Synthesis of polyesters by biocatalysis in ionic liquids

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1. Introduction

Ionic liquids (ILs) have emerged as exceptionally interesting nonaqueous reaction media for enzymatic transformation reactions, and research in this area has increased widely during recent years [1-4]. They are salts entirely composed of ions which are liquids below 100 °C or typically above room temperature. Their interest as green chemicals resides in their high thermal stability and very low vapor pressure, which can be used to mitigate the problem of volatile organic solvent emission in the atmosphere. Moreover, the physical properties of ILs (density, viscosity, melting points, polarity, etc.) can be finely tuned by the appropriate selection of anions and (or) cations [5].

The 1-alkyl-3-methylimidazolium ionic liquids are polar solvents. They are miscible with polar solvents like methylene chloride and water, and inmiscible with hexane (although [BMIM][BF_4] is inmiscible in water). Although polar organic solvents inactivate enzymes, ionic liquids are an exception; this feature extends enzyme-catalyzed reactions to a solvent polarity range that was previously inaccessible. The ability to use solvents with greater polarity increases the solubility of polar substrates, leading to faster reactions and changes in selectivity [6].

Lipase-catalyzed polymerizations provide an environmentally friendly methodology of biodegradable polymer syntheses owing to non-toxic enzyme catalyst and mild reaction conditions [7]. The first report of ring-opening polymerization (ROP) by lipases in the presence of ionic liquids was made by Kobayashi *et al* [7]; they reported the synthesis of PCLs with molecular weights in the range of 430-4200, with long reaction times (24-168 h). In this work, the ring-opening polymerization of ε-caprolactone by *Yarrowia lipolytica* lipase in the presence of ionic liquids was investigated for the first time. The effects of lipase concentration, monomer concentration, reaction time and temperature (60 °C) were evaluated. Results indicate that polyesters with multiphase morphology were obtained.

2. Experimental conditions

1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), 1-buthyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-buthylpyridinium tetrafluoroborate ([BuPy][BF_4]), 1-buthylpyridinium trifluoroacetate ([BuPy][CF_3COO]), 1-ethyl-3-methylimidazolium nitrate ([EMIM][NO_3]) synthesis [8] and lipase production was previously reported by Barrera *et al* [9]. ε-CL (Aldrich Chemicals Co.) was dried over calcium hydride and distilled under reduced pressure before use. In a typical experiment, 1 mL of ionic liquid, 1.0 g of ε-caprolactone (8.76 mmol) and 0.1 g of *Y. lipolytica* lipase were added to a 10 mL vial previously dried and purged with dry nitrogen. Vials were stoppered with a teflon silicon septum and placed in a thermostated bath at predetermined temperatures (60 °C) for 24 h. After 24 h, the enzyme was filtered off. The polymer was extracted by five consecutive extractions with 5 mL toluene. Toluene was removed by evaporation at reduced pressure. Final polymer was crystallized from chloroform/methanol and dried under vacuum. Molecular weights and conversions during reaction were monitored by ^{1}H-NMR.

3. Results and discussion

Experiments were carried out to determine the reaction order of monomer. In the ROP of ε-caprolactone (ε-CL), the effects of lipase concentration, monomer concentration, reaction time and temperature (60 °C) were evaluated. In figure 1 kinetic curves for the enzymatic ROP of (ε-CL) by *Y. lipolytica* lipase in different conditions are shown. It was observed that lipase presented a higher stability in function of time and temperature when it is used in ionic liquids compared to organic solvents, in which the enzyme becomes inactive.

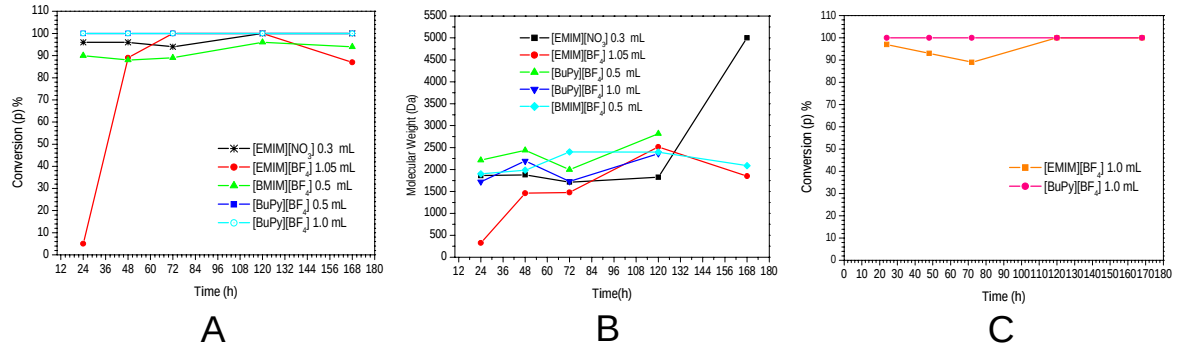

Figure 1. *Kinetic curves for ROP catalyzed by Y. lipolytica lipase in ionic liquids.* ***A.*** *R= 3 mmol ε-CL/50 mg LY/x mL ionic liquid. T= 60 ℃.* ***B.*** *R= 3 mmol ε-CL/50 mg LY/x mL ionic liquid. T= 60 ℃.* ***C.*** *R= 3 mmol ε-CL/100 mg LY/ 1.0 mL ionic liquid. T= 60 ℃.*

Polyesters with molecular weights in the range of 329-8100 Da were obtained; these PCLs presented a bimodal distribution (DSC) and high degrees of crystallinity (WAXS, FT-IR and DSC). This bimodal distribution can be attributed to the presence of two or more different polymer species.

In Figure 2 the ^{1}H-NMR spectrum of the adduct obtained after addition of TFA to HA-PCL is shown. Formation of an ester group leads to the upfield shift for methylene *f* from δ 3.64 to 4.384 ppm [*f*, $-CH_2OCOCF_3$]. Reaction between the HA-PCL carboxylic acid end group and TFA to form anhydride [*a*, $-CH_2CO_2COCF_3$] also occurs. Therefore, derivatization of both PCL end groups in one step can be achieved using trifluoroacetic anhydride.

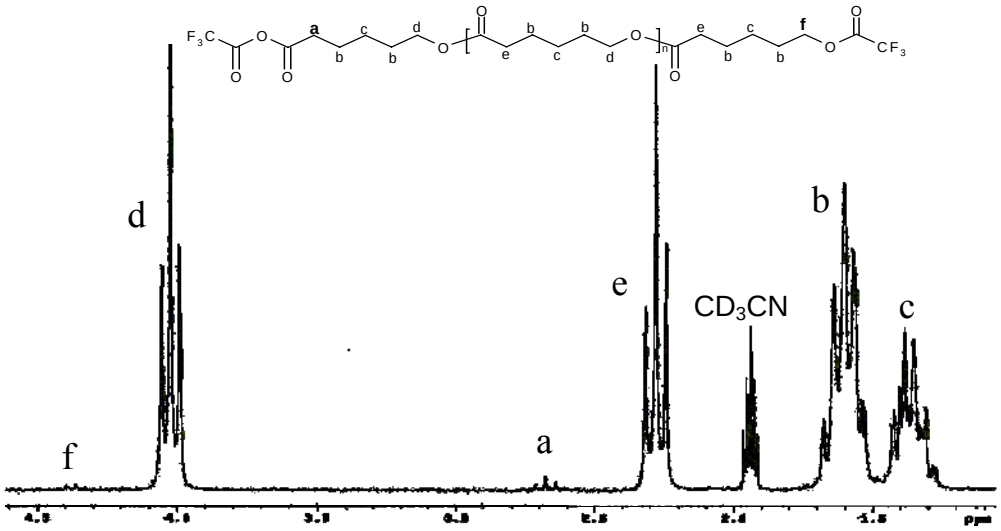

Figure 2. *^{1}H-RMN spectrum for poly(ε-caprolactone) obtained with Y. lipolytica lipase in CD_3CN after derivatization with trifluoroacetic anhydride. Mn(NMR)=8100 Da. R= 2 g ε-CL/100 mg lipase, 1 mL [BuPy][BF_4], T= 60 ℃, t= 24h.*

Peak patterns observed in the CP-MAS spectra for all PCLs are similar to those reported in the literature [10] However, MAS spectra show important differences, in particular for signals due to carbonyl and to the methylene carbon adjacent to oxygen.

In the MAS spectrum, a sharp peak due to carboxyl group (173.7 ppm) separated from a shoulder at 176 ppm (due to a end-group carboxylic acid functionality), can be seen. Peak for carboxylic acid is not clearly observed in the CP-MAS spectra. The most notorious differences are seen in the methylene linked to oxygen zone (carbon *f*, see Figure 3). These differences indicate the coexistence of various types of phases, which are formed during crystallization. Occurrence of co-crystallization is rare in Polymer Science [11] and there are not previous reports on this phenomenon in PCLs.

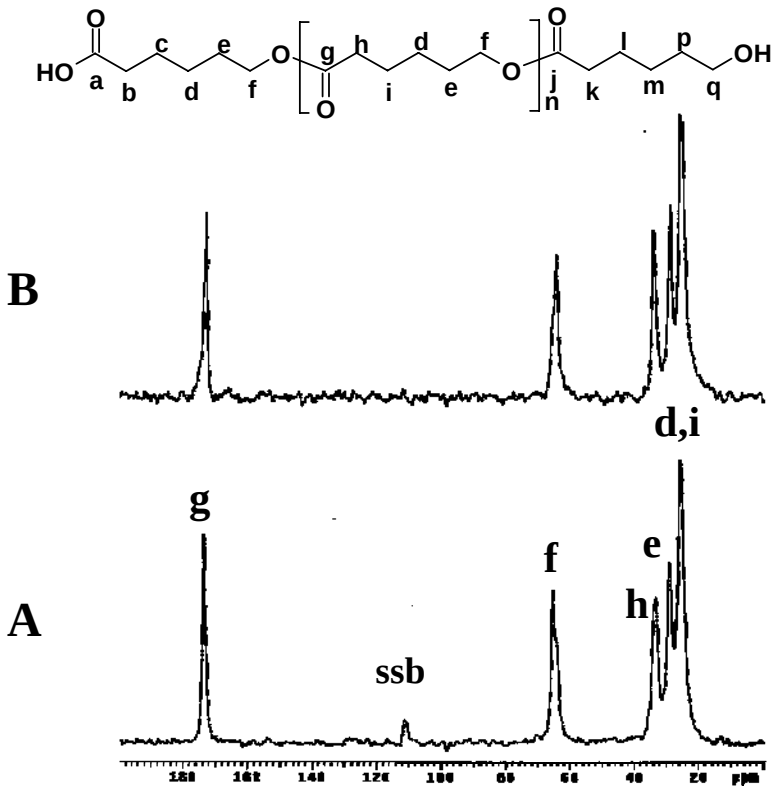

Figure 3. *Solid-state ^{13}C NMR (75.47 MHz) spectrum for poly (ε-CL) obtained with Y.lipolytica lipase in the presence of [BuPy][BF_4]. R= 2 g ε-CL/100 mg lipase, 1 mL [BuPy][BF_4] T= 60 ℃, t= 24h.*
(A) CPMAS spectrum, contact time 1 ms, repetition time 3 s; (B) MAS spectrum with a repetition time of 20 s. Spinning sidebands (ssb).

4. Conclusions

Yarrowia lipolytica lipases are efficient catalyst in the ROP of lactones in the presence of ionic liquids. High molecular weight polyesters in short reaction times are obtained. As expected from the current accepted mechanism for ring-opening polymerization of CL by lipases, final polymers are asymmetric telechelic α-hydroxy-ω-carboxylic acid poly (ε-caprolactones), as determined by proton and carbon-13 NMR.

References

[1] Kragl, U.; Eckstein, M.; Kaftzik, N.*Curr. Opin. Biotechnol*. **13** (2002) 565.
[2] Park, S.; Kazlauskas, R. J. *Curr. Opin. Biotechnol*. **14** (2003) 432.
[3] van Rantwijk, F.; Madeira-Lau, R.; Sheldon, R. A. *Trends Biotechnol.* **21** (2003) 131.
[4] Song, C. E. *Chem. Commun*. (2004) 1033.
[5] De Diego, T., Lozano, P., Gmouh, S., Vaultier, M., Iborra, J.L., *Biomacromolecules* **6(3)** (2005) 1457.
[6] Park, S., Kazlauskas, R., *Current Opinión in Biotechnology* **14** (2003) 432.
[7] Uyama, H., Takamoto, T., Kobayashi, S., *Polymer Journal* **34(2)** (2002) 94.
[8] Barrera-Rivera, K.A., Martínez-Richa, A., *Journal of the Mexican Chemical Society*, Submitted (2007).
[9] Barrera-Rivera, K.A., Flores-Carreón, A., Martínez-Richa, A., *Journal of Applied Polymer Science*, Submitted (2007).
[10] Mathias, L.L.; Colleti, R.F.; Halley, R.J.; Jarrett, W.L.; Johnson, C.G.; Powell, D.G.; Warren, S.C.; Solid-state NMR Polymer Spectra. Collected Volume 1, Hattiesburg, MS: MRD Polymer Press. p. 89.
[11] Inoue, Y.; Solid State NMR of Polymers, edited by I. Ando and T. Asakura, "Biodegradable Polymers", in Solid-state NMR of Polymers. Studies in Physical and Theoretical Chemistry, I. Ando and T. Asakura eds., Vol. 84, Elsevier, 1988, p. 771-817.