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One-Pot Biocatalytic Synthesis of Sugar Based Poly (ε-caprolactone)

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Summary: The one-pot biocatalytic synthesis of amphiphilic polymers consisting of an isosorbide headgroup and a hydrophobic polyester chain is described. The immobilized lipase of *Yarrowia lipolytica* (YLL) catalyzed ring-opening polymerization of ε-caprolactone (ε-CL) initiated by the multifunctional initiator isosorbide. Polymerizations were carried out in bulk at 70, 80, 90 and 120 °C. The highest reaction rates for ε-CL polymerization resulted for YLL immobilized on macroporous resins: Lewatit VP OC 1026 at 80 °C, and the strongly acidic Lewatit K-2629 at 90 °C. Structural analyses were made by MALDI-TOF, ^{1}H, and ^{13}C-NMR.

Keywords: biocatalysis; biodegradable polymers; isosorbide; lipase

Introduction

The ability to attach synthetic polymers onto carbohydrates is a pathway to new applications in the fields of detergents, packaging, and pharmaceuticals. In the case of some aliphatic polyesters, this approach leads to an increment in the biodegradability of the target polymers. Water soluble and dispersible polymers are in great demand for applications as detergents and surfactants. Low molecular weight amphiphilic compounds such as fatty acid esters of carbohydrates also function as useful surfactants. However, selective functionalization of carbohydrates is difficult to achieve, as carbohydrates contain multiple hydroxyl groups with different chemical reactivity. Selective monoacylation of the carbohydrate is only obtained using protective group strategies. Deprotection steps are required and the synthetic scheme becomes complicated. Enzymes are highly selective catalysts and therefore they have been used to regioselectively acylate carbohydrates. Lipase-catalyzed polyester synthesis is an attractive alternative to poorly selective chemical catalysts.[1] In particular, incorporation of aliphatic polyesters to sugars appears to be a promising strategy for the design of new biodegradable amphiphilic structures.[2]

Isosrbide is a rigid, bicyclic diol produced in two chemical steps from dextrose that can be prepared, and is available, on a large commercial scale.[3] Recently, enzyme-catalyzed reactions have been demonstrated to provide high selectivity for the acylation of various carbohydrates. Specifically, lipases and proteases have been successfully used for the acylation of the primary hydroxyl group(s) of sugars (glucose, lactose, maltose, etc.) in polar aprotic solvents.[2]

In this work, studies on the bulk ring-opening polymerization of ε-caprolactone induced by immobilized *Yarrowia lipolytica* lipase (YLL) in the presence of isosorbide is reported. Products were characterized by ^{1}H and ^{13}C- NMR, MALDI-TOF and DSC.

Experimental Part

Lipase Isolation and Immobilization

Lipase production by *Yarrowia lipolytica* was made as previously reported by Barrera *et al.*[4] Lewatit beads were purchased form Sigma-Aldrich. Before immobilization, beads were activated with ethanol (1:10 beads: ethanol), washed with distilled water

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and dried under vacuum for 24 h at room temperature. The beads (1g) were shaken in a rotatory shaker in 15 mL of YLL lipase solution with 0.1568 mg/mL at 4 °C for 14 h. After incubation, the carrier was filtered off, washed with distilled water and then dried under vacuum for 24 h at room temperature.

Polyester Synthesis

Isosorbide (Aldrich) was recrystallized twice from dry acetone. Prior to its use Isosorbide was dried over P_2O_5 in a desiccator for 24 h at room temperature. ε-CL (Aldrich) was distilled at 97–98 °C over CaH_2 at 10 mmHg. In a typical run, x mmol of ε-CL, x mmol of isosorbide and 12 mg of immobilized YLL were placed in a 10 mL vial previously dried. Vials were stoppered with a teflon silicon septum and placed in a thermostated bath at predetermined temperatures and predetermined time. After the reaction was stopped, the enzyme was filtered off and the residue was analyzed for conversions by ^{1}H-NMR. Products were purified by dissolving in chloroform (1 volume), precipitating in methanol (10 volume), and drying in a desiccator at room temperature.

Analysis

Solution ^{1}H and ^{13}C-NMR spectra were recorded at room temperature on a Varian Gemini 2000. Chloroform-*d* ($CDCl_3$) was used as solvent. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) spectra were recorded in the linear mode by using a Voyager DE-PRO time-of-flight mass spectrometer (Applied Biosystems) equipped with a nitrogen laser emitting at $\lambda = 337$ nm with a 3 ns pulse width and working in positive-ion mode and delayed extraction. A high acceleration voltage of 20 kV was employed. 2,5-dihydroxybenzoic acid (DHB) was used as matrix. Samples were dissolved in acetonitrile and mixed with the matrix at a molar ratio of approximately 1:100. DSC thermograms were obtained in a Mettler-Toledo 820e calorimeter using heating and cooling rates of 10 °C/min. thermal scans were performed from 0 °C to 100 °C.

Results and Discussion

Lipase Absorption

Different styrenic and polyesterenic resins with varied physical properties were

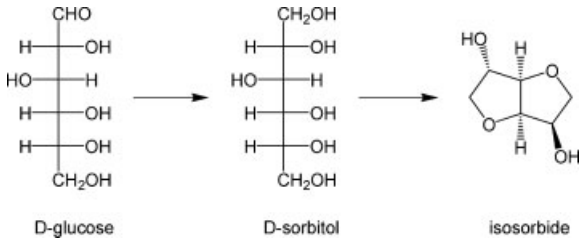
Table 1.
Matrix parameters and loading of *Y. lipolytica* lipase on Lewatit beads.

	Matrix	Matrix active group	Protein Content (mg/g)	Protein adsorption (%)	Surface Area	Pore diameter
Lewatit® K-2629 hydrogen form particles	styrene-divinylbenzene (macroporous)	sulfonic acid	0.1443	92	~40 m^2/g	65 nm
Lewatit® MonoPlus TP 214	styrene-divinylbenzene (macroporous)	thiourea	0.1379	88	~40 m^2/g	65 nm
Lewatit® MP-62 free base	styrene-divinylbenzene (macroporous)	–	0.0784	50	~40 m^2/g	65 nm
Lewatit® VP OC-1026	crosslinked polystyrene (macroporous)	di-2-ethylhexyl phosphate	0.136	87	–	–
Lewatit® VP OC 1064 MD PH	crosslinked polystyrene	None	0.0652	42	very high surface area	–
Lewatit® VP OC 1065 weakly basic	crosslinked polystyrene	benzyl amine	0.1076	69	–	–
Lewatit® VP OC 1163	crosslinked polystyrene microporous	–	0.081	52	1000–1400 m^2/g	0.5–10 nm

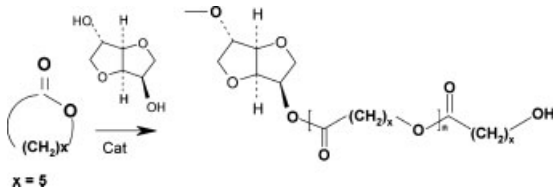
employed as supports for YLL immobilization, and the results for protein absorption were measured and are shown in Table 1. For styrene resin samples (Lewatit K2629, TP214 and MP62), each with 65 nm pore diameter, the saturation time for YLL absorption was ~60 min. For polystyrene resin samples (Lewatit 1026, 1064, 1065 and 1163) the saturation time for YLL absorption was 30 min for Lewatit 1026, the differences in pore diameter and surface area showed similarly rapid YLL adsorption. The enhanced adsorption rates of polystyrenic resins relative to styrenic resins with similar physical properties are attributed to stronger hydrophobic interactions between styrenic surfaces, functional groups and YLL. The dependence of adsorption rate on particle size is due to the pore size that is limiting protein transport to the inside of the particles. In other words, the small size of pores slows protein diffusion into beads so that smaller beads more rapidly were saturated in protein.

Polyester Synthesis

Isosorbide was used as the multifunctional initiator for ε-caprolactone (ε-CL) ring-opening polymerizations (Scheme 2). This provided a novel route for the one-pot



Scheme 1.
Isosorbide synthesis.



Scheme 2.
Yarrowia lipolytica catalyzed polymerization of ε-caprolactone and isosorbide to form biodegradable amphiphilic polyesters.

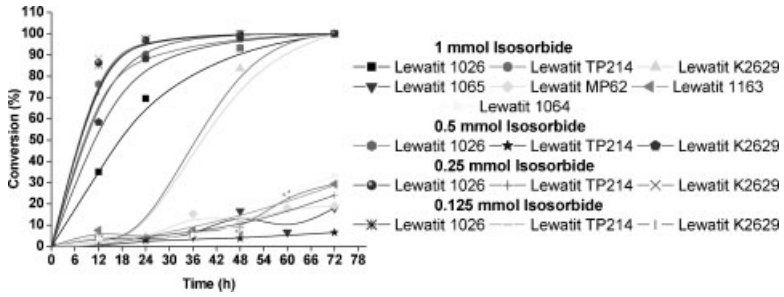


Figure 1.
Monomer conversion as a function of time for the enzyme-catalyzed ε-caprolactone-isosorbide polymerizations at 70 °C. R = 1 mmol ε-CL/12 mg immobilized lipase.

synthesis of oligomers with isosorbide headgroups.

Experiments were performed to determine the reaction order of monomer. The effect of incubation times, isosorbide concentration, resin type and temperature in the polymerization of ε-CL were evaluated. Figure 1–3 show the kinetic studies in function of time and isosorbide concentration. Lowest conversions of ε-CL to PCL-isosorbide were observed when lipase was immobilized on Lewatit TP214 at 70 °C and

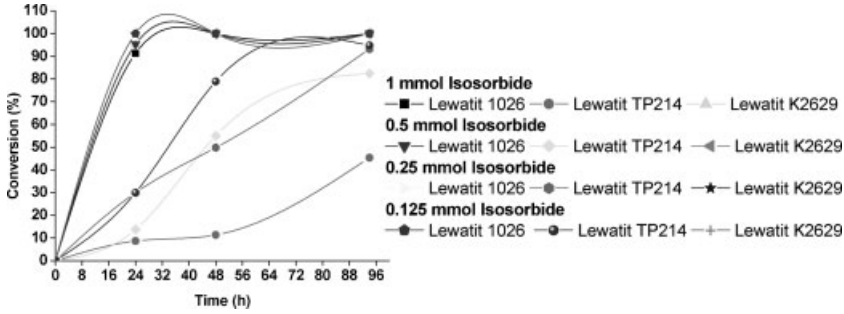


Figure 2.
Monomer conversion as a function of time for the enzyme-catalyzed ε-caprolactone-isosorbide polymerizations at 80 °C R = 1 mmol ε-CL/12 mg immobilized lipase.

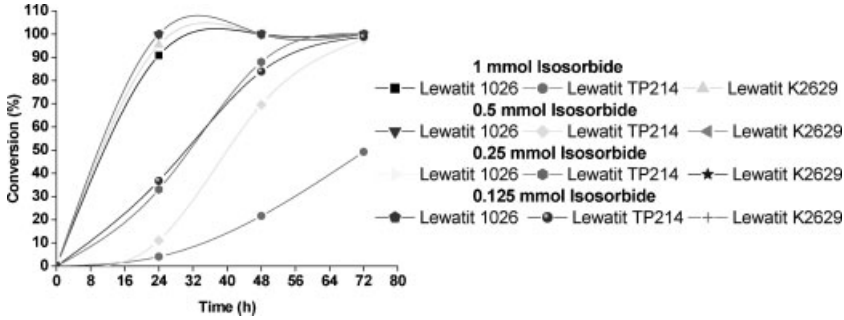


Figure 3.
Monomer conversion as a function of time for the enzyme-catalyzed ε-caprolactone-isosorbide polymerizations at 90 °C. R = 1 mmol ε-CL/12 mg immobilized lipase.

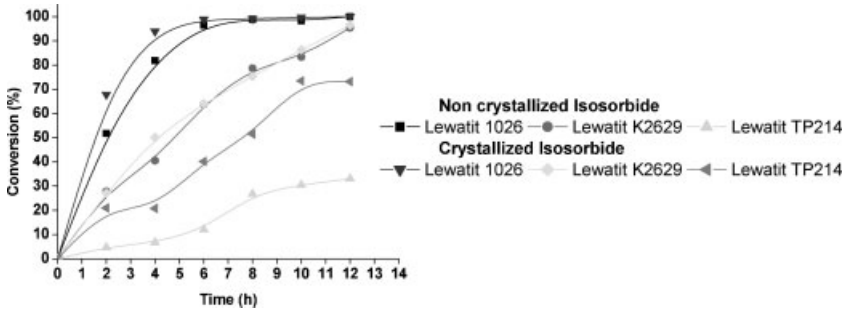


Figure 4.
Monomer conversion as a function of time for the enzyme-catalyzed ε-caprolactone-isosorbide polymerizations at 120 °C. R = 5 mmol ε-CL/1 mmol isosorbide/12 mg immobilized lipase.

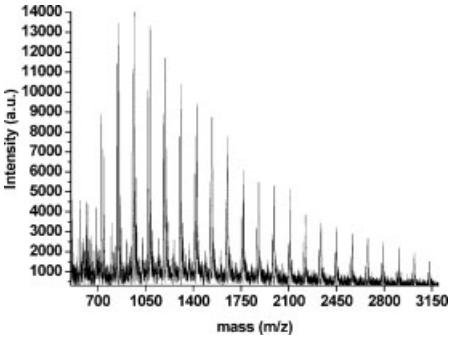
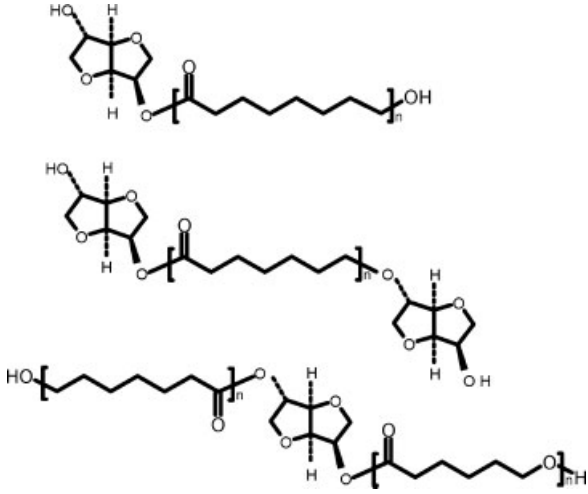


Figure 5.
MALDI-TOF spectrum for PCL-Isosorbide obtained by enzymatic ROP. R = 1 mmol CL/0.125 mmol isosorbide/12 mg YLL 1026 at 80 °C reaction time 94 h. *Mn*(MALDI) = 1068, *Mw/Mn* = 1.51.

0.5 mmol of isosorbide. One of the reasons of this behavior is the distribution of the enzyme into the polymeric resin, which is affecting the specificity of the enzyme. It was observed that higher conversions were obtained when isosorbide concentration was lower, this can be attributed to the fact that all the active sites of the lipase are in the form of enzyme-activated monomer and enzyme-activated initiator and this converts the reaction rate slower. From the results on YLL activity as a function of particle size and surface area for styrenic and polystyrenic resins, the percent surface area occupied by YLL is a critical factor that can be used to improve immobilized YLL activity. Increased percent accessible surface area will increase the probability of collisions between substrates and YLL. As the matrix active group changes in the styrenic resins, a corresponding increase in polyester synthesis reaction rates was observed.

The 100% of the monomer conversion was obtained at all temperatures using Lewatit 1026, TP214 and K2629; this can be explained from the fact that those resins are the ones that contain the highest percentage of protein content which makes them more active. The polymerization rate strongly depends on the enzyme loading. Furthermore, increase in enzyme loading results in a more uniform distribution of enzyme throughout beads.

Previous studies of lipase-catalyzed polymerizations have avoided the use of temperatures >90 °C. This is likely due to concerns over losses in catalyst activity. Figure 4 shows the results obtained in the ε-CL conversion by changing the resin and using crystallized isosorbide and non crystallized in function of time at 120 °C. We can observe that there is not a significant change in polymerization rates by using



Scheme 3.
Species for PCL-Isosorbide present in MALDI-TOF mass spectra.

crystallized/non crystallized isosorbide. It is clearly seen that 100% monomer conversion is obtained by YLL immobilized on Lewatit 1026. This can be explained by the fact that immobilized YLL on polystyrenic resin with a small bead particle size decreases the diffusion constraints that lead to productive collisions between enzyme and substrate, being the percent resin area in which reactions can occur the same.

Characterization

For the enzyme-catalyzed ring-opening of ε-CL by isosorbide at 80 °C for 94 h in bulk, we obtained a product which was purified by crystallization/co crystallization to remove unreacted isosorbide, the resulting end-group "tailored" oligopolyester had a number average molecular weight (*M*n) and polydispersity (*M*w/*M*n) of 1068 and 1.51 respectively, as determined by MALDI-TOF. In the full MALDI-TOF spectrum given in Figure 5, a series of signals dominate, which can be adscribed to the PCL-isosorbide oligomers doped with Na^+ ions. MALDI-TOF spectra show the formation of at least 3 species (Scheme 3). In Figure 6 the expanded view for the 650-1850 m/z fragments is shown, it typifies the asymmetric and disperse oligomer distributions of the polyester products obtained. The mass range below 300 was dominated by peaks resulting form matrix-fragments and metal ions.

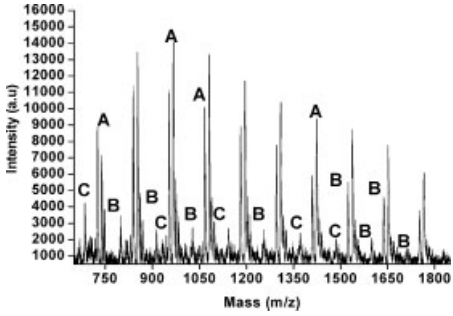


Figure 6.
MALDI-TOF spectra of the PCL-Isosorbide catalyzed by YLL. Expanded view for de 650–1850 m/z fragments. R = 1 mmol CL/0.125 mmol isosorbide/12 mg YLL 1026 at 80 °C reaction time 94 h. *Mn*(MALDI) = 1068, *Mw*/*Mn* = 1.51.

The reaction resulted in poly(ε-CL) chains which were attached by an ester group exclusively to the secondary hydroxyl moiety of isosorbide. The structure of the product was confirmed by nuclear magnetic resonance (NMR) experiments.

During the lipase-catalyzed bulk polymerization of lactones, water is known to act as an initiator.[5–7] Therefore, there is the possibility of competitive initiation between isosorbide and water. If a fraction of the poly(ε-CL) chains were initiated by water, this would result in carboxylic acid terminal chain ends, with a carbonyl signal in the region between 178 and 175.5 ppm.[8,9] Based on assignments of carbon-13 NMR spectrum of PCL, the signal at

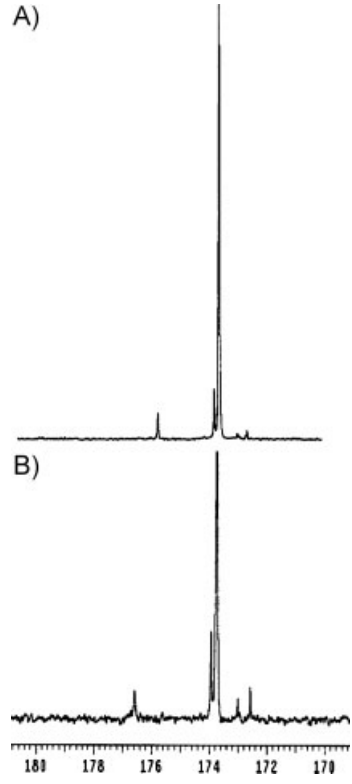


Figure 7.
Spectral regions (170–180 ppm) of the ^{13}C NMR spectra (200 MHz, solvent CDCl3) of (A) PCL-isosorbide. R = 1 mmol CL/0.125 mmol isosorbide/12 mg YLL K2629, T = 90 °C reaction time 24 h and (B) PCL-isosorbide. R = 1 mmol CL/0.125 mmol isosorbide/12 mg YLL 1026 at 80 °C reaction time 94 h.

Table 2.
Calorimetric results on poly (ε-CL)-isosorbide.

Sample	Crystallization	Fusion	Cristallinity
	T °C	T °C	%
PCL-Isosorbide (1026)	8.67–8.64	32.75–32.79	60.83
PCL-Isosorbide (K2629)	21.93–20.99	47.75–47.79	30.05

173.6 ppm is ascribed to the intrachain carbonyl ester group of poly(CL). The presence of a peak in the 175.5-178 ppm region suggested that the initiation of the poly(ε-CL) chains for the reaction that were carried out with YLL in Lewatit K2629 and in Lewatit 1026 occurred by both, water and isosorbide (Figure 7A and 7B). Carboxylic acid peak appears at 176.5 ppm in Figure 7A and 175.8 in Figure 7B. This difference in chemical shifts indicated that in the polymerization carried out with YLL in Lewatit K2629, ring-opening initiated by isosorbide is more common.

The calorimetric data for the obtained oligo-polyesters are collected in Table 2. Figure 8A shows DSC first heating curves for the two samples obtained after crystallization. In Figure 8B cooling and second heating curves for the samples are displayed. We can observe in the second heating that at least two different PCL crystalline phases are

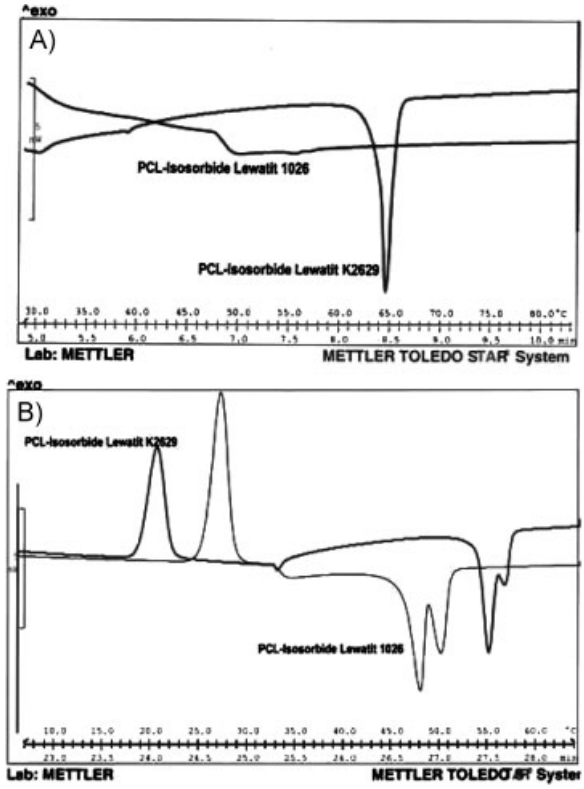


Figure 8.
DSC curves for PCL-isosorbide. R = 1 mmol CL/0.125 mmol isosorbide/12 mg YLL K2629, T = 90 °C reaction time 24 h and PCL-isosorbide. R = 1 mmol CL/0.125 mmol isosorbide/12 mg YLL 1026 at 80 °C reaction time 94 h. (A) first heating and (B) crystallization and second heating.

present (biphasic curves). Phase transitions for sample obtained with Lewatit 1026 are displaced to lower temperatures due to its low molecular weight. This multiphase morphology has been previously reported for low-molecular weight PCLs.[4]

Conclusions

A convenient one-pot biocatalytic synthesis of novel biodegradable amphiphilic oligomers is described. The selectivity of different immobilization matrices and general applicability of the method was also demonstrated by screening a number of commercial matrices with *Yarrowia lipolytica* lipase. Thus, by this strategy, chains were formed having sugar headgroups without using protection-deprotection strategies.

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