### Property Impact of Common Linker Segments in Sequence-Controlled Polyesters

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Property Impact of Common Linker Segments in Sequence-Controlled Polyesters

Jordan H. Swisher*, Jamie A. Nowalk, and Tara Y. Meyer

Heterogeneous “linkers” are incorporated into polymers for a number of reasons, most commonly to facilitate the coupling of the targeted backbone segments. Due to their inclusion in the backbone, these linkers have the potential to affect the overall properties of the copolymer, even when present in relatively low weight percentages. To characterize the degree of impact of some common linkers, a set of polymers that incorporate both degradable sequenced segments and linkers were synthesized and systematically examined. Seven sequence-controlled olefin containing ester macrocycles were prepared, each with a unique central moiety, including a five-carbon alkyl chain, diethylene glycol, a urea, a thioether, a triazole, a bioaromatic, and an extension of the ester sequence. The macrocycles were polymerized via ED-ROMP to yield seven polymers that vary only in the the linker segment. The properties of all polymers were compared to determine the relative dominance of the different linker types. The properties tested in the study included thermal behavior, mechanical characteristics, hydrolytic degradation and film qualities. The thermal and mechanical properties proved to be dependent primarily on the ability of the linker to promote interchain interactions, as well as the weight fraction of the linker, whereas the hydrolytic degradation was dominated by the relative hydrophobicity of the linker groups. In all cases, the linker identity was a significant contributor to the behavior.

Introduction

The preparation and investigation of sequence-controlled polymers (SCPs) is a rapidly growing area of macromolecular research, and the importance of SCPs is ever rising as their unique and desirable properties are discovered.(1) Sequence-control in polymers has been used for information storage,(2-9) precise folding and self-assembly,(10-17) molecular recognition, enhanced photophysical properties,(18-24) and more.(25) In recent studies, for example, we have shown that sequence matters in the hydrolytic degradation of biodegradable polyesters, such as poly(lactic-co-glycolic acid) (PLGA).(26-31) By controlling monomer order, the degradation behaviors were enhanced when compared to random analogues, allowing tunability of degradation rates based upon sequence in addition to composition and molecular weight. The challenge that remains in the field of SCPs is synthetic accessibility and scalability.

When preparing SCPs, it is often necessary to include additional components that are not structurally homogenous with the monomers. Groups such as click-chemistry linkers,(32-35) step-growth polymerization functional handles,(36) polymerization triggers,(37) and other moieties,(38, 39) are often incorporated into the backbone of SCPs. Each of these moieties serve an important role in the preparation of these materials, but when moving toward applications or desired properties, it is important to determine the effect of these backbone segments on the overall behavior of the polymer.

Our interest in this study arose from our prior observation of a linker effect in sequenced polyesters that we assembled using entropy-driven ring-opening polymerization (ED-ROMP).(28) Macrocycle monomers, containing both an ethylene glycol and an α,ω-dialkene linker, were polymerized using ED-ROMP to create polymers incorporating the targeted polyester sequence with molecular weight control. Despite the successes of using ED-ROMP as a general and scalable method...
to prepare SCPs, the incorporation of two conformationally-flexible linkers resulted in a glass transition temperature \( (T_g) \) well below that required for bioengineering applications. This study inspired us to investigate how other common linker segments that could potentially be used in our materials would impact the properties of these polymers and to determine, in particular, whether these linkers would affect the targeted sequence-related degradation (Figure 1).

To quantify the impact of linker structure on mechanical, thermal, and degradation properties, a set of polymers with identical sequence and monomer composition and a variable central linker were prepared via ED-ROMP. In this case, we replaced the ethylene glycol linker from our previous study with a series of alternatives. We chose to study this polyester system because of the high sequence-fidelity associated with ED-ROMP of ester macrocycles, along with our understanding of the synthesis and behavior of related polymers. The linkers in this study include a C5 carbon chain, an ethylene glycol, a urea, an aromatic ester, thioether, triazole, as well as an extension of the ester sequence (additional lactic and glycolic acid). In so doing, we have not only demonstrated the power of the ED-ROMP strategy to create sequenced copolymers that incorporate a range of additional functionality but also investigated the impacts these functional groups have on the properties of the prepared polymers. Herein, we describe the results of this study and discuss their relevance to this approach to preparing SCPs.

**Experimental**

**Materials**

All chemicals were purchased and used without purification unless specified otherwise. See supplementary information for further details.

**Synthesis**

See supplementary information for detailed synthesis and characterization information.

**Methods**

**NMR \( (^1H \text{ and } ^13C) \)** NMR spectra were acquired in CDCl\(_3\), MeOD or d-DMSO (Cambridge Isotope Laboratories, Inc.) using Bruker spectrometers (400, 500 MHz) and calibrated to residual solvent peaks (δ7.26 and δ77.13 for CDCl\(_3\) spectrometers (400, 500 MHz) and calibrated to residual or d-DMSO (Cambridge Isotope Laboratories, Inc.) using Bruker and data.

**Free Radical Polymerization (FRP)** was used with a set of polystyrene standards (2.5, 9, 30, 50, 100 ppm Ru prepared by volume), prepared using a ruthenium standard for ICP (Inorganic Ventures, 1003 ± 5 µg/mL Ru in 10% HCl) diluted in a 5% nitric acid matrix. All standards and unknown samples were measured 6 times and averaged. A 7 min flush time with 5% nitric acid matrix was used between all runs, and a blank was analyzed before each unknown sample to confirm removal of all residual metals from the instrument.

**Differential scanning calorimetry (DSC)** was performed with a TA Instruments Q200 DSC. Standard data were collected with a heating and cooling rate of 10 °C/min under nitrogen flow (20 mL/min) with two heating and cooling cycles from -10 °C – 200 °C. Degraded polymer sample data was collected with only one heating and cooling cycle.

**Thermogravimetric analysis (TGA)** was performed with a TA Instruments Q500 TGA. Standard data were collected by heating from RT to 500 °C with a heating rate of 10°C/min.

**Pd sputter coating** was performed using a Cressington sputter coater. The coater was flushed with argon and evacuated three times and then samples were coated for 60 seconds.

**Scanning electron microscopy (SEM)** was performed on Pd-coated samples using a JEOL JSM 6610 Low-Vac SEM. Images were taken using secondary electron imaging (SEI) with an acceleration voltage of 5 kV under high vacuum.

**Optical profilometry** was performed using a Bruker Contour Elite I optical profilometer. Samples were sliced to create a step indicative of film thickness, and sputter coated with Pd to give uniform reflectiveness, and then analyzed. (See supporting information for optical profiles, Figure S1-7)

**Nanoindentation** was performed using a Hysitron Ti 950 Triboindenter with a Berkovich diamond indenter tip. See supporting information and Figure S8 for methods and for data analysis.

**Film Degradation Study**

Films were prepared by casting 18 µL of a 100 mg/mL chloroform solution of each polymer onto pre-weighed 7 mm aluminum foil discs. Resulting films weighed 1.5 - 2.1 mg on average and ranged from 15-35 µm in thickness. Films were then air dried for 24 h in a fume hood and then dried in vacuo for 72 h. The films were placed into individual pre-weighed vials along with 3 mL of PBS buffer. Vials were then shaken lightly at 37°C until removal. Upon removal, films were rinsed three times with DI water, flash frozen with liquid nitrogen and lyophilized for at least 2 h. Film appearance, mass and molecular weight were monitored with time, and samples were also taken for DSC and SEM as the Ms, of the polymer approached 50% of its original value. At least three films were used for each reported data point before day 70, and associated error bars represent the standard deviation in both the positive and negative direction. On and after day 70, at least 2 films were used for
reach reported data point, and associated error bars represent the range in both the positive and negative direction.

Results and Discussion

Polymer Synthesis

Olefins containing macrocycles were prepared via orthogonal protection, deprotection and ester coupling strategies (Scheme 1). The polymerizations of poly(Alk), poly(EG) and poly(EEG) were performed via the previously reported methods (1 mol% Grubbs 2nd generation catalyst, 0.7 M DCM, R.T. for 4 h).(28) Poly(Urea) and poly(Sy) were prepared in THF at 60 °C due to low conversion at R.T. in DCM. Poly(Thio) and poly(TZ) also required elevated temperatures, in addition to a higher catalyst loading (2.5 mol% and 5 mol%, respectively). All polymerizations resulted in molecular weights below the theoretical molecular weights based on catalyst loadings, and poly(TZ) gave molecular weights below our targeted range (>20 kDa) even at higher catalyst loadings, where the highest molecular weight of poly(TZ) obtained was 10.2 kDa.

The inability of the triazole-containing monomer to form high molecular weight polymer is likely due to complexation of the triazole to the Ru catalyst. As previously reported by Kuhn et al., triazole functional groups have been used as ligands in Ru compounds,(40) and triazole-containing monomers have been reported to give low molecular weights under ROMP conditions.(41) The Lewis basic thioether linker may similarly bind to the Ru metal center, inhibiting the polymerization. The fact that Ru metal is retained after reprecipitation at a higher level in these two polymers relative to the other five polymers supports this hypothesis. As measured using inductively-coupled plasma – optical emission spectroscopy (ICP-OES), the poly(TZ) and poly(Thio) samples exhibited an increase in Ru concentration of 40% and 10%, respectively, after precipitation (Figure S9). For all other linkers, ruthenium concentration remained unchanged or decreased in the precipitated polymer samples. While we have not studied the mechanism of coordination-based disruption in detail, it plausibly involves a combination of intramolecular and intermolecular chelation of functional groups from polymer chains and unreacted monomer to the active site of the catalyst.

Thermal Properties

Differential scanning calorimetry (DSC) did not show sharp crystalline transitions for the seven polymers, although poly(Alk) and poly(EG) display broad thermal transitions between 20 – 60 °C that may correlate to the melting of disperse crystalline domains (Figure 2). Neither annealing nor slower cooling rates sharpened these transitions. Similar broad features are seen in the partly degraded samples of poly(Alk), poly(EG), poly(EEG), poly(Sy) and poly(Thio).

Figure 2. Differential scanning calorimetry thermograms (endo up) of virgin and degraded polymers.

Scheme 1. Example synthetic scheme for the variable linker containing polymers. i (Alk, EG, EEG) – 1 mol% Grubbs 2, R.T., 0.7 M DCM, 4 h, 75%. ii (Urea, Sy) – 1 mol% Grubbs 2, 60 °C, 0.7 M THF, 4 h, 60-80%. iii (Thio) – 2.5 mol% Grubbs 2, 60 °C, 0.7 M THF, 4 h, 76%. iv (TZ) – 5 mol% Grubbs 2, 60 °C, 0.7 M THF, 4 h, 68%.
The first \( T_g \) of the polymers were predicted using the Fox equation wherein the \( T_g \) is estimated by assuming that each component contributes based on its weight fraction and the \( T_g \) of the homopolymer with which it is most structurally homologous.\(^{(42)}\) In this case, we used the \( T_g \) of the homopolymer with which it is most structurally homologous. \(^{(42)}\) For the ethylene glycol and thioether linker,\(^{(43-45)}\) the weight fractions and \( T_g \) values were then used to predict the \( T_g \) values in Figure S10. Not surprisingly, the \( T_g \) was most impacted by moieties that promote interchain interactions, such as the urea and syringic acid linkers. Also, as expected, the weight fraction of the linker segment played a key role.

As for thermal stability, thermogravimetric analysis (TGA) data showed that five of the seven polymers had similar mass loss profiles, with decomposition temperatures \( T_d \) values in the range of 260-280 °C (Figure S11). The nitrogen-containing polymers, poly(Urea) and poly(TZ) had slightly lower \( T_d \) values of 202°C and 240°C, respectively.

**Mechanical Properties**

Mechanical data measured by nanoindentation of soft polymer films has its limitations,\(^{(46)}\) and the correlation of nanoscale mechanical properties to bulk scale mechanical properties is imperfect. For the purposes of this study however, direct comparison of films via nanoindentation sheds light on the relative mechanical properties of the polymers on a small scale, including hardness (H), stiffness (S), Youngs modulus (E), reduced elastic modulus (E’), and maximum applied load \( P_{\text{MAX}} \).

Mechanical data showed that the linker had a substantial impact on the mechanical properties of the polymer set; poly(Sy) has an E of nearly 500 MPa, whereas the other five polymers, excluding poly(TZ), have E’s ranging from 7.2 – 19.4 MPa. Hardness values show a large disparity with poly(Sy) having a H value of 80 MPa, compared to 0.5 – 1.9 MPa for the other five polymers, again excluding poly(TZ).

As shown by the median load displacement curves in Figure 3, it appears we have prepared three different classes of material. Poly(Alk), poly(EG), poly(EEG), poly(Thio) and poly(Urea) all seem to be viscous elastomers, poly(TZ) showed significant sample-probe adhesion but was a more tough material, and poly(Sy) is significantly harder than any of the other polymers.

Sample-probe adhesion is a known source of error in the nanoindentation of soft materials.\(^{(46)}\) The load-displacement curves in Figure 3 show that poly(TZ) was the only polymer to adhere significantly to the probe, where the adhesion force is proportional to the area of the curve with a negative load value. This adhesion force between the indenter tip and the polymer.

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**Table 1. Polymer GPC characterization, thermal properties and mechanical properties.**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>( M_w )(^{a})</th>
<th>( D )(^{a})</th>
<th>Predicted ( T_g )(^{b})</th>
<th>( T_f )</th>
<th>( T_d )</th>
<th>( E ) (S.D.)(^{b})</th>
<th>( E ) (S.D.)(^{b})</th>
<th>( S ) (S.D.)(^{a})</th>
<th>( H ) (S.D.)(^{a})</th>
<th>( P_{\text{MAX}} ) (S.D.)(^{a})</th>
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<tr>
<td>Alk</td>
<td>32.0</td>
<td>1.49</td>
<td>28</td>
<td>19</td>
<td>275</td>
<td>7.2 (0.6)</td>
<td>7.9 (0.6)</td>
<td>0.15 (0.01)</td>
<td>0.47 (0.05)</td>
<td>130 (13)</td>
</tr>
<tr>
<td>EG</td>
<td>38.5</td>
<td>1.45</td>
<td>28</td>
<td>21</td>
<td>266</td>
<td>10.0 (0.5)</td>
<td>10.9 (0.6)</td>
<td>0.18 (0.01)</td>
<td>1.5 (0.1)</td>
<td>283 (18)</td>
</tr>
<tr>
<td>EEE</td>
<td>30.4</td>
<td>1.46</td>
<td>33</td>
<td>31</td>
<td>267</td>
<td>19.1 (0.3)</td>
<td>21.0 (0.4)</td>
<td>0.39 (0.01)</td>
<td>1.7 (0.02)</td>
<td>416 (6)</td>
</tr>
<tr>
<td>Urea</td>
<td>23.1</td>
<td>1.34</td>
<td>41</td>
<td>44</td>
<td>202</td>
<td>19.4 (3.4)</td>
<td>21.3 (3.8)</td>
<td>0.39 (0.07)</td>
<td>1.9 (0.2)</td>
<td>447 (62)</td>
</tr>
<tr>
<td>Sy</td>
<td>32.8</td>
<td>1.40</td>
<td>74</td>
<td>68</td>
<td>276</td>
<td>486 (32)</td>
<td>534 (35)</td>
<td>8.1 (0.6)</td>
<td>80 (2)</td>
<td>13,550 (6)</td>
</tr>
<tr>
<td>Thio</td>
<td>24.6</td>
<td>1.82</td>
<td>27</td>
<td>18</td>
<td>263</td>
<td>16.3 (1.1)</td>
<td>17.9 (1.2)</td>
<td>0.33 (0.02)</td>
<td>1.4 (0.1)</td>
<td>352 (27)</td>
</tr>
<tr>
<td>TZ</td>
<td>10.2(^{1})</td>
<td>1.54(^{1})</td>
<td>44</td>
<td>38</td>
<td>240</td>
<td>…(^{g})</td>
<td>…(^{g})</td>
<td>…(^{g})</td>
<td>…(^{g})</td>
<td>1,040 (200)</td>
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\(^{a}\) Determined by size exclusion relative to polystyrene standards. \(^{b}\) Determined by the Fox equation, see S.I. \(^{c}\) Determined by differential scanning calorimetry using second heating cycle at 10 °C/min. \(^{d}\) Determined by thermogravimetric analysis with heating rate of 10 °C/min. \(^{e}\) Determined by nanoindentation. \(^{f}\) Sample not fully soluble. \(^{g}\) Not reported due to sample-probe adhesion.
sample distorts the calculated H, S, E, and E_r, and for this reason, these values are not reported for poly(TZ). P_max should be independent of the sample-probe interactions, and we see that the poly(TZ) required over 1,000 µN on average to reach 4,000 nm in depth, more than all but poly(Sy). This P_max value is proportional to hardness in all other cases outside of poly(TZ), so we assume that if we were able to optimize indentation to limit adhesion, poly(TZ) would be the second hardest of the set, yet still well below poly(Sy).

The indentation sites on five of the seven polymers were imaged using scanning probe microscopy (SPM) (Figure S12). Poly(Alk) and poly(EG) indentation sites were not located by SPM. As these were the softest of the polymers, it is likely that the indentation sites recovered rapidly. The effect of defects on the measurements could also be directly observed. Scanning electron microscopy (SEM) images of poly(Sy) taken after nanoindentation testing showed the grid of 20 indentations (Figure 4). The image shows 19 pyramidal indentations with similar shape and area, and one indentation that was performed on a defect on the surface, which correlates with the only outlier from the poly(Sy) indentation dataset, indicating that minor surface defects can lead to drastic outliers, and that the Thompson-Tau method was effective at removing misrepresentative data points.

Film Degradation Study
The hydrolytic degradation of the polymers displayed a large dependence on the linker group. As the degradation of these materials depends on the cleavage of the sequenced ester unit, the magnitude of the influence of the linker groups is somewhat surprising. We hypothesize that the increased hydrophobicity of the syringic acid and alkyl chain containing polymers led to an increased lifespan when subjected to in vitro conditions, with poly(Sy) being the least susceptible to hydrolytic degradation (Figure 5). Consistent with this hypothesis, the replacement of one CH_2 for an oxygen per repeat unit in the poly(EG) led to a three-week difference in the time required to reach one half of the polymer’s original M_w. Incorporation of a urea functional...
group had surprising effects when it came to hydrolytic degradation, as it reached one half of its original \( M_n \) the second fastest, behind only the triazole containing polymer. The low solubility of both the virgin Poly(TZ) sample and the degraded film samples in THF suggests the molecular weight data obtained via SEC may not be truly representative, yet the early mass loss of the triazole-containing polymer suggests that it is likely the most susceptible to hydrolytic degradation (Figure S13, \( M_W \) plot Figure S14, additional GPC data Figure S15). This increased degradation rate is likely due to multiple factors, including a lower virgin \( M_n \), increased hydrophilicity and the presence of a weak organic base, which may catalyze the ester cleavage.

Visually, the polymer films changed from transparent on day 0 to cloudy and opaque as the polymers degrade (Figure 6a). This transition occurs concurrently with loss in molecular weight, as poly(Urea) and poly(TZ) became opaque far quicker than either poly(Alk) or poly(Sy). SEM images show that the surface morphology of the virgin films and degraded films are drastically different (Figure 6b). All seven polymers had similar surface morphology at day 0, yet upon hydrolytic degradation, each seems to have its own unique morphology. Though intriguing, this study did not investigate the causes of these morphological differences, nor attempt to classify the morphologies.

As discussed earlier, DSC thermograms of the degraded polymer films showed a range of different degradation properties (Figure 2). Poly(Alk), poly(EG), poly(EEG), poly(Sy) and poly(Thio) showed similar thermal transitions to their virgin polymer counterparts, with shifts of few degrees and rougher baselines. Poly(Urea) and poly(TZ), both of which have very low molecular weights at the time of observation, show clear melting points and no \( T_g \).

**Conclusions**

The structure-property relationship of several common linkers and functional groups were tested. We have limited the variables in this study by controlling sequence and molecular weight (excluding poly(TZ)), to provide a direct comparison of how the nature of certain moieties affect polymer properties. Not surprisingly, we discovered in the preparation of these materials that triazole groups and other Lewis bases interfere with ruthenium catalysed metathesis reactions. No optimization was done to prevent such hinderances, but one could presumably provide a more favourable coordination partner, such as copper in the case of a triazole moiety, to limit the interaction of the Lewis base in the monomer/polymer backbone with the ruthenium catalyst.(47)

In our structure-property investigation, we found significant property differences depending on the linker incorporated. Thermal properties were consistent with the linker’s ability to promote interchain interactions, i.e., hydrogen bonding with urea units, and pi-stacking with bioaromatics. Mechanical properties were dictated by the linker’s rigidity. The rigid bioaromatics in poly(Sy) gave mechanical properties orders of magnitude stronger than the rest, followed by poly(TZ), containing a rigid triazole unit. Hydrolytic degradation seemed to be dominated by the relative hydrophobicity of the polymers, as poly(Sy) significantly outlasted the rest during *in vitro* hydrolysis. The most staggering example of this phenomenon,
though, was that the exchange of one CH$_2$ in poly(Alk) for an oxygen to give poly(EG), nearly halved the lifespan of the polymer.

The data presented suggests that incorporating other functionalities can have large impacts on properties. While it is not surprising that changing the backbone composition of a polymer changes its characteristics, we were surprised at the magnitude of the effect in these systems, particularly the variance in degradation rates as we believed that they would depend primarily on the preserved ester sequence embedded in each monomer. As we discussed in the introduction, linker character is intrinsically important to the SCP field because one primary, scalable route to periodic copolymers is to prepare oligomeric macromonomers and then polymerize them. Moreover, it is common to exploit well-known, high yielding reactions, e.g. click reactions, to assemble the sequenced backbone. Thus, though our study is focused on one-particular class of polymer, we suggest that the results can be extrapolated more generally to any polymerization that creates a periodic SCP separated by structurally dissimilar linkers.

It is important to note that the property changes that are introduced by linkers can also be exploited deliberately. If one understands the impact a linker might have on the properties of a polymer, that understanding can be used to design an optimized material for a targeted application. For example, if one were to desire a degradable material that is tougher than standard PLGAs, but degrades faster, one could incorporate urea/amide linkages, which we have shown strengthens mechanical properties, while promoting degradation.

**Conflicts of interest**

There are no conflicts to declare.

**Acknowledgements**

Scott Crawford and Dr. Michelle Ward for their help acquiring and analyzing ICP-OES data.

**Notes and references**


Linker segments in sequence-controlled polyester backbones significantly affect thermal, mechanical and degradation properties.