A Cutinase with Polyester Synthesis Activity

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New paradigms in polymer synthesis are needed to meet increasing demands for structural complexity without a concurrent increased environmental burden. This requires catalysts that are selective while operating under mild conditions. Some enzymes, in nonaqueous media, have proven to be surprisingly active for a wide range of polyester and polycarbonate synthetic reactions. Reactions leading to polymers include step-condensation, transesterification, and ring-opening polymerization.[1] Surprisingly, the majority of enzymes studied for polymerization reactions have been from the lipase family with Lipase B from *Candida antarctica* as the dominant enzyme. This paper reports for the first time that a cutinase from *Humicola insolens* (HiC) has been found to have this unusual characteristic of catalyzing polyester synthesis. Cutinases are extracellular fungal enzymes whose natural function is catalyzing the hydrolysis of ester bonds in cutin, a lipid−polyester found in the cuticle of higher plants.[2] With molecular weights of around 20 kDa, cutinases are the smallest members of the serine α/β hydrolase superfamily.[3] Thus far, the majority of published work on cutinase-catalyzed biotransformations have focused on degradation of polyesters[4] and on the esterification or transesterification of small molecules.[5] Herein, we report that the cutinase from *Humicola insolens* (HiC), obtained from Novozymes, has promising activity for lactone ring-opening and condensation polymerization reactions.

Previous work has shown that, for polymerization reactions, it is preferable to immobilize enzymes on high surface area supports.[6] This increases the enzymes accessibility to high molecular weight substrates that must diffuse to and from the catalyst active site. Furthermore, it is well-known that immobilization of enzymes on solid supports often increases its thermal stability.[7] Many literature reports on lipase-catalyzed polymerizations use Lipase B from *Candida antarctica* immobilized on Lewatit beads.[8] Hence, as a starting point for studies of HiC activity for polyester synthesis, this enzyme was similarly immobilized by physical adsorption onto Lewatit beads. A study was performed to determine HiC activity for polyester synthesis via condensation reactions. A series of diols and diacids were selected that differ in chain length (see Scheme 1).

Diacids were used in the free acid form without activation by esterification with groups such as vinyl or halogenated alkyl esters. Indeed, many literature reports on lipase-catalyzed polyester synthesis have relied on activation of diacids with such leaving groups.[9] First, polymerizations were performed between adipic acid and diols of differing chain length (see Table 1, entries 1−3).

Table 1. HiC-Catalyzed Polycondensation Reactions between Various Alcohols and Diacids[a]

entry	alcohol	diacid	M_n[b] [Da]	M_w/M_n[b]	DP[c]
1	1,4-butanediol	adipic acid	2700	1.4	13
2	1,6-hexanediol	adipic acid	7000	1.5	31
3	1,8-octanediol	adipic acid	12000	1.6	47
4	1,4-CHDM	succinic acid	900	2.4	4
5	1,4-CHDM	adipic acid	4000	2.3	16
6	1,4-CHDM	suberic acid	5000	2.8	18
7	1,4-CHDM	sebacic acid	19000	1.7	61

[a] Reaction conditions: 1% w/w enzyme, 48 h, vacuum (10 mm of Hg), 70 °C, bulk, quantitative conversion. [b] Determination of conversion and molecular weight by GPC in THF using polystyrene standards. [c] Degree of polymerization (DP) = polymer M_n/molecular weight of repeating unit.

With an increase in diol chain length from C4 to C6 and C8, M_n increased from 2700 to 7000 and 12 000, respectively. The polydispersity (M_w/M_n) values were between 1.4 and 1.6, well below that for statistically random condensation polymerization reactions. In another series of reactions, 1,4-cyclohexanedimethanol (1,4-CHDM) was used as the diol, and the diacid chain length was varied from C4 to C10 (Table 1, entries 4−7, and Figure 1). Inspection of Table 1 shows that M_n values were 900, 4000, 5000, and 19 000 for the C4, C6, C8, and C10 diacids, respectively.

Hence, these experiments show that HiC has good activity for polycondensation polymerizations and that HiC activity is dependent on diol and diacid chain length. Increase in the diol and diacid chain length to C8 and C10, respectively, resulted in polyesters of increased molecular weight. Preference of HiC for longer chain length building blocks is reminiscent of substrate specificity observed for *Candida antarctica* Lipase B-catalyzed polycondensations.[10] Furthermore, cutin, the natural substrate hydrolyzed by cutinases, consists of long chain (e.g., C16, C18) hydroxyacids. 1,4-Cyclohexanedimethanol was found to be a good substrate for HiC-catalyzed polycondensation reactions. This is useful since the cyclohexyl ring can impart greater rigidity to the resultant polyesters. Low polydispersities for some polymerizations in Table 1 indicate HiC is “chain selective”.[11] In other words, chain growth occurs where HiC has higher activity for formation and/or esterifications between oligomers of certain chain lengths. Interestingly, chain selectivity during polymerization reactions was similarly observed for *Candida antarctica* Lipase B-catalyzed polycondensation reactions.[11]

Initial investigations of immobilized HiC thermal stability were conducted by performing polycondensation reactions at different temperatures (see Figure 1). Polymerizations between 1,4-CHDM and diacids with chain lengths C4 to C10 were performed at 50, 60, 70, 80, and 90 °C. Good activity was found over a large temperature range with an optimum at 70 °C. Large decreases in HiC activity occurred for reactions performed at 80 and 90 °C. Since polymerization reactions are often run in viscous reaction media, the ability to perform enzyme-catalyzed reactions at relatively higher temperatures, approaching 100 °C, is desirable.

Inspired by the finding that HiC is active for polycondensation polymerizations, studies were performed to assess HiC activity for lactone ring-opening polymerizations (Scheme 2).

HiC-catalyzed ROP was carried out in either bulk or toluene. To determine the relationship between reaction temperature and enzyme activity for lactone ring-opening polymerizations, ϵ-caprolactone (**1**) in bulk was taken as the model system.

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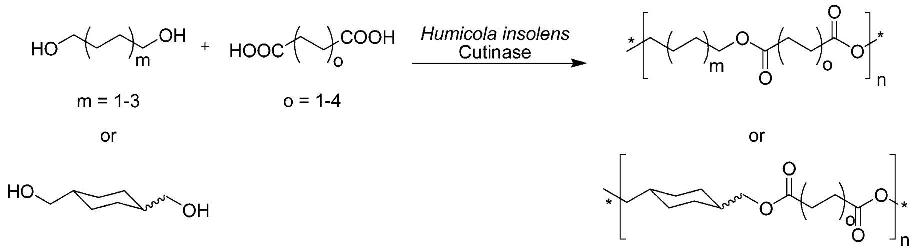
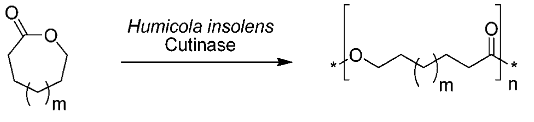
Scheme 1. HiC-Catalyzed Polycondensation Reactions between Diols and Diacids



Scheme 2. HiC-Catalyzed Ring-Opening Polymerization of Lactones



1: m = 1
2: m = 9

Table 2. HiC-Catalyzed Ring-Opening Polymerization of Various Lactones[a]

entry	monomer	solvent	T [°C]	conv[b] [%]	M_n[c] [Da]	M_w/M_n[c]	DP[d]
1	**1**	bulk	50	57	4300	4.2	38
2	**1**	bulk	60	83	6300	3.2	55
3	**1**	bulk	70	>99	16000	3.1	140
4	**1**	bulk	80	14	2500	2.7	22
5	**1**	toluene	70	>99	24900	1.7	218
6	**2**	toluene	70	>99	44600	1.7	186

[a] Reaction conditions: 0.1% w/w enzyme, 24 h, no vacuum, in toluene or bulk. [b] Determination of conversion by ^{1}H NMR ($CDCl_3$). [c] Determination of molecular weight by GPC in THF (entries 1−5) and $CHCl_3$ (entry 6), using polystyrene standards. [d] Degree of polymerization (DP) = polymer M_n/molecular weight of repeating unit.

Inspection of Table 2 , entries 1−4, shows that optimum conversion and polyester molecular weight from **1** were at 70 °C. HiC activity dropped precipitously for polymerizations conducted at 80 °C. These results agree with those described above for condensation polymerizations. By performing poly(ϵ-caprolactone) in toluene instead of in bulk at 70 °C, M_n increased from 16 000 to 24 900 and M_w/M_n decreased from 3.1 to 1.7.

An increase in M_n is expected for solution polymerizations since the solvent decreases the viscosity of the reaction medium, thereby easing diffusion constraints between substrates and the enzyme. However, the decrease in polydispersity for the solution polymerizations is less easily explained. One possibility is that transesterification reactions leading to broader polydispersity occur more rapidly for reactions conducted in bulk.[10] HiC catalysis of ω-pentadecalactone (**2**) polymerization in toluene at 70 °C gave poly(ω-pentadecalactone) with M_n and M_w/M_n of 44 600 and 1.7, respectively. Thus, both 7- and 16-membered lactones **1** and **2** are excellent substrates for HiC-catalyzed polymerizations.

In summary, the cutinase from *Humicola insolens* is a new, promising biocatalyst for polyester synthesis. The immobilized cutinase showed optimal activity at 70 °C and catalyzed a broad range of condensation and lactone ring-opening polymerizations in bulk and using toluene as solvent, yielding high molecular weight polyesters. On the basis of an extensive literature review and experience in our laboratory, we conclude that the activity of cutinase from *Humicola insolens* for the polyester synthesis reactions studied herein is rivaled only by Lipase B from *Candida antarctica*. Work is in progress to better define the activity, stability, and recyclability of *Humicola insolens* immobilized on other solid supports.

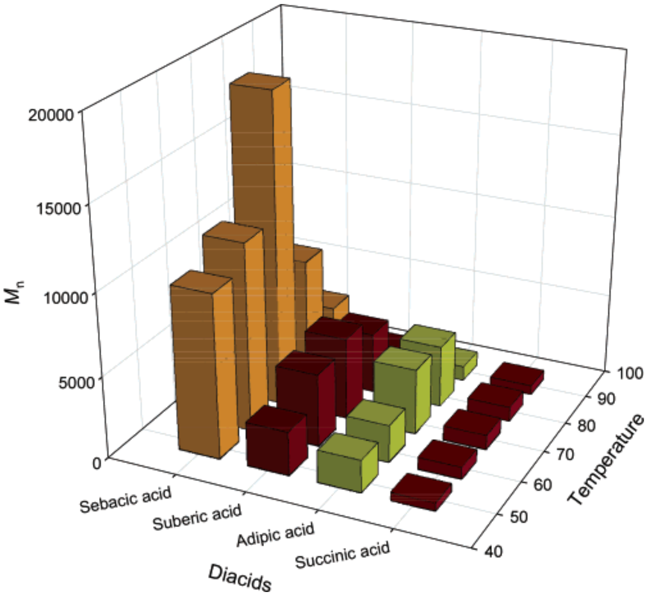


Figure 1. Polycondensation between 1,4-CHDM and various carboxylic diacids (C4, C6, C8, and C10, respectively) over the temperature range of 50−90 °C showing obtained molecular weights (M_n).

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Supporting Information Available: Experimental procedures and characterization of polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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