Ring-Opening Polymerization

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Tunable, Temperature-Responsive Polynorbornenes with Side Chains Based on an Elastin Peptide Sequence**

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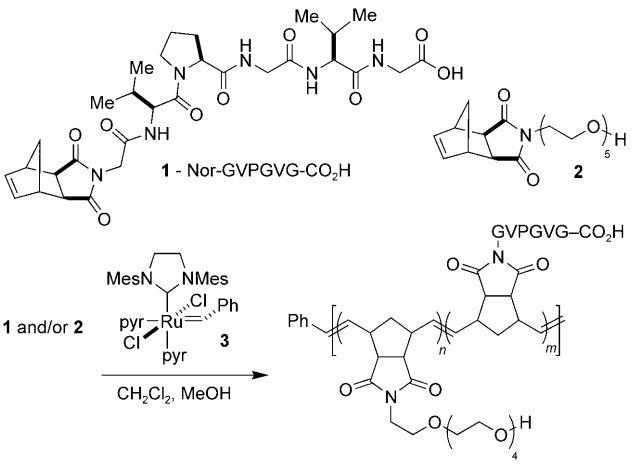
Natural mammalian elastin fibers are cross-linked networks of the protein tropoelastin, which functions as the primary component of human blood vessels. Extensive physical and theoretical studies on this protein have shed light on the mechanism behind its unique elasticity.[1] Tropoelastin is comprised of hydrophobic domains of the repeating amino acid sequence -(VPGVG)$_n$- and domains rich in alanine and lysine residues for intermolecular cross-linking. The hydrophobic domains are conformationally dynamic, and the transition between random coils and tightly wound β sheets results in large changes in the hydration sphere of the protein. This process has been determined to be fundamental to the elasticity of the cross-linked networks.[2] In the absence of chain cross-linking, the change in conformation is manifested by a temperature-dependent phase transition known as a lower critical solution temperature (LCST), below which the protein is soluble and above which it is insoluble. To take advantage of the physical properties of tropoelastin, elastin-like polypeptides (ELPs) have been synthesized by microbial expression systems[3] and have been studied for use as biomaterials.[4] The promise presented by ELPs has inspired us to search for readily accessible synthetic derivatives of these proteins for the development of new materials that promote endothelial cell growth. We hoped to incorporate the elastin amino acid sequence -(VPGVG)- as the side chain on biomimetic polynorbornenes to obtain a synthetic polymer that exhibits the phase-transition behavior of its polypeptide model.

The research groups of van Hest and Cameron have demonstrated that polymers with the -(VPGVG)- elastin sequence as a side chain exhibit LCSTs that are dependent on the concentration, degree of polymerization, and the pH value.[5,6] The polymers were synthesized by using controlled radical polymerization methods to form either ABA block copolymers with low degrees of polymerization (DP < 12)[5] or homopolymers with higher DP values.[6] Recently, Setton and co-workers have shown that dimeric repeat units of -(VPGVG)- attached to norbornene monomers could be polymerized by using ring-opening metathesis polymerization (ROMP) with [(H$_2$IMes)(PCy$_3$)(Cl)$_2$Ru=CHPh] (Mes = 2,4,6-trimethylphenyl, Cy = cyclohexyl) as an initiator. The oligomers (DP < 12) produced exhibited temperature-dependent phase transitions.[7]

The LCSTs in most synthetic elastin-based materials are strongly dependent on the overall molecular weight of the polymer. Limitations in the synthetic methods used for their assembly have meant that high-molecular-weight elastin-based polymers have not been investigated. Our goal was to develop a robust method to synthesize elastin-based polymers in which the LCST was not molecular-weight dependent and could be tuned for any targeted application. We anticipated that by making random copolymers of an elastin-based monomer and a hydrophilic polyethylene glycol (PEG) based monomer by ROMP, the LCST could be manipulated through the ratio of the co-monomers in the feed. Ruthenium-catalyzed ROMP is an ideal method for the assembly of materials incorporating peptidic side chains because of its high level of tolerance towards polar functional groups,[8] and use of [(H$_2$IMes)(pyr)$_2$(Cl)$_2$Ru=CHPh] (**3**; pyr = pyridine) has been shown to produce low polydispersity (PDI) materials through fast initiation.[9]

Our studies commenced with the polymerization of norbornene monomer **1**, which was synthesized by using standard Fmoc-based solid-phase synthesis procedures (see the Supporting Information for details). As shown in Scheme 1, treatment of monomer **1** with initiator **3** in a CH$_2$Cl$_2$/MeOH solvent mixture resulted in rapid polymerization. Methanol was required as a cosolvent to maintain a homogenous solution throughout the polymerization reaction. Other solvent systems or additives, such as CF$_3$CH$_2$OH,



Scheme 1. ROMP of elastin-like monomers with PEG$_5$ comonomers.

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AcOH, or LiCl,[10] that have been reported for ROMP with peptides resulted in either precipitation of the polymeric products or a decreased reaction rate.

The homopolymer of **1** did not exhibit an LCST and was insoluble in aqueous phosphate buffer at pH 2 and 0 °C.[11] The inclusion of a PEG_5 comonomer to reduce aggregation[12] of the VPGVG side chains and increase the hydrophilicity of the polymers resulted in the random copolymerization of **1** and **2** leading to complete consumption of the monomers and the generation of polymers with narrow PDIs (Table 1). Gel-

Table 1: GPC and LCST data for random copolymers from monomers **1** and **2** with the -(VPGVG)- elastin-based sequence and PEG_5.

Polymer	**1**:**2**	$[M]_0/[\mathbf{3}]_0$[a]	M_n(theo, kDa)	M_n(GPC, kDa)	PDI	LCST [°C][b]	T_{onset} [°C][c]
4	1.0	20	10	15	1.04	27	17
5	1.0	40	20	29	1.03	26	17
6	1.0	50	25	37	1.05	26	16
7	1.0	60	30	40	1.04	24	16
8	1.0	80	41	47	1.03	22	16
9	1.0	100	51	64	1.05	21	14
10	1.5	50	27	35	1.03	17	10
11	0.7	50	24	32	1.04	37	29
12	0.5	50	23	37	1.02	44	39

[a] $[M]_0$: total monomer concentration $=[\mathbf{1}+\mathbf{2}]_0$. [b] The LCST is taken as the midpoint on the turbidity profile at 0.5 mg mL^{-1}. [c] Temperature at which the polymer begins to precipitate upon heating.

permeation chromatography (GPC) of the polymers with a multiangle laser light scattering (MALLS) detector showed narrow and monomodal molecular-weight distributions. Furthermore, it was observed that the molecular weight of the polymer increased linearly with an increasing ratio of monomer to initiator, thus indicating a living polymerization process (polymers **4**–**9**). The molar ratio of **1**:**2** could also be varied to produce polymers with differing amounts of the elastin peptide sequence (polymers **9**–**12**).

To determine whether the ROMP polymers produced in Table 1 were random copolymers or possessed gradient composition, the disappearance of each of the monomers during the copolymerization was monitored by ^{1}H NMR spectroscopy. First order kinetics were observed, with the slope of the line [k_{obs} (min^{-1})] shown Figure 1 corresponding to the polymerization rate of each of the monomers. The similar rate of incorporation of **1** and **2** throughout the course of the polymerization strongly indicates a random copolymerization.

Each of the copolymers shown in Table 1 exhibited the temperature-responsive phase behavior characteristic of ELPs. As anticipated, the ratio of the peptidic to nonpeptidic monomers in the feed could be used to control the LCST of the resulting polymer. The temperature transitions were measured by UV/Vis spectroscopy in aqueous phosphate buffer at pH 2. Turbidity measurements on polymers in which the molar ratio of **1**:**2** was varied reveal a large dependence of the LCST on the elastin content (Figure 2a). For polymers of similar molecular weight, the LCSTs increased from 17–44 °C (Table 1, polymers **6**, **10**–**12**) as the elastin content was

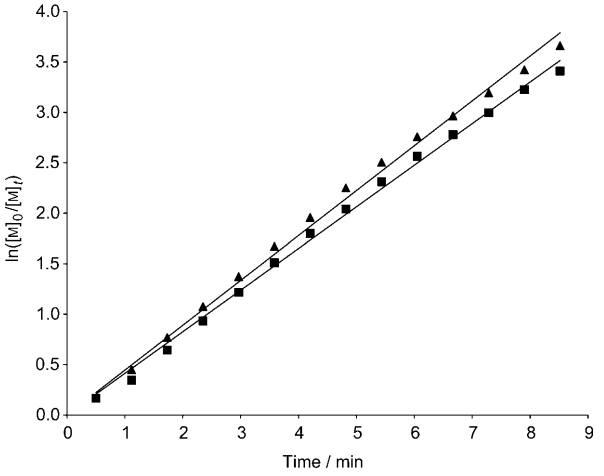

Figure 1. Log plot of the random copolymerization of monomers **1** (▲) and **2** (■) with $[\mathbf{1}]_0=[\mathbf{2}]_0=0.01$ M and $[\mathbf{1}+\mathbf{2}]_0/[\mathbf{3}]_0=40{:}1$ in 4:1 CD_2Cl_2/CD_3OD. A linear least-squares fitting gave the following slopes and R^2 values, respectively: monomer **1**: 0.45, 0.996; monomer **2**: 0.42, 0.997.

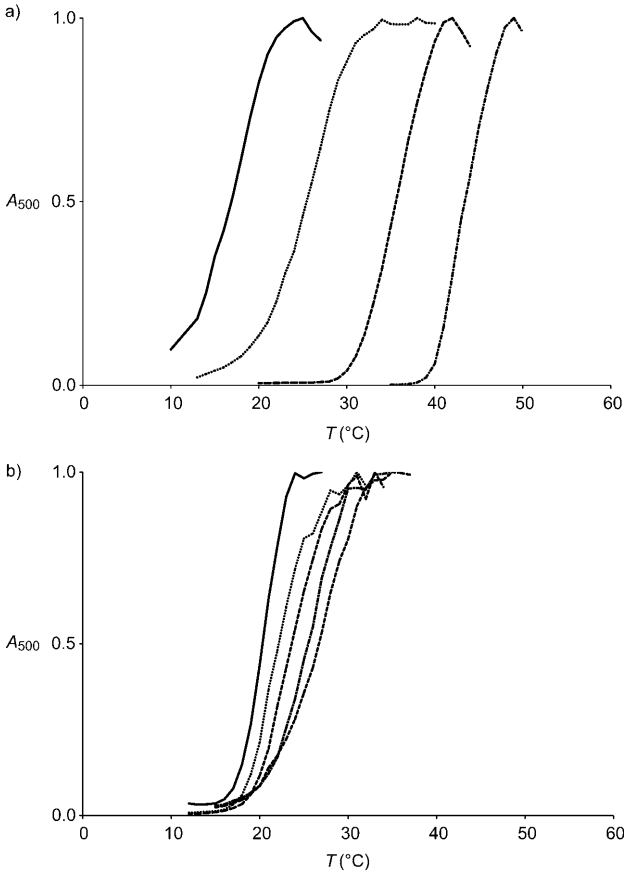

Figure 2. Turbidity measurements for polymers of a) varying elastin content: ——, 60%; - - - -, 50%; •••••, 40%; –•–•–, 33%; and b) varying molecular weight (as determined by GPC): ——, 64 kDa; •••••, 47 kDa; - - - -, 40 kDa; –•–•–, 29 kDa; – – –, 15 kDa. Measurements were taken at pH 2, 0.5 mg mL^{-1}, and heating at a rate of 0.5 °C min^{-1}. Absorbance values at 500 nm have been normalized to 1.

decreased from 60 to 33%. It should be noted that the homopolymer of **2** exhibited an LCST at 95 °C. The temperature-responsive behavior of polymers containing ethylene

glycol units is well-documented and has been shown to be dependent on the number of ethylene glycol units in the side chain and the nature of the backbone of the polymeric material.[13]

A relatively small dependence of the LCST (27–21 °C) on the molecular weight (15–64 kDa) is observed for polymers with the same elastin content (Figure 2b). This minor correlation of LCST with molecular weight is in contrast to results observed for ELPs[14] as well as block[5] and homopolymers[6,7] with elastin-based side chains. In these reports, the LCST was strongly correlated to the degree of polymerization. The relatively small molecular-weight dependence in our elastin-based polynorbornenes can be rationalized by the random composition of the co-monomers in the polymer (Figure 1). Given a specific ratio of **1**:**2**, the repeat frequency of the -(VPGVG)- monomer within the polymer chain should be constant, regardless of the overall degree of polymerization. The independence of the LCST on the molecular weight may be a desired attribute for the use of elastin-based synthetic polymers as biomedical materials because it leads to greater consistency between batches.

A significant concentration effect was observed for all of the random copolymers synthesized. An example of the concentration dependence is depicted in Figure 3, where the

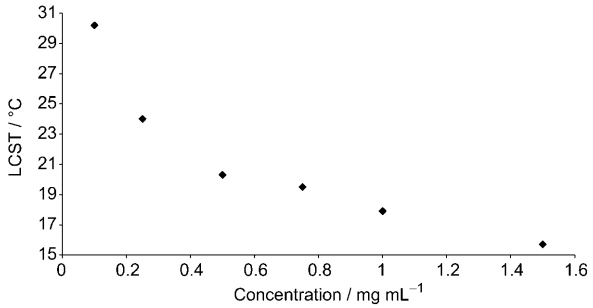

Figure 3. Concentration dependence of the LCST of polymer **9** at pH 2.

LCST of polymer **9** decreases from 30 to 16 °C with increasing polymer concentration in aqueous buffer. A similar dependence on concentration has been observed for ELPs as well as synthetic polymers with elastin side chains, and is thought to originate from the increased intermolecular interactions promoting the transition from random coils to tightly wound β sheets in more concentrated solutions.[6,14,15]

In conclusion, temperature-responsive, elastin-based polynorbornene materials were synthesized by ROMP. A PEG_5 co-monomer was incorporated randomly into the polymer backbone to control the LCST. The polymerization reaction showed living character that was exemplified by a linear dependence of the molecular weight on the ratio of the monomer to initiator. Similar rates of incorporation of each of the monomers were observed in kinetic studies, thus implicating a statistical distribution of the two monomers in the copolymer. The LCSTs were found to be highly dependent on the ratio of the peptidic to PEG_5-containing monomers and on the concentration, but showed only a small dependence on the molecular weight. The polymerization conditions developed in this study are well suited for further studies that will enable the incorporation of monomers containing cell-binding motifs in addition to the elastin-based peptide side chains.

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