INVESTIGATING THE MECHANISM OF ENZYME-CATALYZED RING-OPENING COPOLYMERIZATIONS[†]

Matthew T. Hunley,[a] Nese Sari,[b] and Kathryn L. Beers[a]

[a]Polymers Division, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899-8542
[b]Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD 20850

Introduction

As the research interest in degradable polymers has risen, an ever increasing number of manuscripts report the synthesis of copolymers through ring-opening copolymerization techniques. The incorporation of comonomers can enhance the mechanical performance,[1] tailor the degradation rates,[2] and improve the barrier properties[3] of degradable polymers. To reduce the brittleness of poly(lactic acid) (PLA), the comonomer ε-caprolactone (ε-CL) can be used to tailor the glass transition and crystalline melting temperature.[1] Adding ε-CL to a PLA homopolymer can also increase the permeability of hydrophobic therapeutic molecules for drug delivery applications.[3] The specific sequence of monomer units within the copolymer also acts as a tool to control polymer properties. Li et al.[2] measured the degradation rates of copolymers composed on specific dyad sequences of LA and glycolic acid (GA). The degradation rate of the copolymers was highly dependent on monomer sequence. They measured a dramatic increase in hydrolytic cleavage rates for GA-GA linkages compared to LA-LA dyads.

The copolymerization behavior of lactones and lactides can be unpredictable. For example, the reactivity ratios for the bulk copolymerization of ε-CL and LA using $Al(O^iPr)_3$ are $r_{\varepsilon\text{-}CL} = 0.58$ and $r_{LA} = 17.9$.[4] However, homopolymerization of ε-CL using the same catalyst proceeds three orders of magnitude faster than LA under identical conditions. The mechanism of copolymerization also remains unexplored. To the best of our knowledge, all studies reporting reactivity ratios of enzyme-catalyzed ring-opening copolymerizations apply the terminal model with no analysis of the actual propagation method. In addition, the presence of transesterification reactions at higher conversions complicates the analysis of copolymer composition and monomer sequence distributions.[5]

Recently, we described an *in situ* spectroscopic technique to calculate reactivity ratios for enzyme-catalyzed ring-opening copolymerization of ε-CL and δ-valerolactone (δ-VL).[6] The copolymerization was well described by the terminal model reactivity ratios. In the present study, we investigate the mechanism of copolymerization of ε-CL and δ-VL via lipase catalysis. We used ^{13}C nuclear magnetic resonance (NMR) to quantify the monomer sequence distributions of the poly(ε-CL-*co*-δ-VL) (PCLVL) copolymers.

Results and Discussion

The copolymerization of ε-CL and δ-VL (Figure 1) was chosen due to the similar polymerizability of both monomers under a wide range of conditions and catalysts. Our previous study demonstrated that the copolymer compositions of PCLVL are well described by terminal model reactivity ratios $r_{\varepsilon\text{-}CL} = 0.38$ and $r_{\delta\text{-}VL} = 0.29$ when catalyzed by lipase.[6]

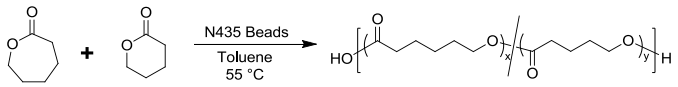

Figure 1. Monomer structure and copolymerization of ε-CL and δ-VL.

Table 1. Kinetic Equations and Reactivity Ratios for the Terminal and Penultimate Copolymerization Models for ε-CL and δ-VL

Terminal Model

~C· + C → ~C·	k_{CC}
~C· + V → ~V·	k_{CV}
~V· + C → ~C·	k_{VC}
~V· + V → ~V·	k_{VV}
$r_C = k_{CC}/k_{CV}$	$r_V = k_{VV}/k_{VC}$

Penultimate Model

~CC· + C → ~CC·	k_{CCC}
~CC· + V → ~CV·	k_{CCV}
~VC· + C → ~CC·	k_{VCC}
~VC· + V → ~CV·	k_{VCV}
~CV· + C → ~VC·	k_{CVC}
~CV· + V → ~VV·	k_{CVV}
~VV· + C → ~VC·	k_{VVC}
~VV· + V → ~VV·	k_{VVV}
$r_{CC} = k_{CCC}/k_{CCV}$	$r_{VV} = k_{VVV}/k_{VVC}$
$r_{VC} = k_{VCC}/k_{VCV}$	$r_{CV} = k_{CVV}/k_{CVC}$

Although the terminal model adequately describes the copolymer composition, it may not represent the most accurate model to describe the copolymerization mechanism. For example, in the free radical copolymerization of styrene and acrylonitrile, the terminal model predicts the copolymer compositions but fails to describe the copolymerization kinetics and the monomer sequence within the copolymer.[7,8] Analysis of the monomer sequence distributions and propagation kinetics indicated that the penultimate propagation model best described the overall copolymerization of styrene and acrylonitrile. Table 1 lists the individual reactions and reactivity ratios for both the terminal and penultimate propagation models. While both models can describe similar copolymer composition data, the sequence distributions for each model differ significantly. The number fractions of ε-CL-centered triads for the terminal model are:

$$N_{CCC} = \frac{r_C^2 f_C^2}{r_C^2 f_C^2 + 2 r_C f_C f_V + f_V^2}$$

$$N_{VCC} = N_{CCV} = \frac{r_C f_C f_V}{r_C^2 f_C^2 + 2 r_C f_C f_V + f_V^2}$$

$$N_{VCV} = \frac{f_V^2}{r_C^2 f_C^2 + 2 r_C f_C f_V + f_V^2}$$

where f_C is the molar feed fraction of monomer 1 and $f_V = 1 - f_C$.[8] Similarly, for the penultimate model,

$$N_{CCC} = \frac{r_{VC} r_{CC} f_C^2}{r_{VC} r_{CC} f_C^2 + 2 r_{VC} f_C f_V + f_V^2}$$

$$N_{VCC} = N_{CCV} = \frac{r_{VC} f_C f_V}{r_{VC} r_{CC} f_C^2 + 2 r_{VC} f_C f_V + f_V^2}$$

$$N_{VCV} = \frac{f_V^2}{r_{VC} r_{CC} f_C^2 + 2 r_{VC} f_C f_V + f_V^2}$$

Conclusions

We used quantitative ^{13}C NMR to experimentally calculate the monomer sequence distribution of PCLVL copolymers. The methylene carbon signals were resolved into four separate peaks based on the individual monomer dyads. The sequence distributions indicated a statistical polymerization process, consistent with the previous analysis of reactivity ratios and copolymer composition. Preliminary experiments indicated the ring-opening copolymerization proceeds via the terminal model. This quantitative technique offers a rapid method to probe the mechanism of copolymerization of lactone monomers.

Acknowledgements. M.H. would like to acknowledge support from the National Research Council Research Associateship Program.

References

(1) Wei, Z.; Liu, L.; Qu, C.; Qi, M. *Polymer* **2009**, *50*, 1423.
(2) Li, J.; Stayshich, R.M.; Meyer, T.Y. *J. Am. Chem. Soc.*, **2011**, *133*, 6910.
(3) McDonald, P. F.; Lyons, J. G.; Geever, L. M.; Higginbotham, C. L. *J. Mater. Sci.* **2010**, *45*, 1284.
(4) Vion, J.-M.; Jerome, R.; Teyssie, P.; Aubin, M.; Prud'homme, R.E. *Macromolecules*, **1986**, *19*, 1828-1838.
(5) T. Kobayashi and S. Matsumura, *Polym. Deg. Stab.*, **2011**, *96*, 2071.
(6) Hunley, M.T.; Bhangale, A.S.; Kundu, S.; Johnson, P.M.; Waters, M.S.; Gross, R.A.; Beers, K.L. *Polym. Chem.*, **2012**, *3*, 314.
(7) Hill, D.J.T.; O'Donnell, J.H.; O'Sullivan, P.W. *Macromolecules*, **1982**, *15*, 960.
(8) Burke, A.L.; Duever, T.A.; Penlidis, A. *Macromolecules*, **1994**, *27*, 386.