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Exploiting Sequence To Control the Hydrolysis Behavior of Biodegradable PLGA Copolymers

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Supporting Information

ABSTRACT: Monomer sequence is a potentially powerful but underutilized tool for the control of copolymer properties. Sequence is demonstrated to dramatically affect the hydrolysis profile for the degradation of poly(lactic-*co*-glycolic acid) (PLGA), a member of the most widely used class of biodegradable polymers employed in biomedical applications. The nearly linear molecular weight loss profile and uniform thermal behavior throughout the course of the hydrolysis differ dramatically from the behavior that is exhibited by random copolymer controls with the same comonomer ratio.

Although Nature uses sequence to great effect in the creation of complex biopolymers from a limited set of monomers, the exploitation of sequence to tune properties is relatively rare in synthetic copolymers.[1,2] Those examples that have been reported, however, suggest the potential power in this approach; they include the demonstration by Lutz that tailored microstructures can be used to gain insights into the folding of synthetic polymers,[3] the work by Kamigaito on creating sequenced copolymers using the RAFT methodology,[4] the use of transition metal catalysts to control tacticity and monomer alternation,[5−7] the exploitation of ADMET by Wagener to prepare polyolefins with periodic side chain placement,[8] and the template-based methodologies employed by Sawamoto to control sequence in radical copolymerizations.[9,10] Our group has also worked in this area, developing synthetic methodologies for the creation of repeating sequence copolymers of poly(lactic-*co*-glycolic acid) (PLGA) and demonstrating that the solution phase conformations as reflected by their NMR spectra are extremely sequence dependent.[11,12] Herein, we report the first use of sequence to control the hydrolysis profile of these biodegradable polyesters.

We have focused attention on PLGAs because we believe that there is a great potential for both scientific and biomedical impact: (1) PLGAs, which are FDA approved for medical use, are the most widely employed biodegradable and bioassimilable polymers, both experimentally and clinically.[13−15] If controlling sequence improves properties, there is a well-established field for application. (2) PLGA hydrolysis patterns typically involve very fast initial hydrolysis followed by a very slow degradation of the residual material.[16,17] This pattern can result in undesirable drug release bursts and/or difficulty in clearing the remaining material from the area after its purpose is served.[18,19] Sequence has the potential to address these challenges. (3) Nearly all PLGAs are random copolymers for which L:G monomer ratio and molecular weight represent the only intrinsic variables that can be modified to tune hydrolysis behavior.[20] Other methods for control either require the introduction of additional monomers or focus on PLGA particle formulation and/or engineering.[21−23] Sequence represents a powerful yet unused tool for creating designer PLGAs with precisely tuned behavior.

A key inspiration for our investigations into PLGA sequences can be found in the elegant work that has been performed by Spassky, Kasperczyk, Coates, and others in controlling and understanding the implications of stereosequence in the lactic acid homopolymers (PLAs).[5] By careful design of catalysts used for the ring-opening polymerization (ROP) of lactides, PLAs exhibiting a range of tacticities have been prepared, and the effects of tacticity on polymer properties, including hydrolysis rates, have been reported.[24−26] While powerful, this methodology is not currently amenable to preparing complex structures and stereosequences for copolymers that include glycolic units.[27,28]

Although we can prepare, using a segmer assembly polymerization (SAP) approach, a wide variety of repeating sequenced copolymers containing both lactic and glycolic units, we have focused our initial hydrolysis studies on the simplest sequence, poly(lactic-*alt*-glycolic acid), which we term **poly LG**.[27,29] This polymer, synthesized as described previously from a dimeric precursor, was prepared in two molecular weights, 16 and 26 kDa (Figure 1).[11] Two random PLGAs were selected for comparison: a 1:1 random copolymer of glycolide and *rac*-lactide produced via ROP by Durect Corp., which we refer to as **R-ROP**, and a random polymer variant produced in our laboratory using by the condensation reaction of the dimeric precursors LG, GG, LL, and GL (L = lactic unit, G = glycolic unit). It should be noted that this second polymer, which we term **R-SAP**, was synthesized from stereopure L-lactic precursors and prepared with the same reaction conditions as the sequenced **poly LG** samples. ^{1}H NMR spectroscopy of the polymers confirmed that all samples consisted of a 50:50 ratio of L to G (Table 1).

Prior to the hydrolysis study, the copolymers were formulated into microparticles with sizes ranging from 2 to 4 μm using a standard emulsion method.[30] These particles were purposely sized to minimize the tendency for autocatalytic burst behavior.[31,32] The initial molecular weight of the polymers in the microparticles was characterized by size exclusion chromatography (SEC) in THF vs polystyrene standards. It is important to note that three of the four samples have approximately the same molecular weight (26−32 kDa) while the fourth, **poly LG(16k)**, is intentionally smaller.

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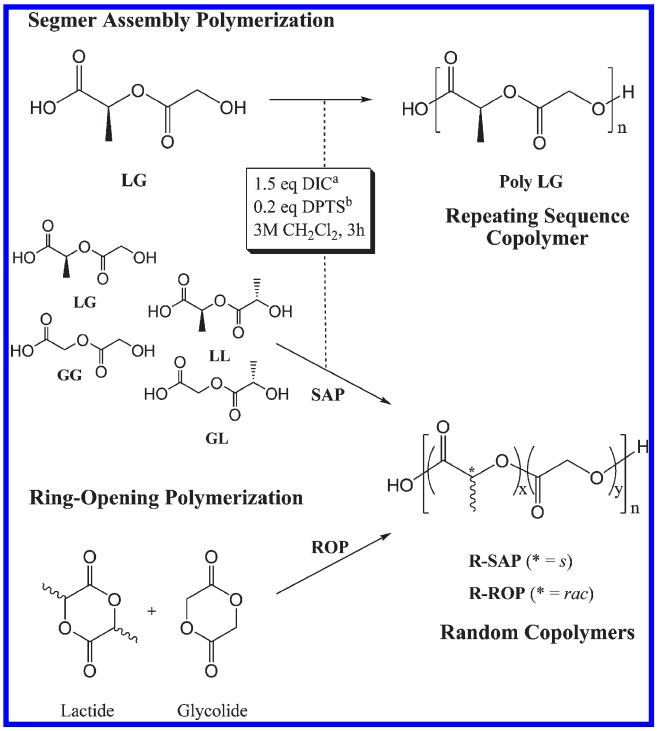

Figure 1. Approaches to sequenced and random PLGAs. [a]DIC = diisopropylcarbodiimide. [b]DPTS = 4-(dimethylamino)pyridinium *p*-toluenesulfonate.

Table 1. Characterization Data for PLGAs

	M_n[a] (kDa)	M_w[a] (kDa)	PDI	particle size[b] (μm)	L:G
R-ROP	31.8	41.3	1.3	2.05	50:50
R-SAP	31.0	40.3	1.3	3.96	50:50
poly LG(26k)	26.5	34.4	1.3	1.84	50:50
poly LG(16k)	15.7	25.1	1.6	2.30	50:50

[a] Number- and weight-average molecular weights determined by SEC in THF vs polystyrene standards. [b] Particle sizes determined by dynamic light scattering.

Given the homology of the polymers, it is expected that the relative molecular weights, as measured by SEC, can be reasonably compared even if the absolute molecular weights differ from those listed.

The *in vitro* hydrolytic degradation behavior of the chosen PLGA polymers was observed at pH 7.4 in a phosphate buffer for 8 weeks. The molecular weights were measured by SEC as a function of time, and the results are plotted in Figure 2. The commercially available random PLGA, **R-ROP**, exhibited a degradation profile that matches the exponential decay typically reported for this class of polymer. The M_n half-life is ~10 days. After the loss of 70% of the initial molecular weight in 4 weeks, the polymer molecular weight does not significantly decrease to the end of the experiment. **R-SAP** also exhibits a rapid decrease in molecular weight (M_n half life = 14 days), although the leveling-off effect is less pronounced. The molecular weight continued to drop even at 8 weeks. It should be noted that by the end of the observation period very little solid PLGA remained in either of the random copolymer samples.

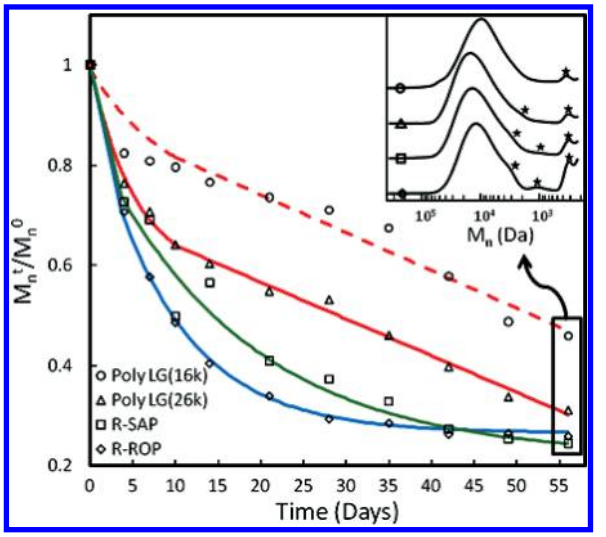

Figure 2. Plot of normalized molecular weight as a function of time for the repeating sequence and random copolymers of poly(lactic-*co*-glycolic acids). Inset: SEC plots for day 56 hydrolysis samples. Asterisks represent low-molecular-weight oligomers.

Although the two random copolymers exhibited similar profiles, their hydrolysis rates were not the same. This discrepancy is not surprising since the copolymers were made by different routes and with a different degree of stereochemical control. In the ROP of glycolide and lactide, there is a significant difference in monomer reactivity that leads to a random but blockier microstructure.[33] NMR analysis shows that the SAP polymer has shorter runs of pure G and pure L blocks. Also, the SAP-prepared copolymer was made from stereopure L-lactic acid. Stereopure PLGAs are known to degrade more slowly than those made from racemic monomers.[28,34]

The sequenced PLGAs degrade with a significantly different and uniform profile. After an initial rapid but minor drop in weight, the loss becomes remarkably linear. The initial weight loss is smaller than that observed for the random controls over the same period: 20% for **poly LG(16k)** and 35% for **poly LG(26k)**. The next phase of the hydrolysis, which exhibits nearly zero-order behavior over the time period under observation, shows a rate of loss of molecular weight that is nearly identical for both the 16 and 26 kDa samples. It should be noted that the data are normalized relative to their original M_n.

The overall rate of degradation is also substantially slower for the alternating sequenced polymer relative to the random analogues. After 28 days, for example, **poly LG(26k)** has decreased in molecular weight to only 14.1 kDa (47% decrease), compared with the 71% decrease shown by both of the random copolymers over the same period. Finally, SEC traces for the sequenced copolymers show a narrower polydispersity and a smaller concentration of oligomers whose molecular weights are discontinuous from the bulk (Figure 2, inset).

The uniformity of the degradation of the sequenced polymers relative to the random copolymers is further demonstrated in the dramatically different thermal behavior observed for the polymers. In Figure 3, differential scanning calorimetry (DSC) studies for the **R-ROP** and **poly LG(26k)** copolymers are presented as a function of degradation time. Although the initial DSC traces of both polymers are similar in that they consist of a single phase transition, T_g, of ~50 °C, the traces obtained after

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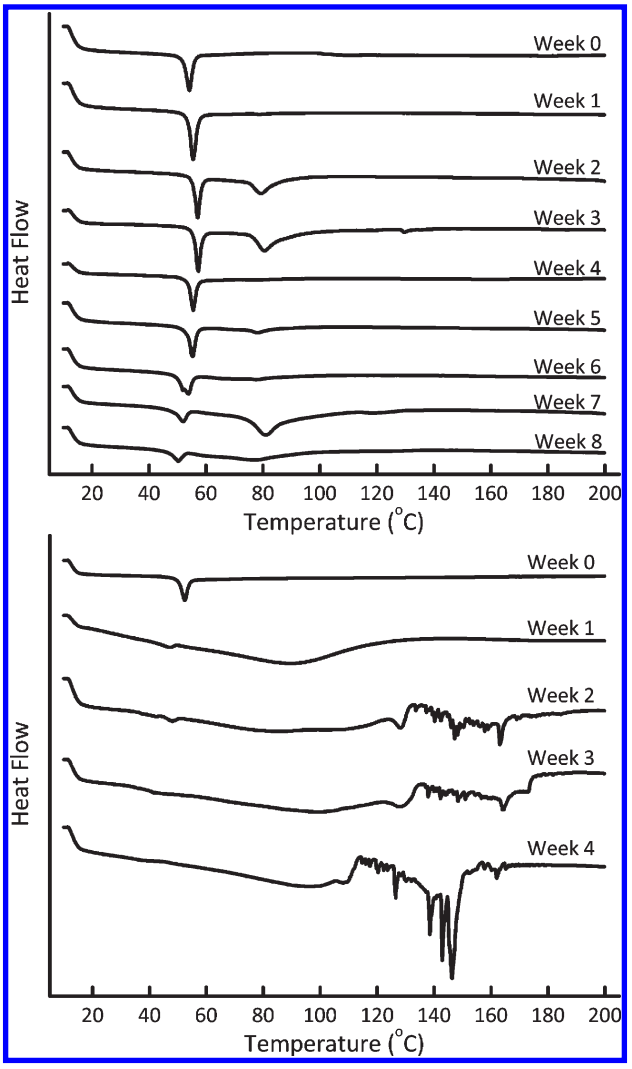

Figure 3. Differential scanning calorimetry thermograms of **poly LG26k** (top) and **R-ROP** (bottom) microspheres during the course of hydrolysis. The first cycle heating flow is shown. The **R-ROP** data are shown only through week 4, as sample size is too small for reliable analysis by week 5.

hydrolysis are significantly different. In weeks 1−4, the random copolymer **R-ROP** exhibits multiple melting transitions ranging from 80 to 160 °C that have been shown in prior studies by multiple groups to be characteristic of crystallized oligomers—those above 120 °C have been specifically assigned as oligomers with a high lactic acid content.[31] Samples isolated after week 4 for **R-ROP** did not give reliable DSC traces due to the fact that little solid material remained in the samples. Thermal data acquired on the small amounts that were collected either showed no transitions or had traces dominated by contaminants (these traces are included in the Supporting Information).

The thermal behavior of the sequenced **poly LG(26k)** reflects the regularity of the polymer. Although the glass transition ($T_g \approx$ 50 °C) shifts and broadens due to the decrease in molecular weight and the presence of oligomers, the persistence of the transition testifies that the material has not undergone a significant transformation. A weak melting transition at 80 °C is also observed in most samples. The constancy of this transition is consistent with the presence of crystalline sequenced oligomers

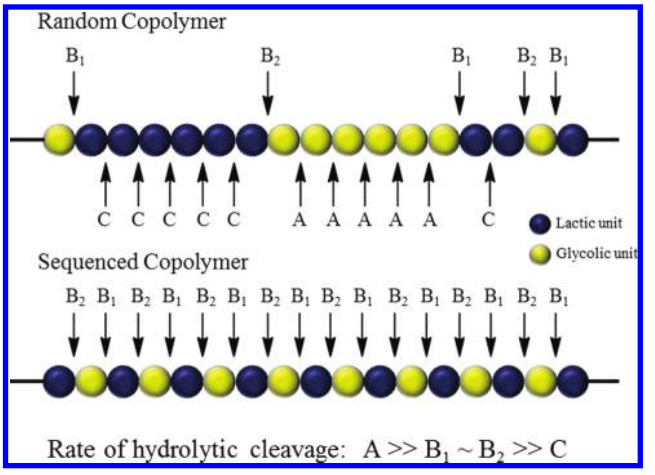

Figure 4. Illustration of the distinct hydrolytic degradation sites in random and sequenced PLGA copolymers.

whose composition does not change over the period of hydrolysis. Peaks in this region are also visible in the **R-ROP** and **R-SAP** samples, but they are broader and shift to higher temperature (or disappear) with time. Transitions above 120 °C, associated with high lactic content oligomers, are noticeably absent from the **poly LG** traces. Interestingly, the DSC traces for the **poly LG(16k)** do not exhibit the 80 °C melting transition despite the homology with the 26k sample. We hypothesize that the lower molecular weight material does not trap and/or promote the crystallization of oligomers as effectively. DSC traces for **R-SAP** and **poly LG(16k)** samples can be found in the Supporting Information.

The key observation that our sequenced PLGA, **poly LG**, exhibits a more gradual and controlled degradation relative to the random analogues can be explained by the uniformity of the cleavage sites. The RSC presents only two types of hydrolysis sites (Figure 4). Nucleophilic attack by water at the glycolic acid carbonyl breaks the C−O bond to the adjacent lactic acid (B_1), while attack at the lactic acid carbonyl should cleave the adjacent glycolic acid (B_2). In contrast, the random copolymers have a wide variety of sites that would be expected to exhibit a more diverse range of reactivity rates with water. At the simplest level, one would suspect that G-G connections will cleave more quickly than G-L/L-G, which should cleave more quickly than the hydrophobic L-L connections. Data on the hydrolysis of random PLGAs are consistent with this hierarchy of rates. Others have reported that the ratio of L to G is known to increase in random copolymers over the course of the hydrolysis,[31] and the observation of crystallized lactic acid oligomers in the DSC traces in this study confirms it.

The homogeneity of polymer chain packing for the sequenced copolymers would also be expected to favor a more controlled degradation profile. There is no opportunity for the formation of glycolic or lactic acid microdomains, as should be possible for a random copolymer bearing even modest blocks of G or L units. The presence of the microdomains would be expected to accelerate or retard hydrolysis of the units within, relative to a free chain, depending on the water affinity of the domain.

Based on this first-level comparison, it is clear that sequence could have significant impact on applications. The lack of quick-cleavage A-type sites could translate into smaller/later burst releases in drug delivery systems, and the overall rate of release

might be more constant. Since the mass loss for the sequenced sample is slower and more gradual, it may be possible to achieve a particular hydrolysis profile with lower molecular weight material. Finally, the ultimate clearance of the polymer from the site may be more predictable if the formation of high lactic content crystalline oligomers is avoided.

In conclusion, the hydrolysis rate for a PLGA with a simple alternating sequence was found to exhibit a dramatically different hydrolysis behavior compared with random analogues. The hydrolysis profile was nearly linear after a small initial weight loss. Future work will focus on characterization of the hydrolysis profiles of other sequences, both *in vitro* and *in vivo*, and on the role of sequence in controlling properties relevant to drug delivery and stem cell scaffolding.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details; plots of molecular weight (not normalized) vs time; DSC thermograms for **R-ROP** (including weeks 5−7), **R-SAP**, and **poly LG(16k)**; and NMR data for polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

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