolymerizations using an aromatic diacid (terephthalic or isophthalic) (**91**, **92**) and an aliphatic diol (1,4-butane- or 1,6-hexanediol) (**79**, **90**). Even at temperatures up to 70 °C, the polymerization of these diacids built from aromatic moieties was unsuccessful. However, using Novozyme-435 as the catalyst, the polymerization of aromatic diacids was accomplished. For example, while the Novozyme-435-catalyzed reaction of isophthalic acid with butanediol yielded oligomers, a similar reaction between the C-6 diol and isopthalic acid at 70 °C yielded a polymer with $M_w = 55 \times 10^3$.



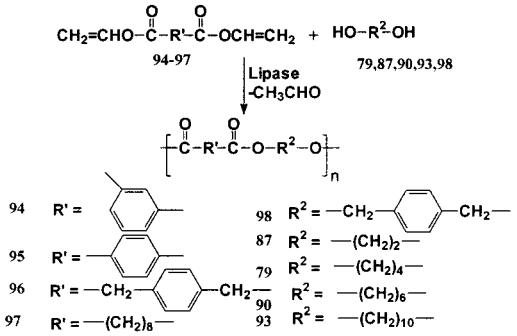
Kobayashi and co-workers[54] studied the potential of carrying out condensation reactions in solventless or bulk reactions. They reported the preparation of aliphatic polyesters with $M_w > 10 \times 10^3$ by reacting sebacic acid (**82a**) with 1,4-butanediol (**79**) in solvent-free system. The reactions were conducted under reduced pressure, using the *C. antarctica* lipase. Binns et al.[55] studied copolymerizations of adipic acid (**85**) and 1,4-butanediol (**79**) using Novozyme-435. They reported that under solvent-free conditions, the mixture was refluxed at 40 °C for 4 h, followed by heating at 60 °C for 10 h under pressure. The polymerization proceeds by a step growth mechanism to give a homogeneous mixture. The GPC of reaction mixture after 4 and 14 h showed very different product distribution, the former showing a discrete array of predominantly hydroxy-terminated oligomers and latter showing the polyesters with weight average molecular weight of 2227 and polydispersity of 1.5. A compilation of lipase-catalyzed AA−BB type condensation polymerizations that have thus far appeared in the literature is given in Table 1.

C. Use of Activated Enol Esters in Condensation Polymerizations

Much of the work carried out on lipase-catalyzed condensation polymerizations has focused on the use of activated diacids such as enol esters. Enol or vinyl esters both accelerate the rate of acyl transfer and shift the reaction equilibrium toward polymer since the byproduct rapidly tautomarizes to a ketone or aldehyde. Acyl transfer using enol esters has been shown to be about 10 times slower than hydrolysis and about 10−100 times faster than acyl transfer using other activated esters.[56] For example, enzymatic hydrolysis of nonactivated esters such as ethyl esters reacts at rates about 10^{-3}−10^{-4} times slower than the corresponding enol esters. Uyama et al.[57] used the *Pseudomonas fluorescens* lipase to investigate polymerizations of divinyl adipate (**27**) with different diols [ethylene glycol (**87**), 1,4-butanediol (**79**), 1,6-hexanediol (**90**), 1,10-decanediol (**93**)]. These polymerizations were performed in isopropyl ether for 48 h at 45 °C. The polymerization of divinyl adipate and 1,4-butanediol catalyzed by *P. fluorescens* in isopropyl ether at 45 °C for 48 h produced a corresponding polyester in 50% yield with weight average molecular weight of 6700 and polydispersity of 1.9. The *P. fluorescens* catalyzed polymerization of divinyl adipate with ethylene glycol, 1,6-hexanediol, or 1,10-decanediol as glycols resulted in polyesters of lower molecular weights (2000, 5900, and 2700, respectively) than the polyester obtained from the polymerization of divinyladipate and butanediol. In an effort to more efficiently conduct lipase-catalyzed polymerizations of divinyl isophthalate (**94**), terephthalate (**95**), *p*-phenylene diacetate (**96**), and sebacate (**97**) diesters, Uyama et al.[58] expanded on the work above. The importance of lipase origin (*C. antartica*, *C. cylinderacea*, *M. meihei*, *P. cepacia*, *P. fluorecens*, and porcine pancreas), temperature (45−75 °C), solvent (e.g., heptane, acetonitrile, cyclohexane, isooctane, THF, and toluene), and effects of chain length in α,ω-alkylene glycols (**79**, **87**, **90**, **93**, **98**) (Scheme 21) were evaluated. Of the lipases screened, lipase CA gave polyesters having the highest molecular weights. Also, nonpolar solvents such as heptane and cyclohexane were preferred. Furthermore, the maximum yields and product molecular weights were obtained at 60 °C. For example, lipase CA catalyzed polymerization of divinyl isophthalate (**94**) and 1,6-hexanediol (**90**) in heptane at 60 °C resulted in polyester formation in 74% yield with M_n and PDI of 5500 and 1.6, respectively, in 48 h.

Russell and co-workers[59] studied the Novozyme-435-catalyzed bulk polymerization of divinyl adipate

Scheme 21. Lipase-Catalyzed Condensation Polymerization of Divinyl Sebacate with Diols of Varying Length



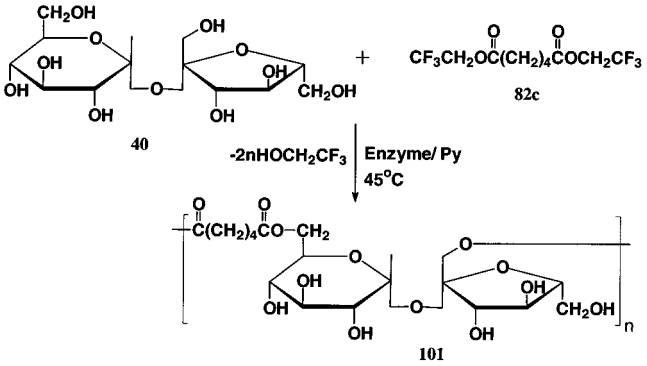
(**27**) and 1,4-butanediol (**79**). The preferred example was a 72-h polymerization at 50 °C that gave the corresponding polyester with $M_w = 23.2 \times 10^3$ g/mol. These workers found that the product molecular weight was decreased when the reaction was conducted without taking proper precautions to exclude reactions of water with reactive divinyl ester groups. They also found excellent agreement between their experimental data and that predicted by a mathematical model.[60] Such agreement allowed Russell and co-workers[61] to conclude that the polymerizations occur by a step-condensation mechanism where the rate of polymerization is a function of substrate molecular weight.

Russell and co-workers[61] also studied Novozyme-435-catalyzed A−A/B−B type condensation polymerizations to prepare aromatic polyesters and polycarbonates. Polymerizations between divinyl esters or dicarbonates with aromatic diols, conducted for 24 h in bulk catalyzed by Novozyme-435 (1/10 wt % of monmers) at preferably 70 °C, gave low molecular weight polycarbonates and polyesters, respectively. The aromatic diols included in their work were as follows: 1,2-benzenedimethanol, 1,3-benzenedimethanol, 1,4-benzenedimethanol, 2,6-pyridinedimethanol or 4,4-isopropylidenebis(2-(2,6-dibromophenoxy)ethanol), and bisphenol A. The M_w of these polycarbonates and polyesters did not exceed 5.2×10^3 and 3.5×10^3, respectively. When various isomers of benzenedimethanol (**98**−**100**) were used, Novozyme-435 exhibited regioselectivity. Thus, *p*-benzenedimethanol (**98**) reacted to a greater extent than the corresponding meta or ortho isomers (**99** and **100**, respectively). The regioselectivity of lipases can be exploited in such work so that selected isomers from complex mixtures are preferentially polymerized.

Russell and co-workers[62] have also studied lipase-catalyzed polymerizations of activated diesters and fluorinated diols. The effects of reaction time, continuous enzyme addition, enzyme concentration, and diol chain length were studied to determine the factors that would limit chain growth. Potential limiting factors considered were enzyme inactivation, enzyme specificity, reaction thermodynamics, hydrolysis of activated esters, and polymer precipitation. As was seen many times above, Novozyme-435 was found to be the effective catalyst. Polymer molecular weight steadily increased and then leveled off at 50 °C after 30 h at $M_w \sim 1773$. Enzyme specificity toward shorter chain fluorinated diols appeared to be a prominent factor that limited chain growth. An increase in the product molecular weight resulted when the fluorinated diol contained an additional CH_2 spacer between CF_2 and hydroxyl groups. For example, no polymer was produced for the reactions of divinyl adipate (**27**) with 1*H*,1*H*,9*H*,9*H*-perfluoro-1,9-nonanediol [$HOCH_2(CF_2)_7CH_2OH$], 1*H*,1*H*,-12*H*,12*H*-perfluoro-1,12-dodecanediol [$HOCH_2(CF_2)_{10}$-CH_2OH], fluorinated oligomers of $M_w \sim 727$ from condensation of 2,2,3,3,4,4-hexafluoro-1,5-pentanediol [$HOCH_2(CF_2)_3CH_2OH$] with divinyl adipate (**27**), and fluorinated polyesters with $M_w \sim 1095$ were produced from reaction of divinyl adipate (**27**) with 2,2,3,3-tetrafluoro-1,4-butanediol [$HOCH_2(CF_2)_2CH_2OH$]. Reactions between divinyl adipate and 3,3,4,4,5,5,6,6-octafluorooctan-1,8-diol [$HOCH_2CH_2(CF_2)_4CH_2CH_2OH$] showed that an additional methylene spacer between the fluorine atoms and the hydroxyl groups has a significant effect on the polymerization in THF and in solvent-free conditions resulting in polyesters of $M_w = 3245$ and 8094, respectively.[62]

Other researchers have exploited enzyme regioselectivity to prepare sugar-based building blocks for condensation[63] and vinyl chain polymerizations.[64] For example, lipases including *C. cylindracea*, PPL, and *P. fluorescens* and proteases including Proleather and protease N were screened as catalysts for the butyrylation of sucrose (**40**).[64] On the basis of this model study, Proleather in pyridine at 45 °C was selected and used to synthesize polyesters (**101**) from sucrose and bis(2,2,2-trifluoroethyl) sebacate (**82c**). By employing this strategy, a sucrose-based polyester was obtained in 20% yield with DP 11 after 5 days (Scheme 22).

Scheme 22. Synthesis of a Sucrose Polyester Catalyzed by Proleather in Anhydrous Pyridine



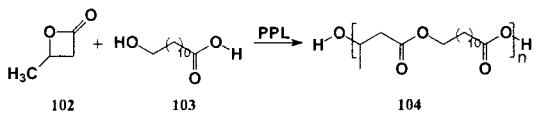
Similar to enol esters, oximes can also be used as irreversible acyl transfer agents for lipase catalysis. Thus, instead of a dienol ester, Athavale et al.[65] polymerized diols with bis(2,3-butanedione monoxime) alkanedioate using lipozyme IM-20. The results obtained by activation with enol esters, and their corresponding oximes were comparable. No attempts were made to analyze the end group of the polyester.

A compilation of lipase-catalyzed condensation polymerizations that have thus far appeared in the literature where activated diesters were used is given in Table 1.

D. Combination of Condensation and Ring-Opening Polymerizations

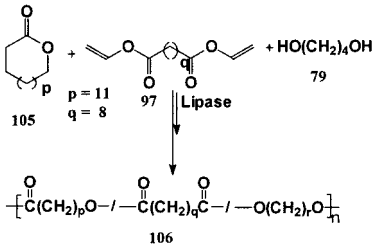
An example of the versatility of lipase reactions is their ability to concurrently catalyze condensation and ring-opening chain-type polymerizations. Jedlinski et al.[66] reported that PPL catalyzes 12-hydroxydodecanoic acid (**103**)/β-butyrolactone (**102**) copolymerizations at 45 °C in organic media (**104**, Scheme 23). Using the preferred solvent (toluene),

Scheme 23. Ring-Opening Copolymerization of β-Propiolactone in the Presence of Hydroxy Acid



after 72 h, the copolymer was formed in 70% yield with M_n of 1 800 and PD 1.1. The electrospray ionization mass spectrum (ESI-MS) of the copolymer showed that the chain segments formed contained various compositions of 3-hydroxybutanoate and 12-hydroxydodecanoate units. Kobayashi and co-workers[8] found that the lipase from *P. cepacia* (lipase PC) catalyzed the concurrent copolymerization of macrolides, divinyl esters, and glycols (Scheme 24). The

Scheme 24. Copolymerization Using Combination of Ring-Opening and Condensation Reactions



copolymerization of PDL (**105**), divinyl sebacate (**97**), and 1,4-butanediol (**79**) in isopropyl ether at 60 °C for 72 h gave a copolymer (**106**) in 80% yield with $M_w = 6.5 \times 10^3$ g/mol. The authors did not provide information on the repeat unit sequence distribution of the products.

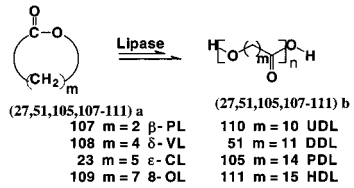
A compilation of examples and literature references that describe lipase-catalyzed polymerizations with concurrent condensation and ring-opening step polymerization reactions is given in Table 1.

IV. Enzyme-Catalyzed Ring-Opening Polymerization

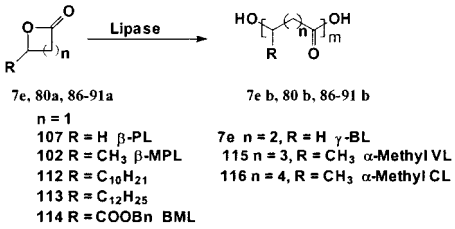
A. Ring-Opening Polymerization of Lactones

In contrast to condensation polymerizations, ring-opening polymerizations of lactones and carbonates do not generate a leaving group during the course of the reactions.[28,29,74,75] This characteristic alleviates concerns that the leaving group, if not efficiently removed, might limit monomer conversion or polymer molecular weight.[76] Research has been conducted on enzyme-catalyzed ring-opening polymerizations of (±)-α-methyl-β-propiolactone (**10**),[1] ϵ-caprolactone (ϵ-CL) (**23**),[28,29,41,42,74,75] β-methyl-β-propiolactone (**102**),[77]

Scheme 25. Lactones That Have Been Studied as Monomers for Lipase-Catalyzed Ring-Opening Polymerizations



Scheme 26. Ring-Opening Polymerization of Substituted Lactones



dodecanolactone (DDL) (**51**),[8,41,78] pentadecanolactone (PDL) (**105**),[40,78−81] β-propiolactone (**107**),[77,82] δ-valerolactone (δ-VL) (**108**),[74] 8-octanolide (8-OL) (**109**),[83] undecanolactone (UDL) (**110**),[8,41,78,79,84] hexadecanolactone (HDL) (**111**),[41] α-decenyl-β-propiolactone (**112**),[85] α-dodecenyl-β-propiolactone (**113**),[85] benzyl-β-D,L-malonolactonate (**114**),[86] α-methyl-ϵ-caprolactone (**116**),[87] α-methyl-δ-valerolactone (**115**),[87] γ-butyrolactone (**7e**),[77,85] 1,4-dioxane-2-one (**117**),[88] and others (Schemes 25 and 26). A compilation of the lactones polymers, the enzymes used, and the corresponding citation(s) is given in Table 2.

Common difficulties reported in the publications listed in Table 2 are low product molecular weights and slow polymerization kinetics. In an effort to overcome these difficulties, our laboratory has investigated reaction parameters such as solvent, temperature, monomer concentration in solvents, enzyme concentration, water content, propagation kinetics, and mechanism of *C. antarctica* lipase (Novozyme-435)-catalyzed ϵ-CL polymerizations.[75,89] It was observed that different combinations of these parameters could be used to control the polymerization rate, molecular weight, and polydispersity.

In contrast to 4-, 6-, and 7-membered lactone polymerizations that can be conducted with good efficiency using traditional chemical initiators and catalysts,[90] the polymerization of macrolactones by traditional chemical methods proceeds slowly to give low molecular weight polymers.[91] Enzyme-catalyzed polymerizations of macrolactones have thus far proved advantageous relative to chemical preparative routes. Kobayashi and co-workers[41,78,79,84] were first to investigate the enzyme-catalyzed polymerization of ω-undecanolide (UDL) (**110**), ω-dodecanolide (DDL) (**51**), and ω-pentadecanolide (PDL) (**105**) (12-, 13-, and 16-membered lactones) (Scheme 25). Screening of enzymes for the polymerization of UDL (**110**), DDL (**51**), and PDL (**105**) using lipases including those from *Aspergillus niger*, *C. cylindracea* (lipase B), *Candida rugosa*, *Rhizopus delmar*, *Rhizopus javanicus*, *P. fluorescens* (lipase P, Cosmo Bio.) *Pseudomonas* sp. (lipase PS, Amano), phospholipase, and

Table 2. Lipase Catalyzed Ring-Opening Polymerizations

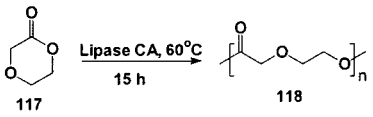
enzyme	monomer/comonomers	refs
Aspergillus niger (lipase A)	ϵ-CL	8, 114
	DDL	8
	PDL	78, 80
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	93
Candida antartica lipase B (Novozyme-435)	ϵ-CL	42, 115, 116
	8-OL	8, 83
	1,4-dioxane-2-one	88
	β-BL(R,RS)	117
	TMC	89, 92
	PDL	80
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	93
	α-Me-γ-VL	87
	α-Me-ϵ-CL	87
	8-OL, 8-OL-*co*-ϵ-CL, 8-OL-*co*-DDL	83
Candida cylinderacea (lipase CCL)	8-OL	83
	TMC	92, 95
	α-Me-β-PL	1
	ϵ-CL	8, 41, 114
	δ-VL	8, 113
	PDL	8, 41, 78
	β-BL (R,RS)	117
	β-PL	82
	DDL	8, 41
	UDL	8, 41
Candida rugosa (lipase AYS)	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	93
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one-*co*-TMC	98
	PDL	78
Mucor javanicus (lipase M, map-10)	β-BL(R,RS)	117
	PDL	80
	TMC	92
Mucor meihei (lipozyme)	β-BL(R,RS)	117
	PDL	78
	TMC	92, 95
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	93
Pencillium roruefortí (lipase PR)	PDL	78
porcine pancreatic lipase (PPL)	DDL	8
	β-BL(R,RS)	117
	ϵ-CL	8, 28, 29, 41, 114
	γ-VL	8, 114
	TMC	92, 95, 30
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	93
	β-BL	77
	α-Me-β-PL	1
	PDL	78
	β-BL-*co*-12-hydroxydodecanoic acid	66
	lactide-*co*-TMC	67
Pseudomonas aeruginosa (lipase PA)	ϵ-CL	8
	DDL	8
	S-MOHEL	8
	8-OL	83
Pseudomonas cepacia (lipase PS, PS-30)	β-BL(R,RS)	117
	δ-DL	116
	δ-DDL	8, 103
	β-BL	77, 116
	ϵ-CL	8, 41, 113, 116
	γ-VL	116
	γ-CL	116
	TMC	92, 95
	PDL	78, 80
	5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one	93
	8-OL	8, 83
	DDL	8
	MOHELs	8
	HDL	41
	PDL-*co*-ϵCL	78
	8-OL-*co*-ϵCL	83
	8-OL-*co*-DDL	83
	α-Me-β-PL	76
Pseudomonas fluoresenes (lipase PF)	ϵ-CL	8, 41, 66, 78, 114
	δ-VL	8, 114
	S-MOHEL	8
	UDL	8, 78
	DDL	8, 41, 78
	PDL	8, 41, 78
	HDL	41
	8-OL	8, 83
	PDL-*co*-γ-VL	78
	PDL-*co*-ϵ-CL	78
	PDL-*co*-DDL	78
	PDL-*co*-UDL	78
	TMC	92

Table 2. (Continued)

enzyme	monomer/comonomers	refs
Rhizopus delemer (lipase RD)	ϵ-CL	114
	PDL	78
Rhizopus japonicus (lipase RJ)	ϵ-CL	41, 114
	γ-VL	114
	PDL	78
HE	PDL	78
PD	PDL	78
PR	PDL	78
CR	PDL	78
clonezyme ESL-001 library	HEC-modified CL	118
Pseudomonas sp. lipase (PSL)	ϵ-CL, β-BL, γ-BL, δ-DCL, δ-DDL, PDL	85
	ethyl 4-hydroxybutyrate, ethyl-6-hydroxyhexanoate, ethyl-3-hydroxybutyrate, ethyl 5-hydroxy-hexanoate, ethyl 5-hydroxylaurate, ethyl 15-hydroxypentadecanoate	
	ϵ-CL-*co*-ethyl lactate, ϵ-CL-*co*-lactide, ϵ-CL-*co*-γ-BL, ϵ-CL-*co*-ethyl 4-hydroxybutyrate, ϵ-CL-*co*-PDL, ϵ-CL-*co*-ethyl 15-hydroxypentadecanoate, ϵ-CL-*co*-lactide-*co*-cyclopentadecanolide	

porcine pancreatic lipase was carried out.[78,79,84] Quantitative conversions of UDL to poly(UDL) (**110b**) were achieved within 120 h using lipase P and PS.[79] The highest number average molecular weight (M_n = 25 000 g/mol) reported by these workers was for poly(DDL) (**51b**) synthesis (75 °C, 120 h) using the immobilized lipase PS from a *Pseudomonas* sp. (lipase PS, Toyobe Co.).[84] Our laboratory evaluated lipase PS-30 immobilized on Celite as a catalyst for bulk PDL polymerization and reported the synthesis of poly(PDL) with M_n = 62 000 and PDI 1.9.[80] Recently, instead of in bulk, Novozyme-435-catalyzed polymerization of PDL was conducted in toluene (1:1 wt/vol). These conditions resulted in 31% monomer conversion within only 1 min and the formation of poly(PDL) (**105b**) with the highest M_n (86 000 g/mol) thus far reported for lipase-catalyzed polymerization.[81] Also, lipase catalysis has been explored for 1,4-dioxan-2-one[117] ring-opening polymerization (Scheme 27).[88] An immobilized form of lipase CA, at 60 °C,

Scheme 27. Ring-Opening Polymerization of 1,4-Dioxan-2-one

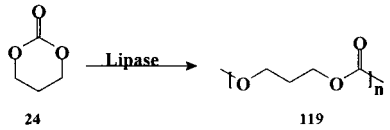


exhibited high catalytic activity for this monomer. The weight average molecular weight of poly(1,4-dioxanone,[118]) was 40 000 after 15 h using 5 wt % of the immobilized lipase CA.

B. Lipase-Catalyzed Poly(carbonate) Synthesis

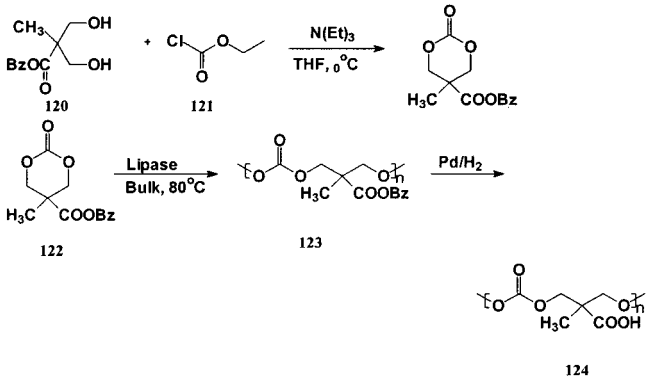
Lipases have also been used to catalyze the ring-opening polymerization of cyclic carbonate monomers.[92−95] Novozyme-435, PPL, PS-30, AK, CCL, MAP, and lipozyme-IM were evaluated by us as catalysts for the bulk polymerization of trimethylene carbonate (TMC, 1,3-dioxan-2-one) (**24**).[92] Of these catalysts, Novozyme-435 has been most effective. In one example, Novozyme-435-catalyzed polymerization of TMC at 70 °C for 120 h gave 97% monomer conversion, poly(TMC) (**119**) with M_n = 15 000, without decarboxylation during propagation (Scheme 28).[92] Similarly Matsumura et al.[95] claimed an ex-

Scheme 28. Ring-Opening Polymerization of 1,3-Dioxan-2-one



traordinarily high molecular weight poly(TMC) (M_w = 156 000) was obtained by using low quantities of PPL (0.1 wt %) as the catalyst at very high reaction temperature (100 °C). The thermal polymerizability of TMC in the absence of enzyme cast questions as to whether the polymerization proceeded by an enzyme-catalyzed mechanism. In contrast, Kobayashi et al.[94] reported the formation of low molecular weight poly(TMC) (M_n = 800) by PPL (50 wt %) catalyzed polymerization at 75 °C. The lipase-catalyzed polymerization of the disubstituted TMC analogue 5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one (MBC, **120** + **121** = **122**) was also studied (Scheme 29).[93] The bulk polymerization, catalyzed by

Scheme 29. Synthesis of Substituted TMC and Its Ring-Opening Polymerization Using Lipase as Catalyst

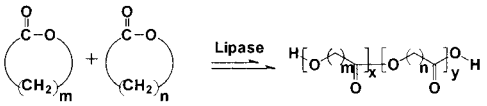


lipase AK (from *Pseudomonas fluorescens*) for 72 h at 80 °C, gave 97% monomer conversion and product (**123**) with M_n = 6100. The benzyl ester protecting groups of poly(MBC) were removed with Pd/C in ethyl acetate to give the corresponding functional polycarbonate with pendant carboxylic acid groups (**124**, Scheme 29).

C. Polyester Synthesis by Lipase-Catalyzed Ring-Opening Copolymerizations

The lipase-catalyzed copolymerization of two or more monomers presents additional challenges and questions. For example, knowledge of the relative reactivity of comonomers as well as the kinetic and thermodynamic distribution of repeat units along chains is particularly important. Since the use of lipase catalysis for lactone polymerization is a relatively new area of study, lactone copolymerizations has thus far received little attention. Namekawa and co-workers[96] first studied the enzyme-catalyzed copolymerization of β-propiolactone (**107**) with ϵ-CL (**23**). Uyama and co-workers[78] performed copolymerizations of PDL (**105**) with DDL (**51**), UDL (**110**), VL (**108**), and CL (**23**) using the lipase from *P. fluorescens*, in bulk for 240 h at 60−75 °C (Scheme 30).

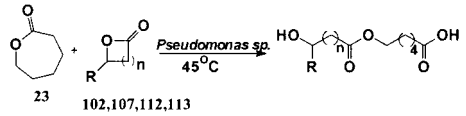
Scheme 30. Ring-Opening Copolymerization of Lactone Monomers



105 m = 14 51. n = 11
105 m = 14 110. n = 10
105 m = 14 108. n = 4
105 m = 14 23. n = 5
109. m = 7 23. n = 5
109. m = 7 51. n = 11

The rates of these reactions were slow and yielded low molecular weight (M_n < 6000 g/mol) copolymers. Kobayashi and co-workers[83] reported copolymerization of δ-valerolactone (**108**) and ϵ-CL (**23**) using the lipase from *P. fluorescens*. They also published the use of a lipase from *C. antarctica* for the copolymerization of 8-OL (**109**) with CL (**23**) and DDL (**51**) for 48 h at 60 °C in isooctane.[83] In this later report, copolymers were formed with percent conversions of ~80% in 48 h at 60 °C with M_n values up to 9000 (0.10 g of lipase/1.0 mmol of monomer). Dong and co-workers[85] reported copolymerizations (bulk, 45 °C, 20 days) of ϵ-caprolactone with some cyclic and linear monomers catalyzed by the lipase from a *Pseudomonas* sp. (40 mg of lipase/0.1 mmol of monomer). Among the copolymerizations performed, that of ϵ-caprolactone (**23**) with pentadecalactone (**105**) gave the highest product M_n (8.4 kDa).[85] The molecular weights of copolymers of ϵ-caprolactone (**23**) with lactones (**102**, **107**, **112**, **113**) were higher than those of copolymers prepared from the corresponding linear hydroxyesters (Scheme 31).

Scheme 31. Ring-Opening Copolymerization of Caprolactone and β-Propiolactone and Its Derivatives



23
102,107,112,113
107 = R = H
102 = CH_3
112 = $C_{10}H_{21}$
113 = $C_{12}H_{25}$

Our laboratory investigated copolymerizations of ϵ-caprolactone (**23**) and pentadecalactone (**105**) (1:1 molar feed ratio, 45 min, 70 °C) catalyzed by Novozyme-435 in toluene. The product, obtained in 88% isolated yield, had an M_n of 20 000 (Figure 1).[81] The

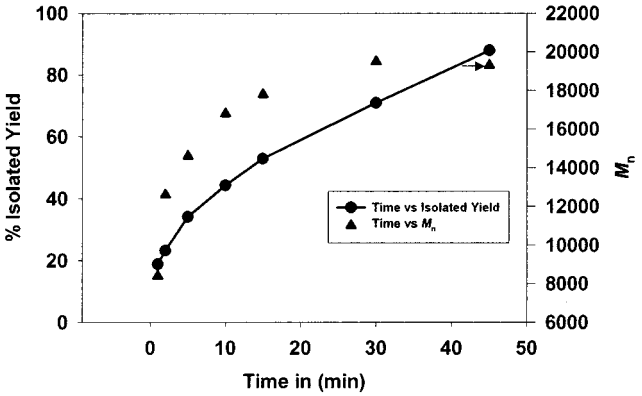


Figure 1. Conversion profile of monomer during the polyesterification of PDL at 60 °C.

reactivity ratios of the two monomers were calculated, and PDL polymerization was found to be 13× faster than that of ϵ-caprolactone. Despite the difference in reactivities of the two monomers, the copolymers formed were random. These results were explained by rapid Novozyme-435-catalyzed polymer−polymer transacylation or transesterification reactions.[81]

D. Poly(ester-*co*-carbonate) Synthesis by Lipase-Catalyzed Ring-Opening Copolymerizations

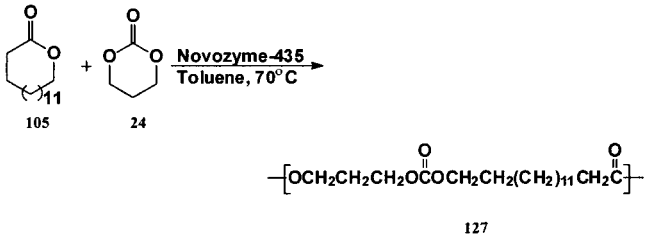
There has been a significant effort to copolymerize TMC with lactones and other carbonate monomers. Matsumura and co-workers[97] performed copolymerizations of lactide (**125**) with trimethylene carbonate (**24**) using porcine pancreatic lipase at 100 °C for 168 h. They claimed that random copolymers were formed (**126**, Scheme 32) with M_w values up to 21 000.

Scheme 32. Ring-Opening Copolymerization of Lactide and TMC

125 24 126

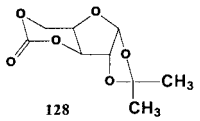
However, since trimethylene carbonate is known to thermally polymerize at 100 °C, the extent of polymerization that occurs due to activation of monomers at the lipase catalytic triad versus by thermal or other chemical processes is questionable.[92] Lipase AK-catalyzed copolymerizations of 1,3-dioxan-2-one (TMC, **24**) with 5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-one (MBC,**122**) were carried out in bulk at 80 °C for 72 h. Although TMC reacted more rapidly than MBC, the product isolated at 72 h appeared to have a random repeat unit distribution.[98] Recently, using Novozyme-435 in toluene at 70 °C, trimethylene carbonate/PDL copolymerizations (**24**, **105**) were performed and gave random copolymers (**127**, Scheme

Scheme 33. Ring-Opening Copolymerization of PDL and TMC



33).[99] Varying the feed ratio of the comonomers allowed regulation of the copolymer composition. The isolated yield and M_n of poly(PDL-*co*-43 mol % TMC) formed after 24 h (feed 2:1 PDL:TMC) was 90% and 30.9×10^3 g/mol, respectively. Thus far, an alternative chemical route to random poly(PDL-*co*-TMC) is not known. For example, PDL/TMC copolymerizations with chemical catalyst such as stannous octanoate, methylaluminoxane, and aluminum triisoproxide resulted either in homo-polyTMC or block copolymers of poly(TMC-*co*-PDL).[99] Chemical catalysts have thus far favored TMC over PDL polymerization. In contrast, by lipase catalysis, PDL was more rapidly polymerized than TMC. Thus, herein lie important differences in the inherent catalytic properties of lipases as opposed to chemical catalysts that can be exploited to give unique copolymers.

Recently, we studied lipase-catalyzed copolymerization of PDL (**105**) with a sugar carbonate (IPXTC, **128**) in toluene at 70 °C. [100] Novozyme-435 was found to be the most effective lipase catalyst based on its ability to form PDL/IPXTC copolymers. For example, by this method, poly(PDL-*co*-19 mol % IPXTC) was prepared in 38% isolated yield in 5 days with M_n = 4070. The copolymer formed consisted of PDL blocks with random interruptions by IPXTC units or short segments.



V. Effect of Reaction Parameters on Polyester Synthesis

To improve propagation kinetics and increase product molecular weight, reaction parameters such as solvent, temperature, monomer/solvent ratio, enzyme concentration, enzyme source, and reaction water content were studied.[43,51,52,59−61,75,78,80,81,84,101−111]

A. Solvent

For any enzyme-catalyzed process in organic media, the solvent plays a crucial role in determining enzyme stability and regulating the partitioning of substrates and products between the solvent and the enzyme. Enzyme specificity can be altered or specifically fine-tuned by proper selection of the organic solvent. The nature of organic solvents is well-known to be crucial for the maintenance of a critical water content necessary for catalytic activity. More hydrophilic solvents tend to strip the essential hydration water from the enzyme, thus distorting its catalytic conformation. Alternatively, more hydrophobic solvents retain enzyme catalytic activity, thus leaving a water layer that adheres to the enzyme surface and acts as a protective sheath.[101] When selecting the solvent for polymer synthesis, it is important to consider the viscosity and solubility of the monomer and corresponding polymer in that solvent. This choice will effect the confirmation of the polymer in solution as well as the diffusivity of the polymer and substrates. The polymer conformation and radius of gyration in a solvent may alter its ability to react with, for example, an activated monomer or chain end. However, deciphering these relationships is difficult since a change in the solvent will simultaneously effect multiple parameters such as enzyme activity and polymer chain extension in that solvent. Recently, Russel and co-workers studied the role of diffusion in biocatalytic polytransesterification. [112]

Knani and co-workers[43] showed that the solvent solubility parameter effects the self-condensation of methyl ϵ-hydroxyhexanoate (**73**). The DP of the polyester decreased with increasing solvent solubility parameter [6−12 (cal/mL)$^{1/2}$]. The DP of the synthesized polyester followed an S-shaped curve relationship with solvent log P ($-0.5 < \log P < 5$). At a log $P \sim 2.5$, the polymer DP increased. Decreasing values of DP in good solvents for polyesters were attributed to better solubility and the removal of product oligomers from the enzyme surface, resulting in reduced substrate concentration near the enzyme. Examples of solvent effect on polyester synthesis by condensation or step-growth polymerization follows. Shuai et al.[113] reported PPL-catalyzed polycondensation of 3-hydroxybutyric acid (**129**) and 12-hydroxydodecanoic acid (**104**) in diethyl ether at room temperature and toluene at temperatures of 55 and 75 °C. The yields of the products varied with solvent and reaction time. The best-isolated yield of 93% resulted from polymerizations in diethyl ether at room temperature (112 h, M_n = 230). The highest M_n of 2900 resulted from polymerizations in diethyl ether at room temperature in toluene at 75 °C (56 h, 36% yields), respectively. Binns et al.[55] studied copolymerizations of adipic acid (**85**) and 1,4-butanediol (**79**) using Novozyme-435. They reported that in solvent-free conditions, the polymerization proceeds by a step-growth mechanism. However, in toluene, they found that transesterification reactions between chains take on greater importance.

Examples of solvent effects on lactone ring-opening polymerizations are described below. Dong et al.[102] studied ϵ-caprolactone (**23**) polymerization at 37 °C for 240 h in a series of solvents (log P from 0.46 to 5.0) using the lipase from a *Pseudomonas* sp. Enzymatic polymerization in solvents with log $P < 2$ (THF, chloroform, 1,2-dichloroethane) gave low monomer conversion and molecular weight. In contrast, for solvents with log P between 2 and 4 (benzene, toluene, diisopropyl ether, and cyclohexane), the monomer conversion and molecular weight was relatively higher. They were not able to directly correlate their results with log P values. Instead, they believe that the solvent dipole moment, substrate or polymer

solubility, and enzyme specificity all are factors that ultimately determine the outcome of solvent effects on polymerization reactions. Similar results have been reported by Kobayashi and co-workers[114] for lipase CC-catalyzed polymerization of ϵ-CL (45 °C for 5−10 days) and γ-valerolactone (45 °C for 1−15 days). In our laboratory, Novozyme-435-catalyzed polymerizations of ϵ-CL at 70 °C were carried out. Solvents having log P values from −1.1 to 0.49 showed low propagation rates (≤30% ϵ-CL conversion in 4 h) and gave products of short chain length ($M_n \leq 5200$ g/mol). In contrast, solvents with log P values from 1.9 to 4.5 showed enhanced propagation rates and afforded polymers of higher molecular weight (M_n = 11 500−17 000 g/mol) (Figure 2).[75]

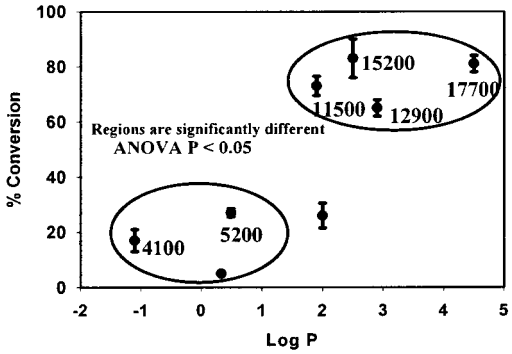
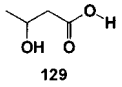


Figure 2. Effect of solvent on the polymerization of ϵ-caprolactone.



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The monomer concentration in solvent is also an important reaction parameter.[81,75] In one study carried out in our laboratory using Novozyme-435 at 70 °C for 4 h, ratios of toluene to ϵ-CL of 0:1 (solventless), 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 4:1, 5:1, and 10:1 (wt/vol) were investigated (Figures 3 and 4).[75] The region of

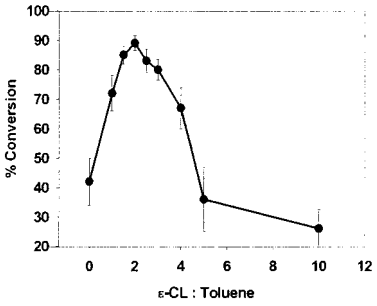


Figure 3. Effect of ϵ-caprolactone concentration in toluene on the polymerization rate.

toluene/ϵ-CL 1.5:2.5 gave substantially increased reaction rates and product molecular weights. For example, solventless and polymerizations in toluene (toluene/ϵ-CL 2:1 vol/vol) gave percent of ϵ-CL conversion, M_n, and polydispersity values of 41 and 85%, 10 800 and 17 200, and 2.1 and 1.8, respectively. Also, variation in the toluene to monomer ratio from 0:1 to 5:1 to 10:1 gave products with polydispersity values of 2.09, 1.52, and 1.38, respectively. This trend in dispersity is explained by a decrease in lipase-

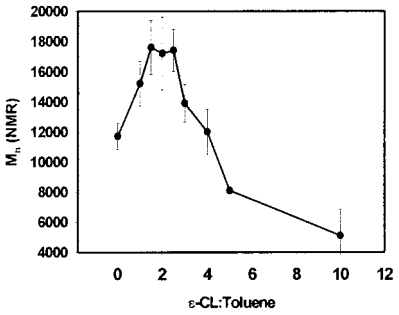


Figure 4. Effect of ϵ-caprolactone concentration in toluene on the polymer M_n.

catalyzed transesterification reactions between chains as the polymer solution concentration is diluted.

B. Enzyme Concentration

Deng et al.[89] reported on how variation in Novozyme-435 concentration affected ϵ-caprolactone polymerizations (bulk, 70 °C). Plots of monomer conversion as a function of reaction time for three different catalyst concentrations are displayed in Figure 5. As anticipated, Figure 5 shows that by

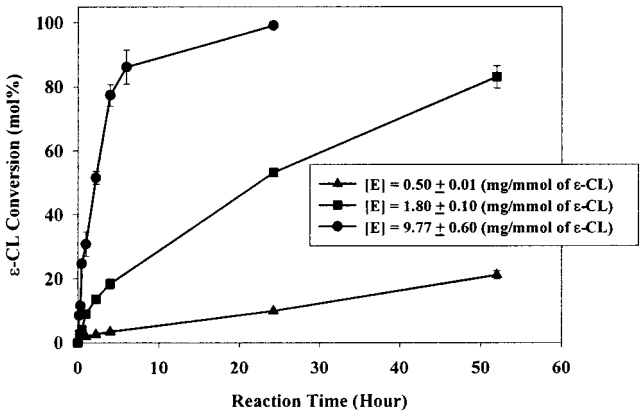


Figure 5. Effect of enzyme concentration on the rate of ϵ-CL polymerization.

increasing the catalyst concentration, the percent of monomer conversion rate increased. The total number of polymer chains per millimole of monomer ([N_p]) increased as the Novozyme-435 concentration was increased. Thus, M_n and Novozyme-435 concentration were inversely related. Russell and co-workers [119] studied the effect of enzyme/substrate concentration on the condensation polymerization of divinyl adipate (**27**) and 1,4 butanediol (**79**). From a series of kinetic experiments, it was determined that, by increasing enzyme/substrate (E/S) concentration, higher molecular weights were attained in a relatively shorter reaction time. This is despite that at higher E/S ratios the extent of hydrolysis of vinyl ester groups was higher. Since the enzyme component of the reaction mixture normally has the relatively highest weight percent of water, it follows that higher E/S ratios will lead to greater reaction water contents and, therefore, greater extents of vinyl ester hydrolysis.

C. Reaction Water Content

The importance of water concentration on various small molecule lipase-catalyzed transformations in

organic media has been well-documented.[5,120] As discussed above, water bound to the enzyme surface plays an important role in maintaining an enzyme's conformational flexibility. Lipase-catalyzed polymerizations create a new dimension to the importance of water concentration in reactions. This is due to their role during chain initiation. The reports below further elaborate on the multiple functions of water in lipase-catalyzed polymerizations.

Studies in our laboratory on PDL polymerization at 70 °C catalyzed by lipase PS-30 (Amano, immobilized on Celite) showed that by lowering the reaction water content, large increases in poly(PDL) molecular weight resulted.[80] This increase in product molecular weight was accompanied by a decrease in propagation kinetics. Furthermore, our analysis of a plot of the total number of chains ($[N_p]$) versus the reaction water content for Novozyme-435-catalyzed ϵ-CL polymerizations showed a positive slope and a linear relationship. Thus, by increasing the water content in reactions, there was a predictable increase in the number of chains.[103] Matsumoto and co-workers[104] used lipase-catalyzed ring-opening polymerization of lactones such as 11-undecanolide (**110**) and 15-pentadecanolide (**105**) to estimate the water content in a reaction mixture. Using the *P. fluorescense* lipase in cyclohexane, at 65 °C, they found that the number average molecular weight values correlated to the water content in the organic solvent. Dong et al.[102] also reported on the function of water during ϵ-CL polymerizations (in bulk, 45 °C, 240 h). They observed that, during the initial stages of reactions, the rate of monomer conversion increased with increases in water content from 0.1 to 1.0, 4.0 and 16.0% (vol/vol) (Figure 6). The plot of M_n as a

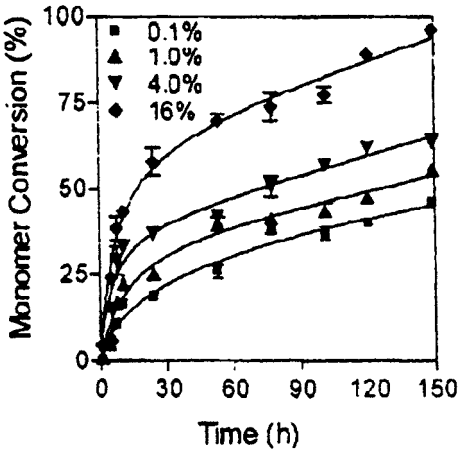


Figure 6. Effect of water content on the monomer conversion of PDL.

function of time (Figure 7) shows that at 16% water, high amounts of low molecular weight product was formed. Also, the profiles for the low molecular weight product at 0.1 and 16% water were nearly identical. The above studies agree that, within a certain range of water contents, the propagation rate increases and the product molecular weight decreases as the reaction water content increases.[102−104] This has been explained as resulting from an increase in the number of propagating chains with increased reaction water.

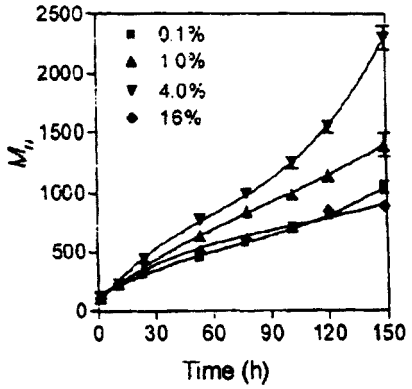


Figure 7. Effect of water content on the polymer M_n of PDL.

D. Reaction Temperature

Attempts to increase the propagation rates of lipase-catalyzed polymerizations by an increase in the reaction temperature must take into account the thermal stability of the lipase. Efforts to increase the thermal stability of enzymes using immobilization techniques, changing the solvent, accessing lipases from extremophiles, and intervening genetically are all relevant to opportunities for operating in vitro polymerizations at higher temperatures. Another general consideration is that lipase-catalyzed polymerizations in solventless or concentrated solutions can encounter diffusion constraints. Work in viscous reaction media can benefit by the ability to operate at higher temperatures.

When chain-type lactone ring-opening polymerization catalyzed by lipases does not have a termination reaction (see section E) and in the absence of diffusion limitations, the number of chain-initiating and -propagating events will control the chain length. In the absence of adding nucleophiles to the polymerizations or in the presence of impurities, water is the prominent initiating molecule. Thus, anything that tends to perturb the concentration of water in the system will also alter the product molecular weight. For example, as the reaction temperature is increased, hydrogen bonding between protein and water will be disrupted. Water that was once enzyme- or matrix-bound may be increasingly accessible. Thus, at higher temperatures, water may become increasingly available in the polymerization to initiate new chains. The studies described below provide experimental data that ultimately must be reconciled with the above or other hypotheses that explain temperature/molecular weight/enzyme activity relationships.

Kobayashi and co-workers[78,84] found large increases in percent of conversions of DDL (**51**) and PDL (**105**) by increasing the reaction temperature from 60 to 75 °C. In their work, the reactions were conducted in bulk where diffusion constraints may be encountered. The lipases used were those from *P. fluorescens* and lipase CC (*C. cylindracea*). For example, using lipase CC, after a 65% PDL conversion at 75 °C and 85% PDL conversion at 60 °C, the product M_n values were 16.2×10^3 and 6.7×10^3, respectively. Thus, by increasing the reaction temperature in viscous reaction mixtures, the mobility of monomer and polymer components is increased and, therefore, so is the product molecular weight.

Our laboratory studied the effects of temperatures ranging from 60 to 105 °C on ϵ-caprolactone polymerization catalyzed by Novozyme-435.[75] To study the reactions as a function of time, toluene-*d* was used as the solvent (ϵ-caprolactone/toluene-*d* 1:5 v/v), and the polymerizations were monitored in situ by NMR. The percent of monomer conversion as a function of time increased with an increase in temperature from 60 to 90 °C (Figure 8). A further increase in the

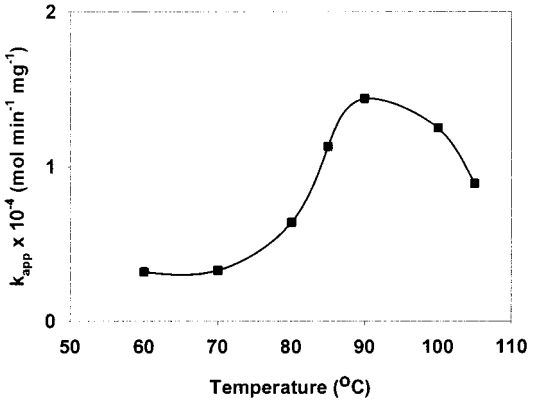


Figure 8. Effect of reaction temperature on the polymerization rate of CL.

reaction temperature from 90 to 105 °C gave decreased reaction rates. Study of the dependence of molecular weight with temperature showed that increasing the temperature from 60 to 105 °C resulted in a decrease in M_n (from ~16 × 10^3 to 7.0 × 10^3) at similar (25%) conversion (Figure 9). It is

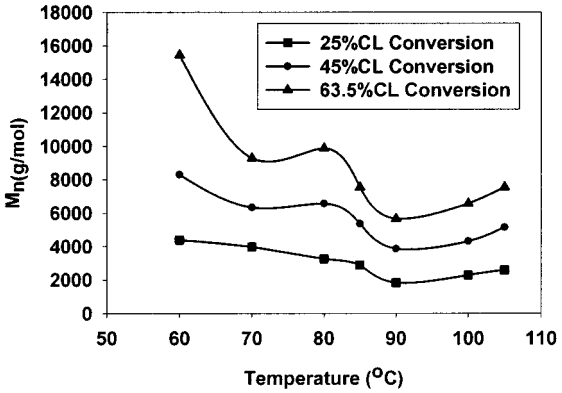


Figure 9. Effect of reaction temperature on M_n of polycaprolactone.

believed that, for solution polymerizations such as ϵ-caprolactone in toluene-*d*, the thermal requirements to overcome diffusional constraints are much lower than was encountered for bulk systems. Thus, at lower temperatures where relatively less water is "free" for chain initiation, higher molecular weight products result. Similar results were also observed for polymerization of PDL (**105**) in toluene.[81]

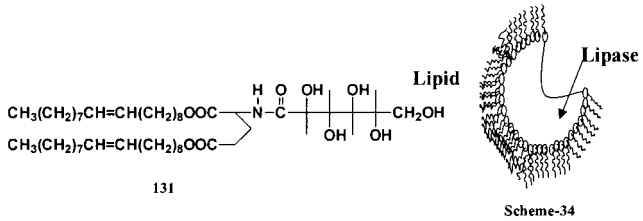
VI. Activation of Enzymes for Polymer Synthesis by Immobilization or Solubilization

Enzyme immobilization can result in improved enzyme stability, recyclability, and activity for various organic transformations.[80,121] An alternative to enzyme immobilization is to solubilize the protein in the organic phase. Techniques to achieve this objective have been reviewed elsewhere;[122] however, a brief description of these methods is provided below for the convenience of the reader.

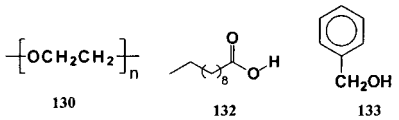
At least two approaches to dissolve enzymes in organic media have been successfully developed. In the first method, Takahashi and co-workers[105] covalently linked poly(ethylene glycol) (**130**) to exterior amino acids of the catalytic protein. Alternatively, the hydrophilic surface of an enzyme was noncovalently associated with a surfactant.[106] These methods result in catalytic proteins that have improved solubility in the reaction medium and, therefore, give higher reaction rates as compared to the enzyme powder.

Association of catalytic proteins with surfactants results in a hydrophobic outer environment that can solubilize protein−surfactant complexes in nonpolar media (Scheme 34).[106−109] Goto and co-workers[110]

Scheme 34. Immobilization of Lipases Using Carbohydrate-Based Surface-Active Compounds



showed that "surfactant coating" can result in lipase esterification rates that are much greater than the corresponding insoluble lipase powder. They also demonstrated that the pH of the buffer solution that is used to form protein−surfactant complex must be carefully regulated to ensure that the complex formed has the desired effect on the lipase activity. Goto and co-workers[110] used the nonionic surfactant glutamate dioleyl ester ribitol amide (**131**). By adjusting phosphate buffer solutions to the mid-pH range, the following lipases were surfactant-coated: lipases from *C. cylindracea*, *Aspergillus niger*, *Rhizopus* sp., *Muccor javanicus*, and *Pseudomonas* sp. (see Scheme 34). By surfactant coating the lipase from *Pseudomonas* sp., the reaction rate for esterification of lauric acid (**132**) with benzyl alcohol (**133**) increased by about 2 orders of magnitude relative to the nonorganic soluble lipase powder. Other studies by Okahata and co-workers[111] showed that dialkyl amphiphilic lipid coated lipases from *Rhizopus delemar*, *Pseudomonas fragi*, *P. fluorescens*, *Rhizopus niveus*, and wheat germ had high activity in organic media for both glyceride ester synthesis[106,111] and resolutions of racemic alcohols.[123]



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The above methods to activate catalytic proteins have been applied to enzyme-catalyzed polymer

synthesis. Noda and co-workers[124] used glutamate dioleyl ester ribitol amide (**131**) to coat and activate lipase PS. By surfactant-coating lipase PS, the percent conversion of PDL as a function of time at 60 °C in cyclohexane increased by a factor >100-fold relative to the nonsurfactant-coated enzyme powder (Figure 10). Furthermore, poly(PDL) formed by using

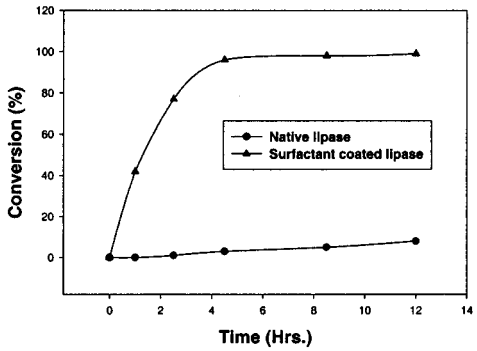
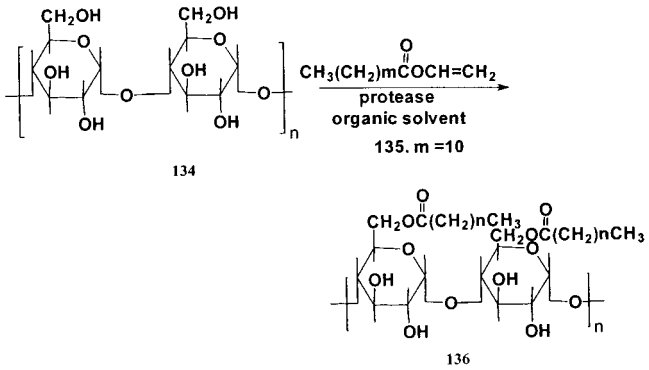


Figure 10. Effect of immobilization (surfactant coated) on lipase-catalyzed polymerization of PDL.

the native lipase PS powder, and the surfactant-coated lipase PS, had M_w values of 9.8×10^3 and 21.3×10^3, respectively. Moreover, by using the surfactant-coated lipase PS in cyclohexane, the polymerization of PDL proceeded to 99% conversion in 72 h at 30 or 45 °C. In comparison with the native lipase PS powder at temperatures up to 60 °C, percent of PDL conversion reached only 41% after 72 h.

Recently, Dordick and co-workers[125,126] observed that the protease *Subtilisin* Carlsberg from *Bacillus licheniformis* can be ion paired with a surfactant and then extracted from aqueous solutions (8.5 mM, HEPES buffer, pH 7.8, containing 6 mM KCl) into organic solvents (e.g., isooctane). This catalyst system retains its activity in isooctane for the acylation of nearly all-available surface primary hydroxy groups of amylose (**136**, thin film or fine powder). By solubilization of the enzyme and an acyl-substrate such as vinyl caproate (**135**), oligo- or polysaccharide substrates (**134**) were the only insoluble reaction component (Scheme 35). An alternative strategy is

Scheme 35. Enzymatic Modification of Amylose



to solubilize the polysaccharide in a polar aprotic solvent and to use the enzyme in its insoluble state. However, the later approach is flawed since it is well-known that catalytic lipases and proteases have poor catalytic activity in polar aprotic solvents. Furthermore, the use of such solvents is generally undesirable.

Our laboratory investigated the affect of enzyme immobilization on ω-pentadecalactone (**105**, PDL) ring-opening polymerization.[80] Solventless polymerizations of PDL at 75 °C using nonimmobilized powdered lipase PS-30 as well as lipase PS-30 that was physically immobilized on Celite-521 were performed. In comparison to nonimmobilized lipase PS-30, the Celite-immobilized enzyme gave a much greater rate of monomer conversion and polyesters of higher molecular weight (Figures 11 and 12, respectively).

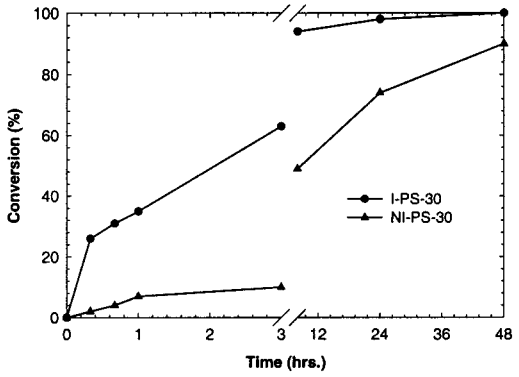


Figure 11. Monomer conversion as a function of time for the bulk polymerization of PDL catalyzed by NI- and I-PS 30 at 70 °C (reaction water content of 0.20% w/w).

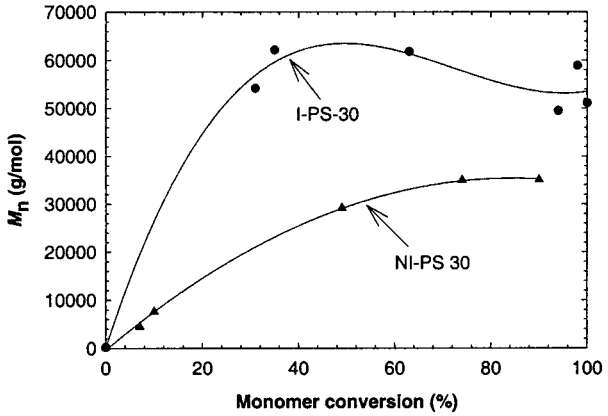


Figure 12. Number average molecular weight as a function of percent conversion for the bulk polymerization of PDL catalyzed by NI- and I-PS 30 at 70 °C (reaction water content of 0.20% w/w).

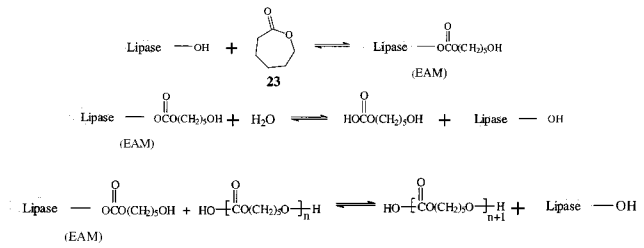
To further illustrate the importance of enzyme activation, Novo Nordisk currently immobilizes lipase B from *C. antarctica* on a polyacrylate resin. This product, marketed as Novozyme-435, has shown extraordinary versatility for a broad range of lactone structural variants.[65,92,127] This has been attributed to the absence of a "lid" that regulates the access of substrate to the enzyme active site.[128] Examples of the exceptional activity of this catalyst for many polymer-related reactions are found throughout this review. Comparative experiments for polyester synthesis using the nonimmobilized form of lipase B from *C. antarctica* have thus far shown reaction rates

that are orders of magnitude lower than for Novozyme-435.

VII. Kinetic and Mechanistic Investigations of Lipase-Catalyzed Ring-Opening Polymerization of Lactones

On the basis of publications from our laboratory[29,129] and others,[84,42] it is believed that lipase-catalyzed lactone ring-opening polymerization proceeds by an enzyme-activated monomer (EAM) mechanism (Scheme 36). For the case of PPL and

Scheme 36. Proposed Mechanism for Lipase-Catalyzed Ring-Opening Polymerization of ϵ-CL



other lipases that contain an active serine moiety, the serine residue participates in the nucleophilic attack on a lactone such as ϵ-CL to form an enzyme-activated monomer (acyl−enzyme intermediate) complex (EAM). For initiation, a nucleophile such as water can react with the EAM complex to form the monoadduct (ω-hydroxyhexanoic acid). If other nucleophiles such as alcohols or amines are added to the polymerization, they can replace water as the initiating species.[29] Changes in the initiator used can result in faster or slower initiation rates and efficiencies. Furthermore, the nucleophile that initiates a chain will ultimately become the group at the chain end. Polymer chain growth or propagation takes place when the ω-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.

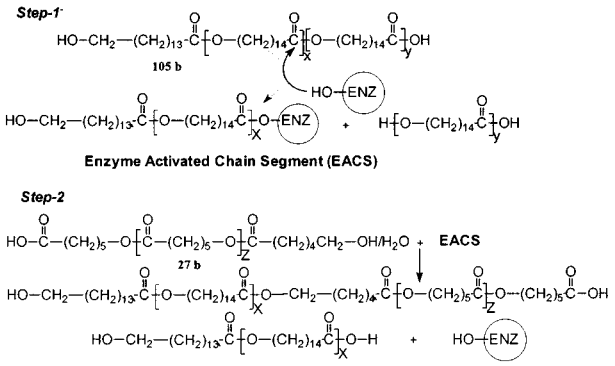
For the ϵ-CL/PPL monomer−enzyme system, three nucleophiles (butanol, water, and butylamine) were used by us to study their affects on chain initiation and propagation.[29] An experimental rate equation was derived, and the living/immortal nature of the polymerization was tested by a classical polymer chemistry 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. Later, Deng and Gross[89] extended these studies to the ϵ-CL/Novozyme-435 monomer−enzyme system. They reported that the rate of chain initiation (k_i) was relatively slow as compared to chain propagation (k_p). To further assess the "living" or "immortal" nature of the polymerization, first-order plots for three catalyst concentrations were constructed after correcting for the amount of monomer consumed in initiation (where N_p is constant).[89] The plot of $\ln\{([M]_0 - [M]_i)/([M_t] - [M]_i)\}$ versus time was linear (r^2 ranged from 0.992 to 0.996), indicating that there was no chain termination and that monomer consumption followed a first-order rate law. The absence of chain transfer and/or chain termination in these reaction systems implied that the product molecular weight may be controlled by adjusting the stoichiometry of the reactants. The extent that the molecular weight distribution of products is broadened by lipase-catalyzed transesterification is discussed below.

Most recently, Henderson and Gross[130] showed that the bulk polymerization of ω-pentadecalactone (PDL) at 50 °C catalyzed by an immobilized lipase from a *Pseudomonas* sp. (I-PS-30) deviated from an *ideal* living system. Analysis of the kinetic data indicated that the polymerizing system consisted of propagating chains that were slowly increasing in number and that M_n was not a simple function of the $[\text{monomer}]_0$ to $[\text{initiator}]_0$ ratio. It was also shown that the M_w/M_n (MWD) becomes narrower with increasing conversion and that only a fraction of the water available in the system was used to initiate the formation of chains. On the basis of these results, comparative studies between slow initiation and slow exchanges involving dormant and active chains were made. The current model used to describe these observations is that which governs the slow dynamics of exchange during propagation. Interestingly, the rate at which monomer was consumed was found to vary from first to second order when examining the kinetics at different stages of the reaction (i.e., 3−13% and 13−40% conversion, respectively). The fact that the concentration of polymer chains ([R ∼ OH]) increased with monomer conversion may explain the increased sensitivity of the reaction rate to monomer concentration as the reaction progresses.

VIII. Transesterification of Polyester Substrates

Lipase-catalyzed transesterification reactions in organic media have been used extensively for the resolution of alcohols and carboxylic acids from racemic low molar mass esters.[131] Later, step-growth condensation polymerizations that involve transesterification reactions between chain ends were used to prepare various polymers including those that are enantioenriched (see section III). Recently, our laboratory reported the ability of Novozyme-435 to catalyze transesterification reactions between preformed polyester substrates.[132,133] In one example, transacylation reactions in bulk at 70−75 °C between polypentadecalactone (**105b**, 4 300 g/mol) and polycaprolactone (**23b**, 9 200 g/mol) were studied. After 1 h, analysis of the product by ^{13}C NMR showed that the mixture of two homopolymers had been transformed into random copolyester.[133] An increase in the molecular weight of the initial homopolymers (polycaprolactone, M_n = 44 000, PDI 1.65; polypentadecalactone, M_n = 40 000, PDI 1.71), under identical reaction conditions, resulted in the formation of a multiblock copolymer (M_n = 64 700, PDI 1.97). An increase in the reaction time from 1 to 24 h resulted in a random copolymer. Thus, the kinetics of Novozyme-435 transacylation reactions was chain-length dependent. From the above observations and the currently excepted mechanisms of lipase-catalyzed polymerizations,[133] the following mechanism for transacylation between preformed polyesters was

Scheme 37. Mechanism for Lipase-Catalyzed Transesterification Reaction between Chains

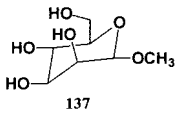


postulated (Scheme 37). In the first step, lipase catalysis results in the cleavage of an intrachain ester linkage. The resulting enzyme-activated polymer complex (EAPC) then reacts with the terminal hydroxyl group at a chain end to form an ester linkage. These reactions continue and thereby reshuffle the repeat unit sequence distribution. In the example above, the redistribution of repeat units occurred to the extent that random copolymer resulted.[132−134]

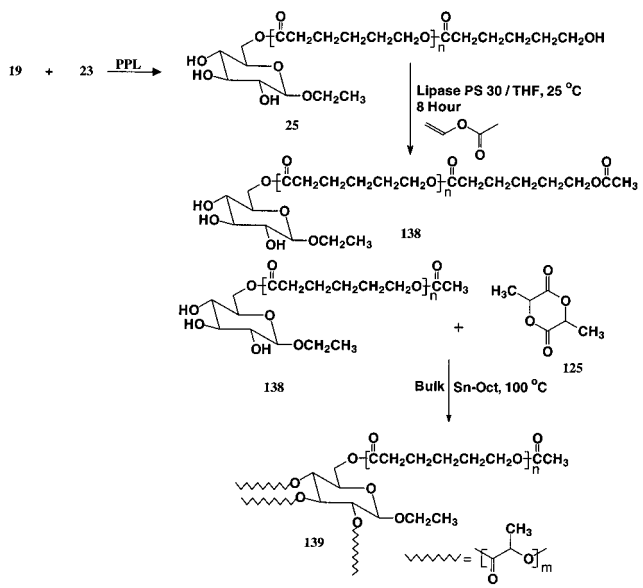
IX. Modification of Carbohydrates

Multifunctional groups on carbohydrate substrates present unique challenges for selective modifications. There is great opportunity to direct selective modifications to create building blocks that are relevant to polymeric systems. Often, traditional chemical methods that lead to selective carbohydrate modification involve tedious introduction and removal of protecting groups.[109] In contrast, enzymes can provide high levels of regioselectivity so that simple one-pot reactions may replace multistep processes.

Our laboratory reported a method where the initiation of lactone or cyclic carbonate ring-opening polymerization occurs selectively from the 6-hydroxyl position of multifunctional alkyl glucosides (Scheme 6). Since α,*β*-ethyl glucose (**19**) and *ϵ*-CL (**23**) are both liquids, this reaction was studied without solvent. For example, at 70 °C for 96 h, PPL catalyzed the highly regioselective initiation of *ϵ*-CL to give 1-ethyl-6-oligo-(*ϵ*-CL)-glucopyranoside [6-oligo(*ϵ*-CL)-EGP] (**25**) (e.g., M_n = 1120 g/mol, M_w/M_n = 2.16).[30] In a separate report published shortly after us, Cordova et al. described a similar result by using Novozyme-435 for acylation of methyl galacto- and glucopyranoside (**137**, **18**).[135] The *ω*-hydroxyl group of oligo(*ϵ*-CL) was regioselectively esterified with vinyl acetate (**41**) using lipase PS-30 (from *P. cepacia*) (**138**).[115] Then, the three free hydroxyl groups located exclusively at the EGP headgroup of the resulting product (**138**) were used as sites for initiation of stannous octanoate-catalyzed L-lactide (**125**, L-LA) ring-opening polymerization. This gave a multi-arm heteroblock copolymer (**139**) with spatially well-defined arms around a carbohydrate core (Scheme 38).

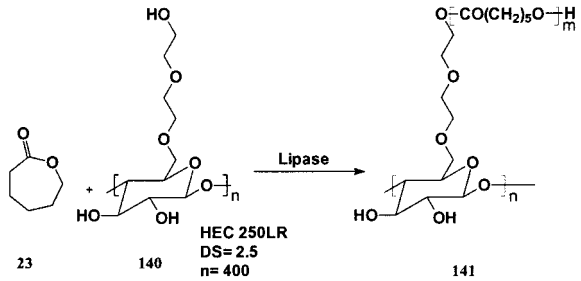


Scheme 38. Chemoenzymatic Process That Was Used To Generate a New Family of Multi-arm Heteroblock Copolymers Having a Sugar Core



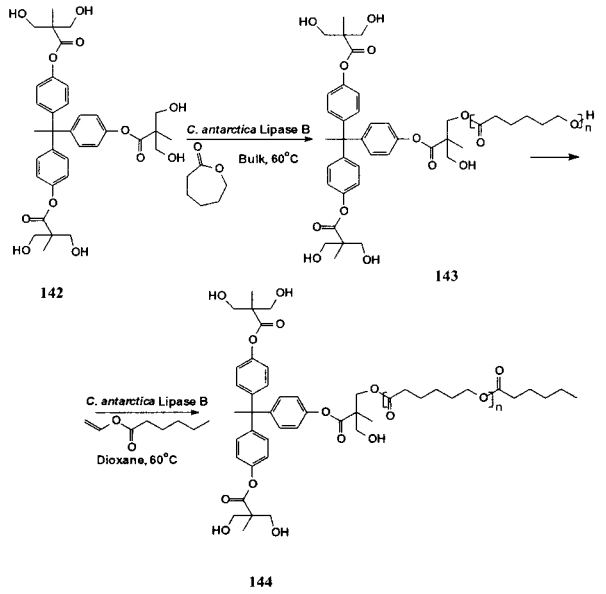
Relative to monosaccharides, polysaccharides are even a more difficult class of materials for chemical and enzymatic processing because of the presence of many reactive hydroxyl groups and their poor solubility in nonaqueous solvents. There are only a few reports on the modification of polysaccharides by enzyme-mediated transformations in organic media.[118,126] For example, hydroxyethylcellulose (**140**) was cast into a film and thereafter modified by enzyme-mediated ring-opening of *ϵ*-caprolactone (**23**) in bulk using lipases derived from PPL and CLONEZYME ESL-001 library.[118] This resulted in the grafting of poly(*ϵ*-caprolactone) from HEC (**141**) with DS values (per anhydroglucose unit) between 0.10 and 0.32 (Scheme 39).

Scheme 39. Enzymatic Modification of Hydroxyethyl Cellulose with Caprolactone



A related but different challenge is the selective modification of the nonnatural hexahydroxy first generation dendrimer built from 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) (**142**). Cordova et al.[136] attempted the selective initiation and propagation of *ϵ*-caprolactone ring-opening from one of the six potential sites of the hexahydroxyl substrate. For this study, lipase B from *C. antartica* (CALB) was chosen as the catalyst.[137,138] They concluded that the polymerization proceeded from only one of the possible six hydroxyl sites (**143**, **144**; Scheme 40).[136]

Scheme 40. Lipase-Catalyzed Selective Initiation of Caprolactone on 1st Generation Dendrimer Generated from 2,2-Bis(hydroxymethyl)propanoic Acid That Has Six Hydroxy Groups

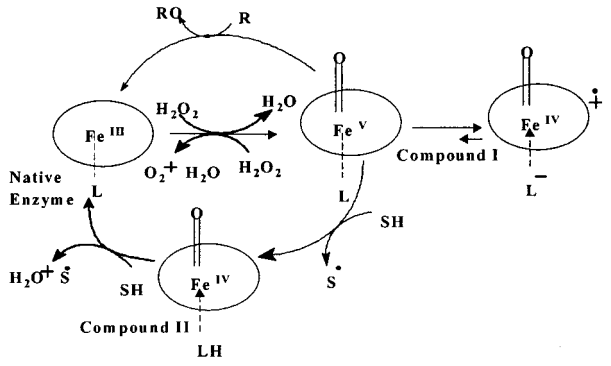


142 143 144

X. Peroxidase-Catalyzed Polymer Synthesis

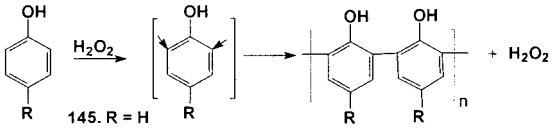
Peroxidases function as oxidoreductases that catalyze the oxidation of a donor using hydrogen peroxide as the oxidizing agent. During this process, two water molecules are liberated (Scheme 41). Novel polyaromatics that are similar in structure to Novalak have been synthesized by oxidoreductase-catalyzed polymerization of phenol, aniline, or related derivatives. The synthesis of Novalak uses phenol and formaldehyde as the key building blocks. Formaldehyde toxicity is a formidable problem in Novalak commercial production. The preparation of polyphenols using horseradish peroxidase (HRP) or related enzymes offers a viable alternative to Novalak-type structures, which circumvents the use of formaldehyde.

Scheme 41. Catalytic Cycle of Peroxidase



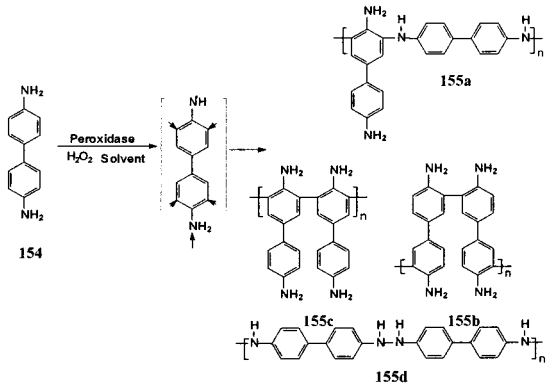
A number of homopolymers and copolymers have been synthesized from substituted phenolic and aromatic compounds (**145**−**154**) using HRP with hydrogen peroxide as the oxidizing agent (Schemes 42−44).[139−142] For example, Kobayashi and co-workers[139,140] reported the HRP-catalyzed polymerization of phenol (**145**) in solvents such as 1,4-dioxane/phosphate buffer (80:20 vol %). The corresponding product was obtained in 72% yield in 24 h at room temperature. The molecular weight of the DMF soluble part of the polymer was analyzed by GPC using DMF/LiCl as eluent to give an M_n and PDI of 35 000 and 3.5, respectively. On the basis of NMR and IR analyses, this product was said to be a mixture of phenylene (**156**) and oxiphenylene units (**157**) (molar ratio of 1.2:1) (Scheme 45). In contrast, the polymer obtained by the enzymatic polymerization of *p*-phenylphenol (**152**) had only phenylene units (**158a**−**c**) (Scheme 46).[139] Soybean peroxidase (SBP)[143] was also found to be a useful catalyst for oxidative polymerizations of phenolics. Comparison of phenol polymerizations catalyzed by HRP and SBP

Scheme 42. HRP-Catalyzed Polymerization of Phenol and Its Derivatives



145. R = H
146. R = CH_3
147. R = CH_2CH_3
148. R = $CH_2CH_2CH_3$
149. R = $CH_2(CH_2)_3CH_3$
150. R = $CH_2(CH_2)_4CH_3$
151. R = $CH_2(CH_2)_5CH_3$
152. R = Ph
153. R = p-tert butyl

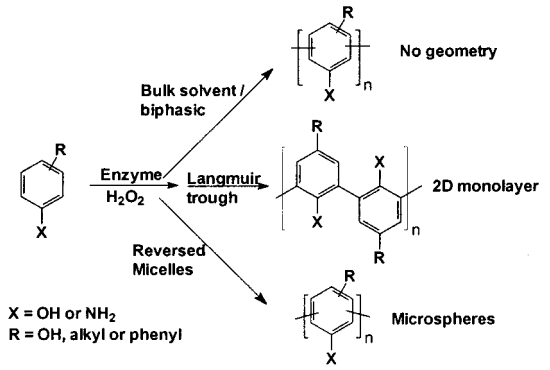
Scheme 43. Simplified Schematic Representation of Polymerization of Biphenyl Amine[a]



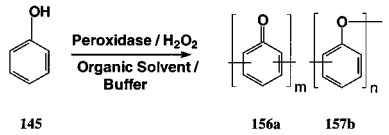
154

[a] Bold arrows (in the phenoxy radical) indicate coupling in the ortho positions. Resonance structures also imply the possibility of para coupling.

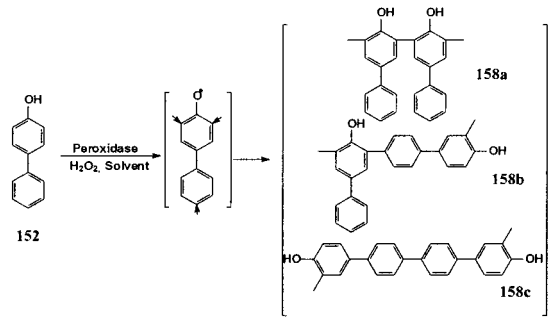
Scheme 44. Peroxidase-Catalyzed Polymerization of Phenol/Aniline in Biphasic/Langmur Trough/ Reversed Micelles System



Scheme 45. Peroxidase-Catalyzed Polymerization of Phenol in 1,4-Dioxane/Phosphate (80:20 vol %) at pH 7.



Scheme 46. Peroxidase-Catalyzed Polymerization of *p*-Phenyl Phenol



showed that the former gave higher product yields and molecular weights. In contrast, SBP was more effective than HRP for the catalysis of *p*-cresol (**146**) polymerizations (**146b**). For example, *p*-cresol polymerizations catalyzed by HRP and SBP in 1,4-dioxane/phosphate buffer (pH 7) at room temperature gave polymer yields and M_n values of 6 and 35% and 4.4×10^3 and 5.1×10^3, respectively.[143] Later, Kobayashi and co-workers[144] found that HRP-catalyzed phenol polymerizations in methanol/buffer gave polyphenols with adjustable levels of phenylene to oxyphenylene units. Thus, by increasing the ratio of methanol to buffer, the ratio of phenylene to oxyphenylene units in the product decreased.[144] HRP-catalyzed copolymerization of phenol with 2,4-dimethylphenol (**159**) produced copolymers with molecular weights lower than that for homopolymers produced under similar conditions.

Pure manganase peroxidase, lignin peroxidase, and laccase all catalyze the polymerization of pentachlorophenol (**160**) and ferulic acid (**161**) mixtures in the presence of hydrogen peroxide and a detergent.[145] This reaction is of interest for the removal of pentachlorophenol from complex wastestreams.

Xu et al.[146] studied the kinetics of phenolic polymerizations catalyzed by peroxidases (HRP and lactoperoxidase) in organic media. Phenols and aromatic amines with electron-withdrawing groups were poor substrates for HRP-catalyzed polymerizations. In contrast, phenols and aromatic amines with electron-donating groups were readily polymerized under identical reaction conditions. Comparison of para- or meta-substituted phenol/aniline (**162**) substrates to that of their ortho-substituted structural isomers showed that the former had higher reaction rates.

Rao et al.[147] found that HRP, when encapsulated in reversed micelles, was effective for catalysis of phenol−analogue polymerizations. The polymerization of *p*-ethylphenol (**147**) in AOT reverse micelles provided a route to regulate the product molecular weight by the adjustment of surfactant concentration in the system. For example, an increase in AOT content by 10-fold led to chains of *p*-ethylphenol (**147**) with higher molecular weight (from 20 000, $n = 166$, to 400 000, $n = 3333$, where n is the number of monomer units) (Scheme 44).[147]

Kobayashi and co-workers[148] also studied HRP-catalyzed polymerization of various *p*-*n*-alkylphenols in aqueous 1,4-dioxane solutions and reversed micelle systems. They observed that in aqueous 1,4-dioxane solutions the yield of *p*-*n*-alkylphenol polymerizations increased as the chain length of the alkyl moiety was increased from methyl to *n*-pentyl (**146**−**149**). Further increase in the *p*-*n*-alkyl chain length from *p*-*n*-pentyl to *p*-*n*-hexyl (**150**) and *p*-*n*-heptyl phenols (**151**) did not result in any further increase in the product yield. In contrast, *p*-*n*-alkylphenol polymerizations conducted in reversed micelle systems showed a maximum polymer yield when the alkyl group was ethyl (**147**). Recently, Tonami et al.[149] reported a chemoenzymatic strategy for the polymerization of *m*-ethynylphenol (**163**) in buffer:methanol (50:50 vol %) at pH 7.0. They found that the phenolic moiety of *m*-ethynylphenol was selectively polymerized by HRP and hydrogen peroxide while leaving the acetylene pendant groups unreacted.

Uyama et al.[150] reported the preparation of polyphenol particles by dispersion polymerizations using HRP in aqueous 1,4-dioxane. These investigators succeeded in obtaining relatively monodisperse particles in the submicron range by including poly(vinyl methyl ether) as a steric stabilizer. Similar particles were also obtained from *m*- (**164**) and *p*-cresols (**146**) and *p*-phenylphenol (**152**).

The HRP-catalyzed polymerization of amphiphilic derivatives of D- and L-tyrosine isomers (**165**, **166**) in micellar solution has been investigated by Sarma et al.[151] These workers observed that the HRP system reacted more rapidly with the D- then the L-isomer. Such stereoselectivity for HRP-catalyzed oxidations was unexpected. In a related study, HRP catalysis was used to covalently link tyrosine (**167**)-containing model peptides.[152] This oligomerization occurred by coupling of the C−C bonds that are at the ortho position with respect to the tyrosine phenol groups. Dimers, trimers, and tetramers (depending on the peptide length and the position of the tyrosine in the peptide sequence) were identified by electron spray mass spectroscopy. These workers noted that the initial products of the oligomerization inhibited further HRP-catalyzed oxidation reactions. This inhibitory effect was not observed when a manganese-dependent peroxidase was used.

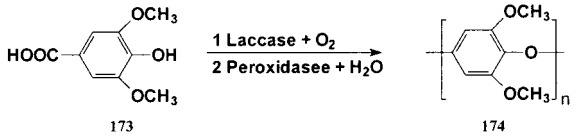
Michon et al.[153] improved the reactivity of poorly accessible tyrosine units in wheat gliadins by an "indirect" catalysis. Thus, by using a fungal peroxidase (manganese-dependent peroxidase), the efficiency of wheat gliadin protein cross-linking via tyrosine−tyrosine aromatic ring condensation in water was improved. These workers obtained similar results with SBP and HRP by the dispersion of the gliadins in water:dioxane (3:1) mixed solvent sys-

tems. Malencik and Anderson[154] have studied dityrosine formation in bovine brain calmodulin through cross-linking and polymerization catalyzed by the *Arthyomyce ramosus* peroxidase.

In an attempt to examine the mechanism of lignin peroxidase, Sheng and Gold[155] used bovine pancreatic ribonuclease A (RNase) as a primary lignin model substrate for oxidative poymerization. They have demonstrated that RNase dimer formation is due to the lignin peroxidase-catalyzed oxidation of tyrosine residues to tyrosine radicals, followed by intermolecular radical coupling.

The bisphenol derivatives 4,4′-biphenol (**168**), bisphenol A (**169**), 4,4′-methylenebisphenol (**170**), 4,4′-dihydroxydiphenyl ether (**171**), and 4,4′-thiodiphenol (**172**) have been enzymatically polymerized using HRP to produce a new class of polyphenols.[156] Kobayashi et al.[156] have reported HRP-catalyzed polymerization of 4,4′-biphenol (**168**) in a mixture of 1,4-dioxane/phosphate buffer. This resulted in a soluble polymer of several thousand molecular weight. Ikeda et al.[157,158] reported that peroxidases (HRP, SBP) or the laccase derived from *Pycnoporus coccineous* catalyzed the oxidative polymerization of 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid) (**173**)[157] and 2,6-dimethyl phenol (**159**).[158] The polymerization of syringic acid proceeded with the elimination of carbon dioxide and water (Scheme 47).[158]

Scheme 47. Regioselective Polymerization of Syringic Acid



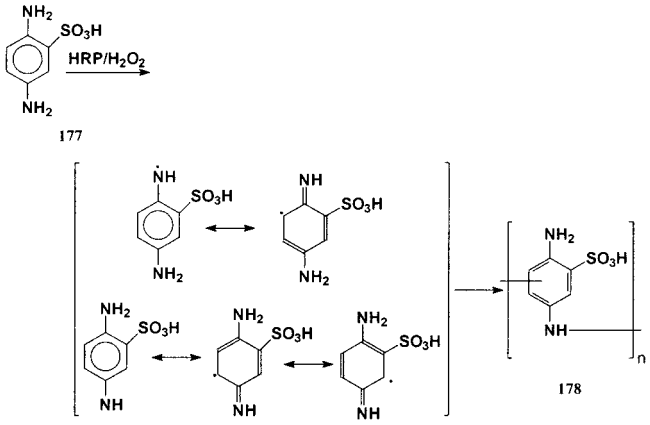
173 174

Peroxidases (HRP and SBP) as well as the laccase derived from *P. coccineous* were effective catalysts for the polymerization. On the basis of NMR and IR analyses, the main chain of the products (**174**) was found to consist of exclusively phenylene oxide units.

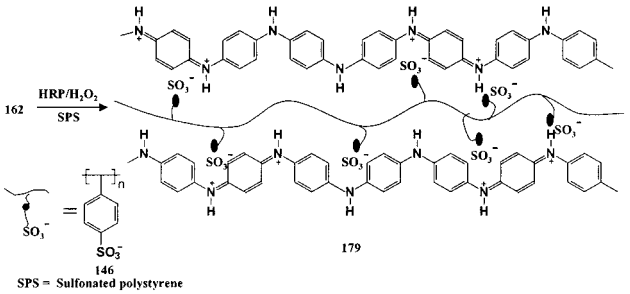
Polymers have been synthesized from substituted aromatic compounds including aniline (**162**), benzidine (**154**), 3-phenylenediamine (**175**), and phenyethylamine (**176**) by using HRP and hydrogen peroxide in dioxane:buffer (85:15) at pH 5.6, 7.0, and 7.5. Peroxidase-catalyzed copolymerizations of phenolics with aromatic amines have been carried out in monophasic organic solvents such as dioxane and acetone with small quantities of water at room temperature.[141]

A novel water-soluble polyaniline, poly(2,5-diaminobenzene sulfonate) (**178**), has been synthesized by HRP-catalyzed oxidative free radical coupling of 2,5-diaminobenzenesulfonate (**177**, Scheme 48).[159] Tripathi and co-workers[160] have used HRP/hydrogen peroxide in the presence of sulfonated polystyrene to produce an irreversibly complexed water-soluble polyaniline.[161] The sulfonated polystyrene is believed to align the monomers prior to polymerization so that the ortho positions of the phenyl rings are blocked. This would prevent the formation of an insoluble branched polyaniline that would have poor electrical properties. Sulfonated polystyrene serves numerous

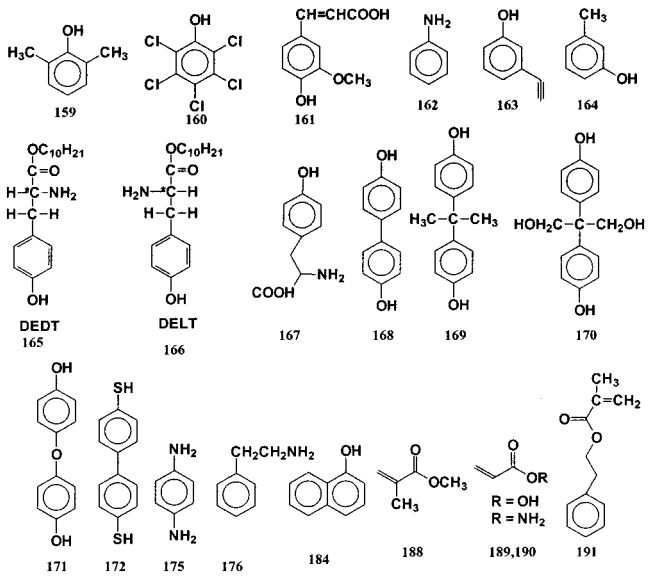
Scheme 48. Peroxidase-Catalyzed Polymerization of 2,5-Diaminobenzene Sulfonate



Scheme 49. Peroxidase-Catalyzed Matrix-Assisted Polymerization of Aniline

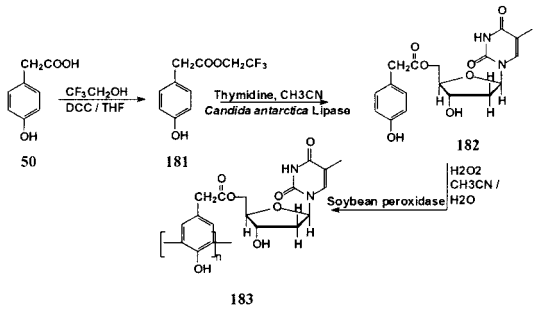


roles in this system since it not only functioned as a template to align the monomers but also served as a polyaniline (**179**) dopant and formed an irreversible complex with polyaniline that renders the product water-soluble (Scheme 49).



Dordick and co-workers[162] have conducted stepwise synthesis using two-enzymes to generate nucleoside-based polyphenols (**183**). The first step was to use the lipase from *C. antartica* in nearly anhydrous acetonitrile to carry out the regioselective acylation of thymidine with a trifluoethyl ester of *p*-hydroxy phenyl acetic acid at the 5′-hydroxyl position (**182**). Subsequently, the SBP-catalyzed polymerization of

Scheme 50. Peroxidase-Catalyzed Phenolic Nucleoside Derivative

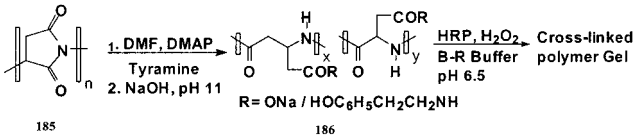


the phenolic nucleoside derivative was carried out (Scheme 50).

Examples of other peroxidase-catalyzed studies and reactions that extend the themes described above are as follows: the use of amphiphilic aromatic monomers to facilitate polymerizations at the air−water interface,[163] enhanced control over the molecular weight of peroxidase free-radical coupling reactions,[164] bioelectropolymerization of phenols,[165] and formation of fluorescent naphthol-based (**184**) polymers via peroxidase reactions.[166]

Our laboratory explored the use of peroxidase-catalyzed reactions as a means to achieve controlled cross-linking of water-soluble polymers. The synthesis of poly(aspartamide) bearing tyramine side groups (**186**) was performed by the ring-opening of polysuccinamide (**185**) with tyramine under basic conditions in DMF. Commercial oxidases including HRP (types I and II), SBP, and *Arythomyces ramosus* peroxidase were evaluated as catalysts to perform selective coupling of the phenol side groups.[167] Different reaction parameters were studied to determine their effect on the rate of gelation and the extent of cross-linking. The parameters evaluated included the d.s. of tyramine side groups, enzyme concentration, buffer pH, reaction temperature, and polyaspartamide molecular weight. All of these factors were of importance in controlling gel formation. An example of preferred reaction conditions for gel formation are as follows: pH 6.5 (Britton−Robinson buffer), 25 °C, use of HRP II as the catalyst, and 90 mol % of tyramine side groups along the chain. These reaction conditions resulted in strong gels after only a 15-min reaction time (Scheme 51).

Scheme 51. Peroxidase-Catalyzed Gelation of Tyramine Polyaspartamide



Recently, HRP has been used to catalyze free radical polymerizations of commodity vinyl monomers. The potential of such reactions with vinyl monomers including methyl methacrylate (**188**), acrylamide (**189**), 2-hydroxyethyl methacrylate (**52**), and acrylic acid (**190**) was first recognized and reported by Derango et al.[168] In a related study, Kobayashi and co-workers[169] reported the HRP-catalyzed polymerization of phenylethyl methacrylate (**191**). Later, HRP-mediated free radical polymerizaton of acrylamide was shown to take place in the presence of β-ketones as initiators for free radical polymerization.[170,171] Our group has investigated the polymerization of methyl methacrylate mediated by HRP in a mixture of water and water-miscible solvents at ambient temperatures.[172,173] By these methods, highly stereoregular polymers with predominant syndiotactic sequences (0.82−0.87 syn-diads) and M_n values that range from 7500 to 75 000 g/mol were prepared.

Further work has been carried out in our laboratory to determine how various reaction parameters can be adjusted to regulate HRP-catalyzed vinyl monomer polymerizations. Thus far, we have reported on polymerizations of acrylamide in concentrated emulsion,[174] aqueous conditions, in the absence and in the presence of surfactants (cationic and anionic) and polymerizations of methyl methacrylate in emulsifier-free emulsions.[175]

XI. Challenges

This review provides numerous examples of the significant advancements that have been made over only the past several years in (i) lipase-catalyzed formation of polymers consisting of ester and carbonate linkages, (ii) lipase- and protease-catalyzed routes to specialty monomers, (iii) peroxidase-catalyzed formation of polyaromatics, and (iv) peroxidase-mediated polymerizations of vinyl monomers. However, a number of problems still exist in transferring these methods from the laboratory to industrial processes. Their remains a need to further improve the catalytic activities displayed by enzymes when used in nonaqueous media. To circumvent this problem, larger amounts of enzymes than desired are currently needed to ensure that the reaction kinetics proceed within a practical time scale. The development of highly stable catalysts, suitable supports, and processes that facilitate the recycling of the enzymes will surely help to alleviate difficulties associated with lower than desired enzyme activity. Not all polymer-related enzyme-catalyzed transformations will allow the incorporation of enzyme recycling steps. For example, solventless polymerizations of polyesters that form highly viscous or even cross-linked products may not permit the isolation and reuse of the catalyst. Thus, for some enzyme-catalyzed reactions, the enzyme may remain in the final product. In such cases, 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 the industry. Much closer attention will need to be made by researchers on assessing and improving the productivity of enzymatic catalysis in terms of units of polymer obtained per unit of enzyme used. Also, researchers must continue to define where enzymes provide significant advantages relative to traditional chemical processes. The mile-stick will always be comparisons between costs of chemical processes and those of biocatalytic processes. 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. New products from enzyme catalysis that provide important performance benefits and are not accessible by existing chemical methods must be identified and developed.

In writing this review, the authors have gained a better understanding of the general need to better define the reaction conditions used for enzyme-catalyzed transformations. Without such information, it is difficult to make quantitative assessments of research reports that incorporate meaningful values of catalyst efficiency. More information is needed on the kinetics of enzyme-catalyzed polymerizations as a function of well-defined experimental parameters. A better understanding of reaction mechanisms will identify current barriers and limitations in enzyme-catalyzed reactions. Challenges exist in many areas including a general need to work with enzymes of greater purity, determining water content in reaction systems, design of immobilization matrixes that are tailored to the specific catalyst and reaction target-(s), developing a better understanding of catalytic protein activity as a function of interactions with surfactants and immobilization surfaces, using molecular modeling to help optimize catalyst−substrate−matrix interactions, develop probes on a molecular level that provide knowledge of enzyme orientation and binding at surfaces, and surely many other ideas not considered by the authors.

Nevertheless, improvements in the kinetics of enzyme catalysis in organic media are occurring at a rapid pace due to new methods for enzyme activation and application of genetic techniques to improve enzyme activity on nonnatural substrates in nontraditional environments (see below). On the basis of the impressive progress that has already been achieved over only the past 6 years, there is much reason to have great optimism that we are only at the very beginning of a revolution where in vitro enzyme catalysis will be incorporated into numerous polymer-related industrial processes.

XII. Additional Options: Enzyme Evolution

Aside from solvent engineering and physical techniques for enzyme activation in nonaqueous media, another option to address the need for more active enzymes in organic solvent or solventless polymerization reactions is to employ recent developments in enzyme evolution. Genetic diversity can now be rapidly generated within the test tube followed by rapid screening to identify new versions of starting genes and the encoded enzymes. This idea of directed evolution has been pioneered in the past few years through the use of DNA shuffling and error-prone PCR.[176,177] Successful implementation of this concept has resulted in modifications to a single gene in the case of error prone PCR or to explore combinations of related genes in the case of DNA shuffling. For example, moxalactamase activity was selected by shuffling four cephalosporinase genes that resulted in up to a 540-fold increase in activity in comparison to an 8-fold enhancement if only one of the four genes was shuffled.[178] This was found after only one round of shuffling and helps illustrate the power of these new techniques. While oligonucleotide-directed random mutagenesis is more commonly used, the addition of homologous recombination of DNA in vitro provides a new key component to this process. The massive parallel approach offered with DNA shuffling and error-prone PCR appears to provide a more diverse set of new clones from which to select improvements in a target function. Error-prone PCR is based on low fidelity-based extension by polymerase, resulting in the introduction of a random low level of point mutations, while DNA shuffling can introduce the block mutations that appear important in evolution. With appropriate screens, new variants of enzymes for polymerization reactions could be selected.

XIII. Acknowledgment

We acknowledge the efforts of many researchers throughout the world and in our own laboratory that have carried out the research that made the writing of this review possible. We also gratefully acknowledge the financial support and encouragement of the Industrial Members (BASF, Cargill-Dow, DSM, Eco-Synthetix, Maxygen, Nalco, Novo Nordisk) of the National Science Foundation Industrial−University−Cooperative−Research Center at the Polytechnic University (Brooklyn, NY).

XIV. Abbreviations

δ-VL	δ-valerolactone
β-BL	β-butrolactone
α-Me-γ-VL	α-methyl-γ-valerolactone
α-Me-ϵ-CL	α-methyl-ϵ-caprolactone
8-OL	octanolide
β-PL	propiolactone
UDL	undecanolactone
TMC	trimethylene carbonate
δ-DL	decanolactone
HDL	hexadecanolactone
HEC	hydroxyethyl cellulose
PDL	pentadecalactone
CL	ϵ-caprolactone
DDL	dodecanolactone

XV. References

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