

Enzyme-catalysis breathes new life into polyester condensation polymerizations

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Traditional chemical catalysts for polyester synthesis have enabled the generation of important commercial products. Undesirable characteristics of chemically catalyzed condensation polymerizations include the need to conduct reactions at high temperatures (150–280 °C) with metal catalysts that are toxic and lack selectivity. The latter is limiting when aspiring towards synthesis of increasingly complex and well-defined polyesters. This review describes an exciting technology that makes use of immobilized enzyme-catalysts for condensation polyester synthesis. Unlike chemical catalysts, enzymes function under mild conditions (≤ 100 °C), which enables structure retention when polymerizing unstable monomers, circumvents the introduction of metals, and also provides selectivity that avoids protection–deprotection steps and presents unique options for structural control. Examples are provided that describe the progress made in enzyme-catalyzed polymerizations, as well as current limitations and future prospects for developing more efficient enzyme-catalysts for industrial processes.

Introduction

Living organisms generally synthesize macromolecules by *in vivo* enzyme-catalyzed chain growth polymerization reactions using activated monomers that have been formed within cells during complex metabolic processes. In many cases, microorganisms accomplish polymer syntheses that are impractical or impossible with conventional chemistry. Microbial polymers, such as polyhydroxyalkanoates and xanthan, are synthesized from renewable, low-cost feedstocks, and the polymerizations operate under mild process conditions with minimal environmental impact. Microbial synthesis of polymers has become increasingly powerful owing to the ability to regulate metabolic pathways and integrate engineered enzymes that allow the synthesis of a broader diversity of products [1]. Nevertheless, the inherent complexity of metabolic pathway engineering, a restricted physiological set of conditions (temperature, pH, aqueous environment) under which enzymes function, and difficulties in isolating polymers from fermentation media and/or cells have led to severe limitations in the range of industrially useful materials that can be produced via whole-cell biocatalysis. To broaden the participation of microbes in industrial polymer production, a notable trend has emerged that involves

engineering metabolic routes to monomers [2–4]. Important recent examples include the microbial synthesis of 1,3-propanediol, 3-hydroxypropionic acid (a precursor to acrylic acid), succinic acid and 1,4-butanediol. In one example, PPT [poly(propylene terephthalate)], a polymer used in textiles and carpeting, was produced from 1,3-propanediol and terephthalic acid monomers [3].

Polycondensation is the primary route by which commercial polyesters are synthesized. Poly(ethylene terephthalate) (PET), used in fibers, films and blown bottles, is the largest volume commercial polyester. Unsaturated, aliphatic, and aliphatic aromatic copolymers also constitute a large volume of polyester-containing commercial materials. Furthermore, polyol-polyesters are important polymer precursors for the manufacture of polyurethanes. This review focuses on the use of isolated immobilized enzymes as catalysts for polyester synthesis via condensation polymerizations. Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are the predominant enzymes that have thus far been used for these cell-free polymerizations. In nature, lipases catalyze the hydrolysis of fatty acid glycerol esters. By shifting the equilibrium of lipase-catalyzed reactions, by working in non-aqueous media, and by removing condensation by-products, bond formation occurs instead of bond breaking. Lipase-catalyzed polycondensations are useful for a broad range of substrates, such that many dicarboxylic acids (and their derivatives), glycols, and oxyacids (and their esters) are suitable monomers.

Chemical synthesis of polyesters by condensation reactions are catalyzed by a wide array of catalysts, such as acetates of manganese, zinc, calcium, cobalt and magnesium, antimony oxide, and titanium oxides, at reaction temperatures ranging from 180 to 280 °C. By conducting reactions at high temperatures, one can polymerize high-melting monomers and polymers and also reduce their viscosities, in turn decreasing diffusion constraints during chain growth. Thus, the current lack of enzymes with adequate stability at temperatures >100 °C is limiting, excluding their use for certain targets under bulk reaction conditions (i.e. PET, which melts at 270 °C). However, high reaction temperatures also cause undesirable side reactions, such as dehydration of diol and β -scission of polyesters to form acid and alkene end groups. High reaction temperatures are particularly problematic when using thermally or chemically unstable monomers. Thus, the requirement to conduct chemically catalyzed condensation

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polymerizations at high temperatures limits the range of polymers that can be formed. Functional groups that are unsuitable for high temperature polymerizations with chemical catalysts include siloxane, epoxy and vinyl moieties.

Other than temperature concerns, residual metals in these catalysts are difficult to remove and can cause undesirable effects on the environment upon disposal. Furthermore, chemical condensation catalysts generally lack selectivity that, for example, leads to uncontrolled branching of polyesters when using monomers with functionality ≥ 3 . However, the lack of selectivity of traditional chemical catalysts has the advantage of functioning on a broad range of monomers, some of which are poor substrates for currently available enzyme-catalysts. New versatile condensation catalysts are needed that catalyze reactions at mild temperatures and provide chemo-, regio- and enantioselectivity. The fact that natural enzymes can provide these characteristics has fueled their use for enzyme-catalyzed polymerization reactions. Advantages of enzymes as catalysts for polyester synthesis are summarized in Box 1.

Comprehensive reviews [5–10] have been written that describe the broad range of cell-free, enzyme-catalyzed polyester syntheses performed since 1984, when lipase-catalysis was first reported for the synthesis of oligoesters (trimers to heptamers) [11]. This paper focuses on seminal work over the past few years that have significantly advanced the field of *in vitro* enzyme-catalyzed polyester condensation reactions.

Lipase-catalyzed polymerization

In most cases, studies on immobilized lipase-catalyzed polymerizations have used *Candida antarctica* lipase B (CALB) because of its high regio-, chemo- and enantioselectivity and its high thermal stability and activity [7] (Table 1). The primary immobilized CALB catalyst used is Novozyme 435 (N435), which consists of CALB physically

Box 1. Considerations when selecting an enzyme or chemical catalyst for polyester synthesis by condensation reactions

Advantages of enzyme-catalysts for polyester synthesis include:

- derived from renewable resources;
- high enantio- and regioselectivity, the latter of which enables one-pot reactions that circumvent protection-deprotection chemistries;
- recyclable to reduce catalyst cost when immobilized;
- function in a broad range of environments, such as solvents, biphasic organic solvent-aqueous mixtures, reversed micelle systems and supercritical fluids;
- can be stabilized to work at surprisingly high temperature (e.g. $>100\text{ }^{\circ}\text{C}$);
- sustainable (come from and are degraded by microbes);
- replace toxic heavy metal catalysts currently used in polymer chemistry;
- naturally operate at mild temperatures owing to their extraordinary ability to lower the activation energy of chemical reactions, thereby reducing process energy consumption;
- retain structures of thermally or chemically sensitive moieties in monomers as they are converted to polymers. Steric hindrance at active sites enables the synthesis of linear or nearly linear chains when using monomers with functionality ≥ 3 .

adsorbed within a macroporous poly(methyl methacrylate-co-butyl methacrylate) resin.

Moving away from activated ester monomers

Early work assumed that activation of carboxylic acids by electron withdrawing groups was needed to perform enzyme-catalyzed polycondensations with diols and polyols [40]. However, it is now clear that activation of acid groups is not necessary for lipase-catalyzed polymerizations [12–16]. Furthermore, reaction times have decreased from days to hours. For example, N435-catalyzed bulk polymerizations of 12- and 16-C ω -hydroxyacids have been performed at ambient pressure, producing polyesters in only 4 h with DP_{avg} 90 (M_n up to 23 000, non-fractionated) [15]. Bulk condensation polymerizations of adipic acid and octanediol, also catalyzed by N435, generated non-fractionated polyesters with $M_n \cong 15\ 000$ in 8 h [16].

Concurrent ring-opening polymerization (ROP) and condensation reactions

An example of the versatility of lipase-catalyzed polymerization reactions is the ability to concurrently catalyze condensation and ring-opening chain-type polymerizations [17–19]. However, several reports involved long reaction times (72 h), yielding low molecular weight substances (80% yield with M_w 6500 g/mol [17] and 70% yield with M_n of 1800 [18], respectively). Slow reaction times can be attributed to: the use of lipases from *Pseudomonas cepacia* and porcine pancrease (instead of immobilized CALB [17,18]); working at relatively low temperatures 45–60 $^{\circ}\text{C}$ [17,18], instead of at $\sim 90\text{ }^{\circ}\text{C}$, which increases diffusion constraints; using polar solvents (isopropanol) [17] instead of working in bulk or in low polarity media (e.g. toluene, diphenyl ether); and selecting monomers with secondary hydroxyl groups (β -butyrolactone) [18] that propagate slowly by lipase-catalysis. The most recent report [19] described the use of N435 to prepare terpolymers from ω -pentadecalactone (PDL), diethyl succinate (DES) and 1,4-butanediol (BD) in a two-stage process with diphenyl ether as the solvent (Figure 1a). In the first stage, reactions were conducted at low vacuum (600 mmHg) for 18–24 h to minimize monomer loss via evaporation as non-volatile oligomers formed. In the second reaction stage, the pressure was decreased to 1–3 mm Hg, and reactions were conducted at 95 $^{\circ}\text{C}$ for 52 h, yielding near-random PDL-DES-BD terpolymers (Table 1; Figure 1a).

Prepolymers have also been prepared by combined ROP and condensation polymerizations. For example, using N435 as a catalyst, PDL was combined with glycidol and adipic acid to form epoxide-terminated prepolymers [20]. One-pot reactions went to completion within 24 h at 60 $^{\circ}\text{C}$. By changing monomer stoichiometry, epoxide-functionalized prepolymers were obtained with degrees of polymerization (DP) of 4, 6 and 10. The resulting prepolymers were copolymerized cationically with a cycloaliphatic diepoxide to high conversion, yielding crosslinked materials that formed durable films [20]. Similarly, poly(ω -pentadecalactone) (PPDL) macromers with terminal acrylate groups have been prepared by a combination of ROP, transesterification and condensation steps [21]. N435-catalyzed

Table 1. Enzyme-catalyzed polyester condensation polymerizations

Enzyme	Monomer	Condition	Solvent	M_w	M_n	Yield (%)	Ref.
N435	PDL, DES, BD	95 °C, 76 h	Diphenyl ether	77000			[19]
	PDL, glycidol, AA	60 °C, 24 h	Toluene		1400–2700	≥95	[20]
	MCH, MUA, PDL	90 °C, 24 h	Bulk		1900	87	[21]
	MCH, VA, PDL	90 °C, 6.5 h			1800	57	
	EGDMA, PDL	90 °C, 24 h			4000	71	
	AA, OD, glycerol	70 °C, 42 h	Bulk	75600		90	[23]
	TMP, OD, AA	70 °C, 42 h	Bulk	2700–26100		40–94	[14]
	AA, OD, erythritol/xylitol/ribitol/ D-glucitol/D-mannitol/D-galactitol	90 °C, 46 h	Bulk	11000–73000			[24]
	AA, OD, L-MA	80 °C, 48 h	Bulk	7400			[25]
	HD, DMMS	70 °C, 48 h	Bulk	14000		92	[31]
	HD, L-DMM	70 °C, 24 h		16300			
	HD, D-DMM	70 °C, 24 h		<400			
	PEG-1500, 5-DMAIP	90 °C, 48 h	Bulk		23000	93.5	[37]
	DMA, diamine disiloxane	70 °C, 12 h	Bulk		2100		[33]
	DEA, OD, Si-NH ₂	70 °C, 70 h	Bulk		6720–7500		[34]
	1,3-bis(3-Carboxypropyl) tetramethyldisiloxane, OD	70 °C, 24 h	Bulk	22000	11700		[35]
	Unsaturated/epoxidized α,ω -DMDA (C18, C20, C26), PD	70 °C, 4 h	Diphenyl ethyl	1900–3300		59–67	[40]
	Unsaturated/epoxidized α,ω -DMDA (C18, C20, C26), BD	70 °C, 4 h	Diphenyl ethyl	7900–11600		72–84	
	Isopropyl aleuriteate	90 °C, 21 h	Toluene, 2,4- dimethyl- 3-pentanol		5600		[43]
	<i>cis</i> -9,10-Epoxy-18-hydroxyoctadecanoic acid	85 °C, 3 h	Bulk	15000			[44]
Unsaturated/epoxidized α,ω -diacid (C18, C22), diols	90 °C, 48 h	Bulk or diphenyl ether	25000–57000		60–89	[13]	
PPL	C12- ω HA, β -BL	45 °C, 72 h	Toluene		1800	70	[18]
	bis(2,2,2-Trichloroethyl) <i>trans</i> -3, 4-epoxyadipate, BD	Ambient temperature, 5 d	Ethyl ether	7900			[28]
Lipase PC	PDL, DVS, BD	60 °C, 72 h	Isopropyl ether	6500		80	[17]
Lipase CA	DVS, glycerol, unsaturated fatty acids	60 °C, 24 h	Toluene		3600–9100		[39]
	DVS, glycerol, epoxidized fatty acids	60 °C, 24 h	Toluene		4200–6500	66–83	
IM-PC	Methyl ricinoleate	80 °C, 7 d	Bulk	73200			[42]
	Ricinoleic acid	80 °C, 7 d	Bulk	8500			
HiC	1,4-CHDM, SA	70 °C, 48 h	Bulk		19000		[48]
	C16- ω HA	70 °C, 8 h	Diphenyl ether		40400		[49]
	OD, C13-diacid				11000		
PHA synthase	3-HB-CoA	Room temperature, 12 h	PBS	106000	59000		[51]

Abbreviations: AA, adipic acid; BD, 1,4-butanediol; β -BL, β -butyrolactone; C12- ω HA, 12-hydroxydodecanoic acid; CHDM, 1,4-cyclohexanedimethanol; DEA, diethyl adipate; DES, diethyl succinate; DMA, dimethyl adipate; DMAIP, dimethyl 5-aminoisophthalate; DMDA, α,ω -dicarboxylic acid methyl esters; DMM, dimethyl malate; DMMS, dimethyl 2-mercaptosuccinate; DVS, divinyl sebacate; EGDMA, ethylene glycol dimethacrylate; 3-HB-CoA, 2-hydroxy butyrate-coenzyme A; HD, 1,6-hexanediol; HiC, cutinase from *Humicola insolens*; IM-PC, lipase from *Pseudomonas cepacia* immobilized on ceramics; L-MA, L-malic acid; Lipase CA, *Candida antarctica* lipase; Lipase PC, *Pseudomonas cepacia* lipase; MCH, 6-mercapto-1-hexanol; MUA, 11-mercapto-1-undecanoic acid; OD, 1,8-octanediol; PBS, phosphate buffer solution; PD, 1,3-propanediol; PDL, ω -pentadecalactone; PHA synthase, polyhydroxyalkanoate synthase; PPL, porcine pancreatic lipase; SA, sebacic acid; Si-NH₂, α,ω -diaminopropyl)polydimethylsiloxane; TMP, trimethylolpropane; VA, vinyl acrylate.

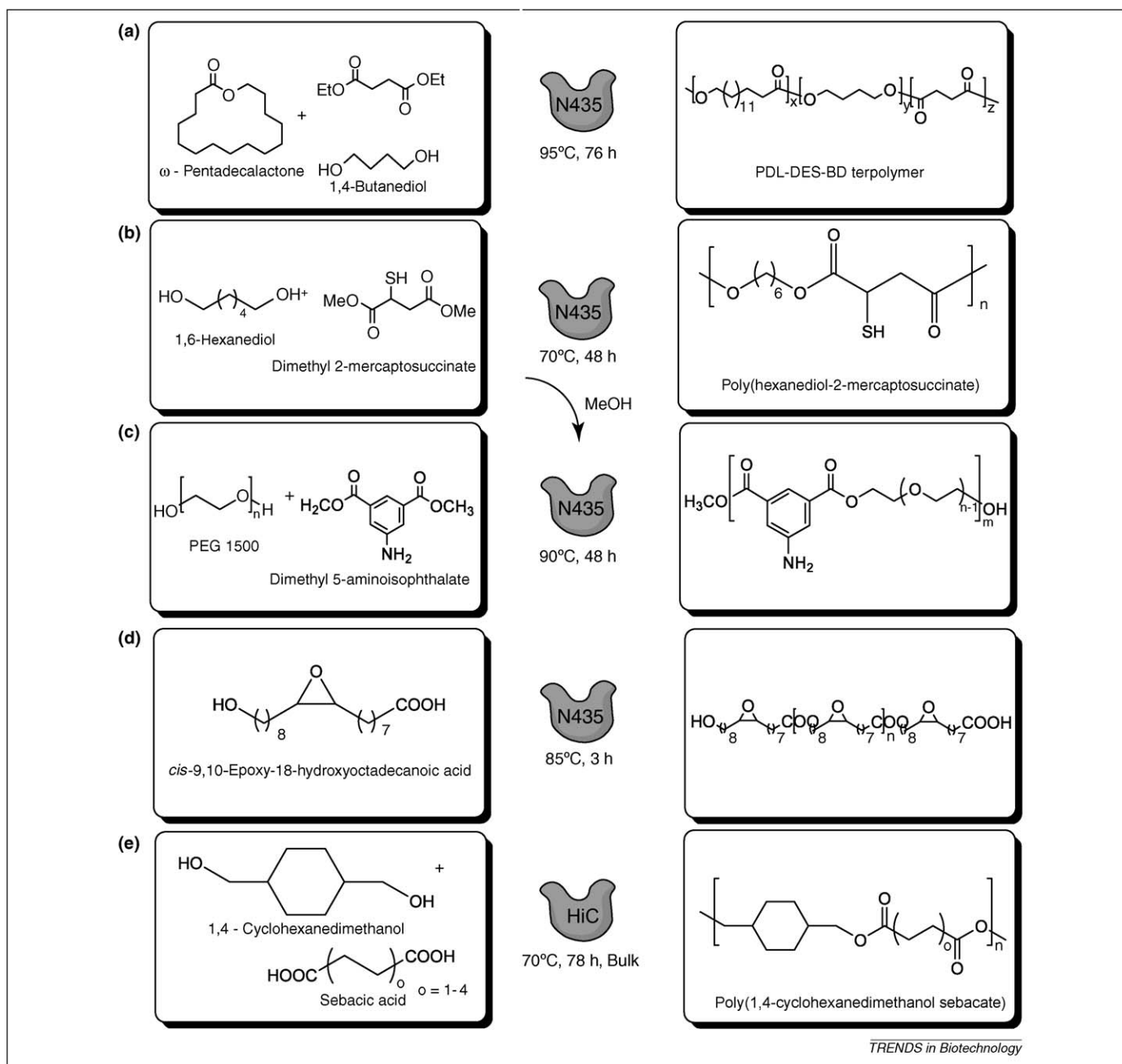
reactions between ethylene glycol diacrylate and PDL (1:10 equivalents) were performed at 90 °C without predrying to ensure that water would initiate chain formation. Reduced pressure was applied after 2 h for a total of 20 h to evaporate water and shift the equilibrium towards formation of α,ω -diacrylate-functionalized PPDL. In a final example, N435 catalysis was used to catalyze the ROP of PDL (5 eq.) where 6-mercapto-1-hexanol was the initiator and termination occurred by a condensation step between terminal ω -hydroxypentadecanoate units and 11-mercapto-1-undecanoic acid [21].

Polyol-polyesters

Enzyme-regioselectivity enables the conversion of multifunctional monomers (functionality ≥ 3) to linear or near-linear homo- and copolymers. Nearly two decades ago, it

was reported that, by using the protease Proleather, condensation polymerizations in pyridine between sucrose and bis(2,2,2-trifluoroethyl) sebacate proceeded with high regioselectivity, giving sucrose oligoesters (DP 11) in 20% yield (45 °C, 5 days) [22]. This inspired subsequent work by others that demonstrated such copolymerizations with polar multifunctional polyols could be performed under bulk reaction conditions, without activation of carboxylic acids.

To avoid the use of deactivating polar-aprotic solvents, polyols were combined with monomers to form monophasic liquids at temperatures sufficiently low to maintain immobilized CALB activity (≤ 95 °C). For example, N435-catalyzed bulk polycondensations were implemented at 70 °C under vacuum (40–60 mmHg) using adipic acid (A), 1,8-octanediol (O) and glycerol (G) as co-monomers



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Figure 1. Representative enzyme-catalyzed reactions showing reactants (left) and products (right). **(a)** N435-catalyzed preparation of terpolymers from ω -pentadecalactone (PDL), diethyl succinate (DES) and 1,4-butanediol (BD) in a two-stage process with diphenyl ether as the solvent [19]. **(b)** N435 chemoselectivity has been explored by performing N435-catalyzed polycondensations between 1,6-hexanediol and dimethyl 2-mercaptosuccinate stereoisomers, producing the polyester poly(hexanediol-2-mercaptosuccinate) [31]. **(c)** CALB-catalyzed copolymerizations of PEG1500 and dimethyl 5-hydroxyisophthalate have been performed using a chemo-enzymatic methodology [37]. **(d)** N435-catalyzed bulk polymerizations of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid, a naturally occurring fatty acid monomer, resulted in a polyester [44]. **(e)** HiC-catalyzed polycondensation of 1,4-cyclohexanedimethanol and sebacic acid has resulted in poly(1,4-cyclohexanedimethanol sebacate) [48].

[monomer feed ratio (A:O:G) 1:0.8:0.2 mol/mol] [23]. Initially, the reaction media was a two-phase liquid; however, within 60 min, the media became monophasic with suspended N435. Products at 45 min and at 2 h had little or no unreacted monomers as well as low M_n and M_w/M_n . Extension of polycondensations to 6 h and 18 h resulted in substantial increases in M_n as well as a broadening of the molecular weight distribution. Furthermore, CALB regioselectivity circumvented branching during chain formation for polymerizations up to 18 h. However, as the reaction time extended towards 42 h, products became increasingly branched as reactions moved from kinetic

to thermodynamic control. Thus, at 42 h, a hyperbranched polymer with 19 mol% dendritic glycerol repeat units was obtained in 90% yield with high M_w and M_w/M_n . Even with branching, the product remained soluble in many organic media.

In another study [14], N435-catalyzed terpolymerizations of trimethylolpropane (TMP), 1,8-octanediol and adipic acid were performed in bulk at 70 °C for 42 h under vacuum (40–60 mmHg). Variation of TMP in the monomer feed led to copolymers with degrees of branching (DB) varying from 20 to 67%. In one example, a hyperbranched copolyester with 53 mol.% TMP-adipate units was formed

in 80% yield and degree of branching 36%. As above, steric constraints imposed by CALB resulted in the formation of soluble branched polyesters. Chemical polymerizations with multifunctional monomers, such as glycerol or TMP, are normally plagued with formation of insoluble gels when reaction conditions are not strictly controlled (i.e. reaction time, monitoring of viscosity changes).

Given the well-known sensitivity of enzyme activity as a function of substrate structure, a study was undertaken to exploit CALB selectivity by using various alditol monomers to 'tune' polyol-polyester branching and, in turn, polymer properties (e.g. viscosity) [24]. CALB-catalyzed bulk terpolymerizations of adipic acid, 1,8-octanediol and a series of alditols (erythritol, xylitol, ribitol, D-glucitol, D-mannitol and D-galactitol) were studied, with all substrates polymerized forming polyol-polyesters with M_w values ranging from 11 000 (galactitol) to 73 000 (D-mannitol). There was no correlation between sugar reactivity and its chain length. Copolymers from D-mannitol had the largest degree of branching and, therefore, the greatest propensity for combined reactivity at both primary and secondary hydroxyl groups. Explanations for this difference in reactivity between sugars were proposed [24], resulting in the realization that an expanded set of alditol substrates will be needed to further understand this lack of correlation.

Malic acid (MA) is a natural AB_2 monomer used to prepare functional polyesters. By chemically catalyzed ROP, (*R,S*)- β -benzyl malolactone has been homo- and copolymerized to prepare MA-containing materials [21]. However, protection-deprotection steps involved are tedious. Enzyme-regioselectivity offers the potential to develop simple and direct routes to prepare MA copolymers. N435-catalyzed copolymerization of adipic acid, 1,8-octanediol and L-MA was recently investigated [25]. Reactions were conducted with 20% (w/w), relative to monomer) N435 for 48 h in bulk under reduced pressure (20–40 mmHg). By using 20 mol.% L-MA in the monomer feed at 80 °C, a copolyester was formed in 91% yield. Most importantly, NMR studies revealed N435 was strictly selective for esterification of L-MA carboxylic groups, leaving hydroxyl pendant groups unchanged.

Chemo- and enantioselective reactions

Most chemical condensation polymerization catalysts are not selective enough to resolve racemic monomer mixtures. Box 2 explains the use of enzymes for the preparation of racemic monomers. A hallmark of enzyme catalysis is enantioselectivity, which makes enzymes (i.e. lipases and cutinases) particularly well-suited for kinetic resolution polymerizations. Research on enzyme-catalyzed kinetic resolution polymerizations has focused primarily on problems in ROP reactions, such as the kinetic resolution of racemic ϵ -caprolactone monomers [26,27]. Two decades ago, the copolymerization of bis(2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate with 1,4-butanediol, using PPL as a catalyst, was reported to give a polyester with M_w 7900 g/mol and an optical purity in excess of 95% [28]. This, and other early work [5–9], provided a roadmap for others that have continued to build an understanding of how enzyme catalysis can be more efficiently used to prepare a wide range of chiral polymers (Box 2).

Box 2. Dynamic kinetic resolution (DKR)

DKR by iterative tandem catalysis (ITC) can be used to convert racemic monomers. Imperfect enantioselectivity during condensation polymerizations of A_2/B_2 comonomers has caused deviations from the required 1:1 stoichiometry, resulting in polyesters of low molecular weights. Furthermore, even when enantioselectivity is nearly absolute, ~50% of the monomer remains unused. To address these problems, ITC has been used to perform DKR [29]. Reactions were catalyzed by the concurrent action of an enantioselective acylation catalyst, N435, and a racemization catalyst, Ru[Shvo], to form enantio-enriched polymers from racemic AB monomers. A series of AB monomers differing in chain length with secondary hydroxyl (at $\omega-1$ position) and methyl ester moieties were prepared and subsequently used for polymerizations conducted in toluene at 70 °C. Dimethyl pyridine (DMP) was added to suppress dehydrogenation reactions. Furthermore, reactions were performed under reduced argon pressure (210 mmHg) to remove methanol while minimizing Ru(Shvo) deactivation owing to O_2 exposure.

In a preferred example, an N435-catalyzed polymerization of methyl-7-hydroxyoctanoate for 169 h resulted in 99% monomer conversion, product $M_p = 16\ 300$ g/mol, with an enantiomeric excess of 92%. DKR was also used to prepare high-enantiomeric-purity copolyesters using difunctional acyl donors and acceptors (i.e. an A_2/B_2 system) by the concurrent action of a Noyori-type ruthenium racemization catalyst and N435 [30]. Based on the high enantioselectivity of CALB for secondary benzylic alcohol group esterifications (reactivity ratio $[R]/[S] \approx 1 \times 10^6$), racemic α,α' -dimethyl-1,4-benzenedimethanol was selected as the diol, and dimethyl adipate as the acyl donor. Thus, at the start of the reaction, α,α' -dimethyl-1,4-benzenedimethanol consisted of an $[S,S]/[R,R]/[R,S]$ diastereomeric mixture in a molar ratio of 1:1:2 and an $[R]/[S]$ ratio of 1:1. As the reaction proceeded, N435 catalyzed the gradual incorporation of all $[R]$ -isomers into propagating chains. Concurrently, the Noyori-type ruthenium racemization catalyst converted free hydroxyl-functionalized $[S]$ -isomers into their $[R]$ -configurations, which were then esterified to methyl adipate units at chain termini by N435 catalysis. By 70 h, hydroxyl group conversion reached 92%, the ratio of $[R,R]/[R,S]$ was 16:1 (i.e. $[R]/[S] = 33:1$) and M_w reached 3400 g/mol ($M_w/M_n = 1.6$). The authors suggested that the above polymerizations could be improved by further suppressing side reactions and by using a faster and more stable racemization catalyst. Nevertheless, DKR, when applicable, is an elegant and effective route to reach 100% monomer conversions from racemic monomers to prepare high enantiomeric purity polyesters.

N435 chemoselectivity has been explored [31] by performing N435-catalyzed polycondensations between 1,6-hexanediol and dimethyl 2-mercaptosuccinate stereoisomers (Table 1; Figure 1b). Reactions between equimolar quantities of D,L-dimethyl-2-mercaptosuccinate and 1,6-hexanediol were conducted in bulk at 70 °C for 48 h using large quantities of N435 [30% (w/w), relative to monomer], producing the polyester, poly(hexanediol-2-mercaptosuccinate). Twenty-four-hour polymerizations were also performed under identical conditions to compare the reactivity for dimethyl esters of L-2-mercaptosuccinate, D,L-2-mercaptosuccinate and D-2-mercaptosuccinate. However, no noticeable difference was observed in molecular weights of polymers obtained from these mercaptosuccinate stereoisomers. Hence, it was concluded that N435 was non-selective for these enantiomeric substrates, and instead was chemoselective, leaving thiol groups unreacted [31]. The availability of thiol pendant groups allowed rapid crosslinking in dimethylsulfoxide (DMSO) by air to form disulfides.

Enantioselectivity of N435 has also been explored, specifically during copolymerizations of 1,6-hexanediol

with dimethyl L- or D-malate. The polyester synthesized in bulk using dimethyl L-malate had the highest molecular weight ($M_w = 16\,300$ g/mol) after precipitation, whereas dimethyl D-malate formed only oligomers ($M_w < 400$ g/mol). Thus, in contrast to mercaptosuccinate stereoisomers, CALB is selective between malate stereoisomers.

End-group functionalized silicones

Enzymes are excellent alternatives to traditional chemical catalysts, particularly when reactions require mild conditions owing to the presence of substances that are intrinsically unstable at elevated temperatures or in the presence of acids, bases or other chemical reagents. An interesting example is the preparation of siloxane-based copolymers that, when synthesized using chemocatalysts, often undergo uncontrolled redistribution and side reactions. 'Sweet silicone' bolamphiphiles have been prepared using N435 as the catalyst [32]. The regioselectivity of N435 enabled exclusive formation of ester bonds between carboxylic acid end-functionalized organosilicones and the hydroxyl group at position C6 of α,β -ethyl glucoside. Furthermore, biocatalytic methods have been developed to prepare a wide variety of polyesters and polyamides with organosilicone segments [33–35].

Poly(ethylene glycol) (PEG)

PEG is a neutral, water-soluble polymer with extraordinary biological properties, finding a niche in various applications, including drug delivery, cell encapsulation and conjugation to biomacromolecules to improve *in vivo* half-life [36]. This has motivated research to define conditions by which enzyme-catalyzed polycondensations can be efficiently used to build important new PEG-containing functional polymers. For example, using a chemo-enzymatic methodology, CALB-catalyzed copolymerizations of PEG1500 and dimethyl 5-hydroxyisophthalate have been performed in bulk at 90 °C (Table 1; Figure 1c) [37]. The product yield/ M_n after 12 and 48 h reaction times were 22%/37 000 and 93%/23 000, respectively. CALB-catalyzed polymerizations proceeded slowly when the 5-hydroxy of dimethyl 5-hydroxyisophthalate monomers was alkylated with chain lengths C-5 and longer, demonstrating how bulky pendant groups along chains cause steric constraints at the CALB active site, thus decelerating further chain growth.

It has been hypothesized that, by converting PEG chain ends to structures that mimic fatty acids, PEG building blocks would be better recognized as substrates by CALB during condensation polymerizations [38]. Hence, a series of PEG200 diacids (i.e. $\text{HOOC}-(\text{CH}_2)_x-\text{O}-(\text{CH}_2\text{CH}_2\text{O})_n-(\text{CH}_2)_x-\text{COOH}$) were synthesized, where x was 1, 4, 5, 7, 9 or 11. Copolymerizations of these diacids were conducted in bulk, under vacuum, at 70 °C for 24 h. Increasing the α,ω -carboxyalkyl methylene spacer length (x) from 1 to 5 resulted in an increase in DP_{avg} from 4 to 25. The reactivity of PEG400, PEG600 and PEG1000 during CALB-catalyzed condensation polymerizations was similarly increased by introducing 5-C carboxyalkyl spacers at PEG chain termini. Thus, introducing fatty acid-like terminal groups at PEG chain termini proved to be an effective strategy to

increase PEG reactivity for CALB-catalyzed polymerization reactions.

Fatty acid-derived monomers

Fatty acids are a diverse family of biobased building blocks. Typical chain lengths vary from 12 to 22 carbons, with variable degrees and patterns of unsaturation. Furthermore, many fatty acids are biosynthesized with hydroxyl groups at various positions along carbon chains. Chemical modification of fatty acids further diversifies their structures. Hydrolysis of fatty acids esters (e.g. triglycerides) is a natural function of lipases; the research below supports the conclusion that, under reverse equilibrium conditions, lipases are also excellent catalysts for converting functionalized fatty acids to polymers.

CALB-catalyzed copolymerization of glycerol, divinyl sebacate and fatty acids (e.g. oleic, linoleic and linolenic acids) has resulted in synthetic polymers with unsaturated pendant groups [39]. These polymers had a high proportion of trisubstituted glycerol units and M_n values that ranged from 3000 to 7000. After epoxidation of fatty acid side chains, the resulting polyesters were thermally cured to give transparent high gloss films. In another example, a Grubbs catalyst was first used to perform metathetical condensation converting 9-decenoic, 10-undecenoic and 13-tetradecenoic acid methyl esters to their corresponding long-chain, symmetrically unsaturated α,ω -dicarboxylic acid methyl esters (C-18, C-20, C-26) [40]. After epoxidation, N435-catalyzed copolymerization of the dicarboxylic acids with 1,4-butanediol in diphenyl ether yielded polyesters with M_w 7900–11600.

Polymers of ricinoleic acid (RA) have also been prepared by enzyme-catalysis. RA [(Z,R)-12-hydroxy-9-octadecenoic acid] possesses a secondary hydroxyl group and is unsaturated. Mild reaction temperatures of enzyme-catalyzed condensation reactions are appealing for RA polymerizations to avoid problems of discoloration, dehydration, free radical coupling, and other side reactions that might occur using chemocatalysis at higher temperatures. However, esterification of secondary hydroxyl groups by lipase-catalysis is generally slow, and RA must be rigorously purified to remove fatty acid impurities that lack both carboxyl and hydroxyl moieties. Given these constraints, bulk polymerizations at 70 °C have been performed [41] using technical grade RA and immobilized CALB. Poly(RA) synthesis proceeded slowly, reaching M_n 724 in 7 days. The use of other lipases was explored to identify a better enzyme-catalyst for polycondensation of RA and its corresponding methyl ester [42]. Polymerizations of methyl RA were performed at 80 °C for 7 days, with 50% (w/w) *Pseudomonas cepacia* lipase immobilized on ceramics (IM-PC) and molecular sieves to absorb methanol thereby shifting the reaction equilibrium. Polymer isolation by precipitation gave poly(RA) with M_w 73 200 in 88% yield, suggesting that the monomer used was of high purity. The activity of IM-PC for RA polymerization is interesting given that lipases generally have low reactivity for polymerization of monomers where reactions proceed via esterification of secondary substituted hydroxyl groups.

An alternative route to functional polyesters with fatty acid-like main chain units has been described for the

formation of poly(aleuriteate), a potential prepolymer for polyurethane synthesis [43]. Aleuritic acid, which has two secondary and one primary (ω -position) hydroxyl groups, was derived from naturally occurring ambrettolide (17-oxacycloheptadec-6-en-1-one). Isopropyl aleuriteate was prepared and its polymerization studied using N435 in a mixture of dry toluene and dry 2,4-dimethyl-3-pentanol at 90 °C and 413 mmHg for 21 h. After precipitation in pentane, poly(aleuriteate) was isolated in moderate yield. Interestingly, the authors found from NMR studies that the polymerization was highly selective for primary ω -hydroxyl groups, with no observable esterification at secondary hydroxyl positions. Furthermore, when isopropyl aleuriteate was polymerized using $\text{Ti(IV)(}n\text{-BuO)}_4$ at 200 °C and reduced pressure (5.0×10^{-2} mbar), the product obtained was discolored, had poor solubility and formed ester bonds at both the primary and secondary hydroxyl moieties.

Another naturally occurring fatty acid monomer is *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid, a major constituent of dry outer bark (100 g/kg) and 40% of aliphatic suberin monomers in *Betula verrucosa* [44]. N435-catalyzed bulk polymerizations of *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid (Table 1) resulted in a polyester after 3 h (Figure 1d). Owing to the mild polymerization conditions, analysis of polymers by NMR showed that the *cis*-epoxy groups remained intact.

ω -Carboxy fatty acid monomers have also been synthesized by feeding fatty acids to *Candida tropicalis* ATCC20962 and related engineered strains [13]. For example, bioconversions of oleic (OA), erucic and epoxy stearic acids by *C. tropicalis* ATCC20962 in shake flasks produced 1,18-*cis*-9-octadecenedioic, 1,22-*cis*-9-docosenedioic and 1,18-*cis*-9,10-epoxy-octadecanedioic. These diacids were then reacted with diols using N435-mediated catalysis to prepare unique functional polyesters. For example, M_w for N435-catalyzed synthesis of poly(ω -carboxyl-OA-co-1,8-octanediol) increased from 16 000 to 27 000 to 35 000 g/mol at 6, 12 and 24 h, respectively. Unsaturated and epoxy moieties on bio-based diacids retained their structure during polymer synthesis.

Other enzyme families used for polyester synthesis

Cutinases

The vast majority of reported cell-free, enzyme-catalyzed polyester syntheses have used immobilized CALB. This decision was either based on screening or simply owing to its previous success on related substrates. Cutinases are extracellular fungal enzymes whose natural function is catalyzing the hydrolysis of cutin, a lipid-polyester found in the cuticle of higher plants [45]. Thus far, the majority of published work on cutinase-catalyzed biotransformations has focused on cutinase activity for polyester hydrolysis [46] and on esterification or transesterification of small molecules [47]. We have discovered recently that the cutinase from *Humicola insolens* (HiC) has striking activity for both ring-opening and condensation polyester synthetic reactions [48]. HiC-catalyzed polycondensation of 1,4-cyclohexanedimethanol and sebacic acid resulted in poly(1,4-cyclohexanedimethanol sebacate) (Figure 1e), with M_n and M_w/M_n values of 19 000 and 1.7, respectively.

The thermal stability of HiC is lower than N435 because it loses substantial levels of activity at temperatures above 70 °C. Nevertheless, at present, HiC activity for cell-free polyester synthesis is rivaled only by CALB.

Systematic studies have been performed using immobilized HiC as the catalyst, with three series of substrates varying in ω -hydroxyalkanoic acid (ω -HA), α,ω -*n*-alkane diol and α,ω -*n*-alkane diacid chain lengths [49]. Comparison of immobilized HiC- and N435-catalyzed C16- ω -HA homopolymerizations in diphenyl ether produced polymers with M_n 40400 and 25500 g/mol, respectively. Furthermore, immobilized HiC and N435 each catalyzed the copolymerization of 1,8-octanediol and C13-diacid in bulk, yielding copolymers with M_n values of 11 000 and 9600 g/mol, respectively. Variation of substrate chain lengths showed that immobilized HiC has higher chain length selectivity than N435. Therefore, N435 was a more promiscuous catalyst relative to immobilized HiC, remaining active on a broader set of substrates. For example, HiC activity with respect to ω -HA substrates with 6-, 10-, 12- and 16-C was C16 > C12, where C10- ω HA and C6- ω HA were not polymerized. By contrast, N435 activity for ω -HA substrates was C16 = C12 > C10, where C6- ω HA was not polymerized.

It is too early in the study of cutinase-catalyzed polyester synthesis to say whether this enzyme family will be generally better or worse than lipases for polymer synthesis. More probably, they will provide complimentary benefits that are useful for different polymerization challenges. Nevertheless, further research on cutinases will certainly result in important new enzymes useful for a wide-range of polyester synthesis reactions.

Poly(hydroxyalkanoate) synthases

In addition to lipase-catalyzed polycondensation polymerizations, bacterial synthase or polymerase (PHA synthase; EC 2.3.1) catalysts are highly active for *in vitro* polycondensation polymerizations of (*R*)- β -hydroxyalkanoate monomers. Poly(hydroxyalkanoate)s (PHAs) are well-known biodegradable polyesters commercially produced via fermentation [50]. PHA synthases catalyze polycondensation polymerizations of (*R*)-hydroxyalkanoate monomers activated by formation of their corresponding coenzyme A (CoA) ester derivative (reviewed by Ref. [7]). Efforts to use lipase-catalysts to polymerize (*R*)- β -hydroxyalkanoate monomers from their corresponding lactones have required long polymerization times giving low-molecular weight chains [49]. Hence, relative to lipases that generally have poor activity for polymerization of monomers from secondary substituted hydroxyl moieties, PHA synthases have a unique capability to polymerize a specific type of such monomers. Herein, we restrict our discussion to recent advances in PHA synthases that focused on exploring their activity for catalyzing PHA synthesis from biomaterial surfaces.

His₁₀-tagged PHA synthase from *Wautersia eutropha* H16 was been site-specifically attached to lithographically patterned surfaces. Non-specific binding of the synthase was inhibited by adding bovine serum albumin (BSA) during enzyme immobilization. *In situ* enzymatic surface-initiated polymerization (ESIP) was subsequently

performed by immersing the enzyme-coated surface in an aqueous solution of 3-hydroxybutyrate-coenzyme A (3-HB-CoA). This resulted in the fabrication of spatially ordered polyhydroxybutyrate (PHB) polymeric structures with a maximum height of 1 μm and with homogenous surface coverage (Table 1). Furthermore, the addition of biotinylated BSA has allowed incorporation of biotin functional groups throughout PHB matrices [51]. This enabled further functionalization of matrices to display biologically active molecules. Thus, the resulting biomaterial surfaces prepared by enzyme-catalysis are envisioned to have numerous biomedical and biotechnological applications.

Concluding remarks

Enzyme-catalyzed polymerization reactions are already poised for use in commercial process to produce polymers for cosmetic and medical applications. Immobilized enzyme catalytic systems provide an important option to polymer chemists. Enzyme catalysts are advantageous versus chemical catalysis in challenging syntheses, such as polymerizing multifunctional monomers without the use of protection-deprotection catalysts, reducing process energy consumption, polymerizing monomers that decompose or undergo side-reactions at elevated temperatures, and when seeking to prepare enantiopure polymers. Box 3 discusses the future challenges for commercial production of polyesters via cell-free enzyme catalysis. Much progress has been made in recent years to reduce reaction times, define the activity of CALB with a broad range of monomer substrates (Table 1), and to begin exploring structure-activity relations with other enzymes, such as cutinases.

The literature revolving around enzyme-catalyzed condensation polymerizations is now sufficiently extensive such that limitations restricting use of this technology for commercial processes can be readily identified. With an increased realization by the scientific community of the

many benefits that enzyme-catalyzed condensation reactions have to offer, there will be an increased focus on improving catalyst efficiency [53]. One important route by which this will be accomplished is by applying rapidly emerging biotechnological tools to engineer proteins. Methods include directed evolution using stochastic methods, semi-rational design methods such as combinatorial active site saturation tests (CASTing) [54], pre-selecting mutants *in silico*, machine-learning-based methodologies, known as protein sequence-activity relation algorithm (ProSAR) [55,56], and much more. Such work will enable improvements in enzyme activity so they can be used at much lower concentrations in reactions. Furthermore, enzyme-engineering will improve catalyst thermal stability so that, when needed, enzyme-catalyzed reactions can be conducted at temperatures well above 100 °C. A focus on methods that improve enzyme and immobilization matrix stability as well as engineering processes to facilitate catalyst recycling will be essential to lower catalyst cost. In conclusion, the many benefits that enzyme catalysis provides in condensation catalysis will drive future development of the science and engineering solutions that are necessary to develop commercially viable processes.

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Box 3. Future challenges for commercial production of polyesters via cell-free enzyme catalysis

- Increased enzyme activity will decrease the amount of catalyst used in reactions. Typically, 10% (w/w, relative to monomer) of immobilized catalyst (10% of which is protein) is used for polymerizations. An attainable target that would provide an option to leave catalysts in products (particularly desirable when separation of catalyst from highly viscous reaction media is required) is to decrease enzyme concentration by more than 100-fold.
- Develop immobilized catalysts with improved recyclability in a wide range of common commercial manufacturing systems, such as stir tank reactors. This particular problem has been described, and one potential technological solution has been offered [52]. However, improving catalyst immobilization will require multiple design solutions that encompass improved enzyme stability, mechanical integrity of immobilization matrices, and engineering approaches that allow the separation of enzymes from viscous media without the need for solvent.
- Increase enzyme thermal stability to facilitate conducting reactions at higher reaction temperatures. This will enable: (a) solvent-free (i.e. 'green') systems with monomers and polymers that melt at higher temperatures, and (b) decreased diffusion constraints, which generally limit molecular weights attained.
- Identify other enzyme catalysts. Because enzymes are selective, there is a need for multiple enzyme-catalysts to satisfy the requirements for the broad range of monomer substrates and polymerization challenges.

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