Elastin-Based Side-Chain Polymers: Improved Synthesis via RAFT and Stimulus Responsive Behavior

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ABSTRACT: Elastin-based side-chain polymers (EBPs) were prepared by the polymerization of a methacrylate derivative of the pentapeptide valine−proline−glycine−valine−glycine (VPGVG) using reversible addition−fragmentation chain transfer (RAFT) polymerization. The polymerizations proceeded in a controlled manner, yielding polymers with a narrow molecular weight distribution (polydispersity indices 1.03−1.23) and molecular weights in good agreement with those predicted from the initial monomer:initiator ratio for the conversion obtained. The dithioester end groups of the resulting polymers were removed by reaction with azo initiator-derived radicals. The lower critical solution temperature (LCST) behavior of the series of EBPs so obtained was investigated in solutions of varying pH (1.5−5.1) and polymer concentration (0.11−0.97 mg/mL) and for polymers of different degrees of polymerization (29−88 repeating units). These EBPs behaved similarly to linear polypeptides, known as elastin-like peptides (ELPs); the transition temperature decreased with increasing polymer concentration and molecular weight. Unlike ELPs, but in common with previously reported EBPs, a strong dependence of transition temperature on pH was observed due to the presence of the carboxylic acid from the C-terminal residue in the peptide side chains. Significant differences between the EBPs described here and those reported earlier were found, however, regarding the transition temperature at a given pH and its variation with molecular weight. These variations are attributed to differences in architecture between the polymers described here (higher molecular weight homopolymers) and those reported earlier (A−B−A triblock copolymers with short EBP A blocks and a PEG B block).

Introduction

The structural protein elastin[1,2] is found in a variety of mammalian tissues, including the skin, lungs, and ligaments where it serves as an elastomeric material.[3] The un-cross-linked precursor protein of mammalian elastin is tropoelastin, which has a primary structure characterized by repeating pentapeptide sequences, the most common of which is VPGVG (where V = valine, P = proline, and G = glycine).[4] Linear polymers of this pentapeptide sequence (i.e., poly(VPGVG)$_n$), which are known as elastin-like peptides (ELPs), have been prepared and their solution behavior, conformation, and mechanical properties studied extensively.[5,6] These ELPs display reversible lower critical solution temperature (LCST) behavior, which is accompanied by a conformational change from an extended to a compact structure as a result of dehydration of the valine side chains upon increasing temperature.[7] These interesting LCST properties make ELPs potentially useful as, for example, species to aid protein purification.[8] Furthermore, their (presumed) biocompatibility lends them to biomedical applications such as drug delivery[6,9] and as substrates for cell adhesion.[10] However, the synthesis of the linear polypeptides, by either solid-phase peptide synthesis or recombinant methods, is nontrivial. Consequently, recent work has described the preparation of synthetic polymers with pendant VPGVG peptide sequences as readily accessible ELP analogues. These have been termed elastin-based side-chain polymers (EBPs) by van Hest et al.[11,12] A methacrylate derivative of VPGVG (MA-VPGVG) was polymerized by atom transfer radical polymerization (ATRP) using a difunctional PEG macroinitiator to yield ABA triblock copolymers, where the B blocks contained VPGVG side chains.[11] Studies of the solution behavior of these polymers indeed confirmed similar self-assembly and LCST properties to ELPs, including similar dependencies of the transition temperature on solution concentration and molecular weight.[12] One notable difference, however, is that EBPs are responsive to pH as well as temperature due the C-terminal glycine $-CO_2H$ group. Therefore, the transition temperature increases with pH up to a given threshold that depends on the molecular weight and concentration.

ATRP is a robust and versatile technique,[13] useful for the synthesis of a wide range of bioinspired functional polymers.[14] However, one of the disadvantages of ATRP, which may limit

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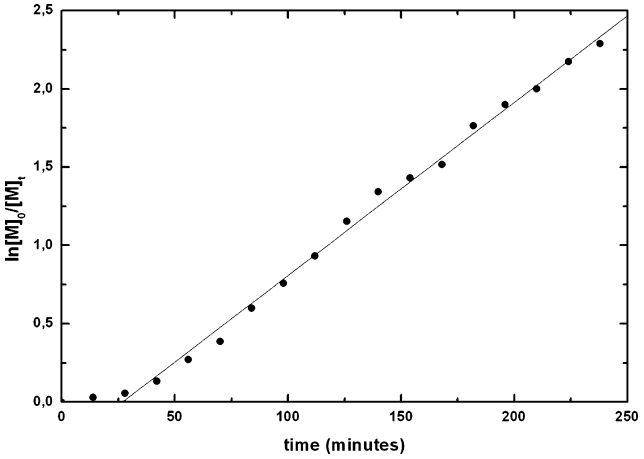

Figure 1. Linear plot of $\ln[M]_0/[M]_t$ vs time for the RAFT polymerization of a methacrylate derivative of VPGVG (MA-VPGVG). Conditions: temperature = 70 °C; [MA-VPGVG] = 0.264 M; **[1]** = 6.6 mM; [V-501] = 3.3 mM; DMSO-d_6. See Scheme 1 for relevant structures.

its widespread industrial utilization, is that the transition metal residues have to be removed from the reaction mixture after polymerization and preferably recycled.[15] Another possible drawback in the present context is the fact that Cu species, the most common transition metal employed in ATRP, have been shown to be toxic for some bioapplications.[16] However, the recent development of ATRP variants employing much lower copper concentrations[17,18] may go some way to overcoming these limitations. Nonetheless, taking into consideration the limitations of the previously described synthesis of EBPs by ATRP (DP_n limited to around 10, relatively poor control and poor quality SEC data)[12] inspired us to investigate the possibility of synthesizing similar EBPs using reversible addition−fragmentation chain transfer polymerization (RAFT). (The possible interactions between amide residues and copper catalyst may also have contributed to the relatively poor control experienced previously.) RAFT[19] is arguably the most versatile controlled/"living" free radical polymerization technique, being applicable to the widest range of monomers under a large number of experimental conditions.[20] One particularly important feature of RAFT is that it allows the synthesis of polymers with terminal or internal thiocarbonylthio functionality that can ultimately be reduced, producing an ω-terminal thiol. This feature of RAFT makes it a useful synthetic tool allowing for potential tailored design and preparation of novel polymeric bioconjugates.[21] In addition, the thermally and photochemically unstable dithioester moiety can be removed fully from the polymer chain end by reaction with an excess of radical initiator, allowing the introduction of a wide range of chain-end functionalities to the polymers.[22]

In this article, we focus on the preparation of homopolymer EBPs by RAFT in order to compare them directly with the published data on linear ELPs.[23] Our intentions are to (i) prepare higher molecular weight EBPs than previously, (ii) investigate the extent of control achieved, and (iii) determine whether the stimulus responsive character of these polymers is affected by the same parameters as linear poly(VPGVG)[23] and those block copolymer EBPs reported previously.[12] For this purpose, a variety of polymers with different degrees of polymerization (DP) have been prepared. The effect of pH, polymer concentration, and DP on the phase transition temperature has been examined.

Experimental Section

General Procedures. NMR spectra were recorded using a Varian Inova 500 spectrometer at 499.87 (^{1}H) and 125.67 MHz (^{13}C) (^{1}H decoupled at 500 MHz) or using a Bruker Avance 400 at 400.13 MHz (^{1}H). NMR spectra were analyzed using the MestreC software. Electrospray LC/MS analysis was performed using a Shimadzu LC/MS 2010A system. Infrared spectroscopy (KBr disc) was conducted on a Nicolet Nexus FT-IR. Aqueous size exclusion chromatography (SEC) was performed using a triple detection method (with angular correction), and measurements were performed on a Viscotek TDA 301 triple detection SEC fitted with two (300 × 7.5 mm) GMPWxl methacrylate-based mixed bed columns with an exclusion limit of 5×10^7 g mol^{-1}, having refractive index (RI), viscometer, and right angle laser light scattering (RALLS) detectors. The eluent used was a buffered aqueous solution containing 0.2 M $NaNO_2$ and 0.01 M NaH_2PO_4 at a flow rate of 1.0 mL min^{-1} and at a constant temperature of 30 °C. Calibration of the RALLS detector response was achieved using a single narrow molecular weight distribution poly(ethylene oxide) (PEO) standard (Polymer Labs) of M_n = 82 500 g mol^{-1} and a d*n*/d*c* value of 0.133 mL g^{-1}. Absolute molecular weights were determined using Omnisec 4.0 software for Windows with a d*n*/d*c* value of 0.121 mL g^{-1} for poly(MA-VPGVG) (determined online using the RI detector response from a number of different, volumetrically measured, concentrations of the pure polymer).

Turbidimetry experiments were carried out using a Varian CaryBio-100 UV−vis spectrophotometer, equipped with multicell, thermoelectric temperature controller. PBS buffered aqueous solutions at different pH and concentration of the polymer were prepared in glass cuvettes, sealed with a Teflon stopper and heated at a rate of 1 °C/min. The measurements were carried out at a fixed wavelength of 480 nm. All the plots represent an average of at least two measurements. In order to calculate the phase transition temperature, all the experimental data were normalized to 1 using Microcal Origin 6.0 software for Windows, and the transition temperature was considered to be that corresponding to an absorption of 0.5.

Reagents. Fmoc-Gly-"Wang" resin (Fmoc = 9-fluorenylmethoxy carbamate; Novabiochem, 0.55−0.77 mmol/g), H-Gly-2-chlorotrityl resin (Novabiochem, 0.59−1.00 mmol/g), Fmoc-valine (Fmoc-Val-OH) (Novabiochem, >99%), Fmoc-glycine (Fmoc-Gly-OH) (Novabiochem, >99%), Fmoc-proline (Fmoc-Pro-OH) (Novabiochem, >99%), 2-isocyanatoethyl methacrylate (Aldrich, 98%), *N*,*N*-diisopropylcarbodiimide (DIPCDI) (Aldrich, 98%), 1-hydroxybenzotriazole hydrate (HOBt) (Fluka, 98%), trifluoroacetic acid (TFA) (Aldrich, 98%), DMSO-d_6 (Aldrich, 99.9%), 2,2,2-trifluoroethanol (TFE) (Aldrich, 99%), and 4,4′-azobis(4-cyanopentanoic acid) (Fluka, >98%) were all used as received. (4-Cyanopentanoic acid)-4-dithiobenzoate was synthesized according to a literature procedure.[24]

Synthesis of Methacrylate-Functionalized VPGVG. Methacrylate-functionalized VPGVG was synthesized by standard solid-phase methods using a "Wang" or "Cl-Trityl" resin as described previously.[11] A representative procedure is given: 1 g of the Fmoc-Gly functionalized resin (loading 0.55 mmol g^{-1}) was swollen and filtered three times in 9 mL of DMF. Next, 10 mL of DMF containing 20% v/v piperidine was added to remove the Fmoc group. A positive Kaiser test indicated completeness of this reaction.[25] The next amino acid was coupled by adding a mixture of 0.571 g (1.65 mmol, 3 equiv) of Fmoc-Val-OH, in 10 mL of DMF, with 0.232 g (1.82 mmol) of DIPCDI and 0.303 g (1.98 mmol) of HOBt. The mixture was shaken for 30 min, after which it was washed thoroughly with DMF. A negative Kaiser test indicated the completeness of the reaction. This procedure was repeated with the following three amino acids: Fmoc-Gly-OH (0.502 g, 1.65 mmol, 3 equiv), Fmoc-Pro-OH (0.568 g, 1.65 mmol, 3 equiv), and Fmoc-Val-OH (0.571 g, 1.65 mmol, 3 equiv). While still on the resin, the Fmoc protecting group on the terminal valine was removed, and the free amine was subsequently coupled with 0.290 g (1.65 mmol, 3 equiv) of 2-isocyanatoethyl methacrylate in

Table 1. Data for the Polymerization of a Methacrylate Derivative of VPGVG (MA-VPGVG)[a]

polymer	$[M]_0/[\mathbf{1}]_0$	$[\mathbf{1}]_0/[\text{V-501}]_0$	conv[b]	$M_{n,th}$ (kg mol^{-1})[c]	$DP_{n,th}$[d]	$M_{n,SEC}$ (kg mol^{-1})[e]	PDI (SEC)[f]
A	34:1	2.5:1	0.85	15.7	29	25.1	1.03
B	42:1	2.3:1	0.81	18.4	34	25.9	1.06
C	88:1	2.1:1	0.87	41.5	76	54.9	1.11
D	83:1	2.0:1	0.73	35.4	65	57.9	1.19
E	117:1	1.8:1	0.75	47.7	88	62.5	1.23

[a] All experiments were carried out at 70 °C. [b] Fractional conversion determined by NMR. [c] Theoretical number-average molecular weight determined from $[M]_0/[\mathbf{1}]_0$, at the given conversion. [d] Theoretical degree of polymerization determined from $([M]_0/[\mathbf{1}]_0) \times$ conv. [e] M_n from SEC. [f] Polydispersity index from SEC.

dichloromethane (DCM). After 1 h the resin was thoroughly washed with DCM and Et_2O. The resin was allowed to dry, and the methacrylate-functionalized VPGVG was cleaved from the resin using 90% TFA/water solution in the case of "Wang" resin or DCM/TFE/AcOH (3:1:1) in the case of "Cl-Trityl" resin. The obtained monomer was precipitated into Et_2O and freeze-dried from aqueous acetic acid (10%). From 10 g of Fmoc-Gly functionalized resin, ~0.296 g (92%) of peptide was obtained.

IR (KBr) ν/cm^{-1}: 3318 (NH str); 2964 (CH str); 1718 (ester C═O str); 1644 (amide C═O str); 1555 (NH bend); 1173 (C−N str).

^{1}H NMR (400.13 Hz, DMSO): δ 0.8−0.9 (12H, m, CH_3-*i*Pr); 1.8−2.1 (9H, m, CH-*i*Pr, CH_2-Pro and CH_3-MA); 3.3−3.4 (8H, m, H_2O + CH_2-Gly); 3.5−3.6 (1H, m, CH-Val); 3.7−3.8 (4H, m, CH_2-Et); 4.04 (2H, t, J = 5.4 Hz, CH_2-Pro); 4.1−4.2 (2H, m, CH_2-Gly); 4.31 (1H, dd, J = 4.3, 8.9 Hz, CH-Val); 5.68 (1H, s, CH-MA), 6.05 (1H, s, CH-MA); 6.1−6.2 (2H, m, NH); 7.61 (1H, d, J = 8.9 Hz, NH); 8.17 (1H, t, J = 5.7 Hz, NH); 8.31 (1H, t, J = 5.9 Hz, NH).

LS-MS: *m*/*e* 583 (MH^+); 605 (M^+−H + Na); 627 (M^+ −2H + 2Na).

RAFT Polymerization. Synthesis of poly(MA-VPGVG) Dithiobenzoate. Polymerizations were carried out using 4-cyanopentanoic acid dithiobenzoate (**1**) as RAFT agent and commercially available 4,4′-azobis(4-cyanopentanoic acid) (V-501) as the radical source. All experiments were conducted in Schlenk tubes sealed with a Young's tap. An NMR spectrum was recorded at the beginning of the experiment to calculate the conversion. The polymerization solutions were degassed with 3−4 freeze−evacuate−thaw cycles and transferred to an oil bath preheated to 70 °C. For the kinetic experiments, the reaction was run under the same conditions in a dry NMR tube fitted with a Young's tap, spectra being acquired every 14 min. After reaction, the solution was quenched by cooling in ice−water for 10 s, and another NMR spectrum was recorded for conversion purposes. The polymer was recovered by precipitation in excess THF followed by centrifugation and freeze-drying from water.

In a typical experiment, to a solution of MA-VPGVG (433 mg, 0.80 mmol) in 2.20 mL of DMSO-d_6 were added 0.41 mL of a 0.06 M solution of **1** (6.6 mg, 0.02 mmol) and 0.42 mL of a 0.02 M solution of V-501 (2.73 mg, 0.01 mmol) both prepared in DMSO-d_6. After quenching the reaction and purification, the title compound was recovered as a pink-white powder by freeze-drying from water (dark, 2 days). Yield: 210 mg, 57%. M_n (SEC) 25 100; PDI (SEC) 1.03; UV (MeOH): λ_{max} = 504 nm.

^{1}H NMR (299.95 MHz, DMSO): δ 0.7−0.9 (12H, m, CH_3-*i*Pr); 1.6−2.0 (10H, m, CH-*i*Pr, CH_2-Pro, CH_3-MA and CH_2-MA backbone); 3.1−4.4 (15H, m, H_2O + CH_2-Gly, CH-Val, CH_2−Et, CH_2-Pro, CH_2-Gly); 6.1−6.3 (2H, m, NH); 7.5−7.7 (1H, m, NH); 8.0−8.4 (2H, m, NH).

End Group Modification. Synthesis of Poly(MA-VPGVG). The dithioester moiety was removed using the procedure described by Perrier et al.[22] In a typical experiment, poly(MA-VPGVG) dithiobenzoate (210 mg, 0.01 mmol) and V-501 (98 mg, 0.34 mmol) were dissolved in 5 mL of MeOH. The polymerization solutions were degassed with three freeze−evacuate−thaw cycles and transferred to an oil bath preheated to 80 °C. After reacting for 3 h, the solution was quenched by cooling in ice−water for 10 s, and the polymer was recovered as a white solid by precipitation in excess THF followed by centrifugation and freeze-drying from water. Yield: 172 mg, 82%. M_n (SEC) 23 500; PDI (SEC) 1.05.

Results and Discussion

Synthesis of EBPs: Polymerization and End-Group Modification. The VPGVG methacrylate monomer was prepared using the solid-phase procedure described previously by the group of van Hest.[11] In our case, both Fmoc-Gly-Wang and Fmoc-Gly-"Cl-Trityl" preloaded resins were employed, leading in both cases to high yields of the monomer. The product was purified by precipitation into Et_2O, and its spectroscopic data were compared to those previously reported, being in all cases identical. Previously, van Hest et al.[11] reported that the concentration of the polymerization mixture was kept low (0.25 M) in order to prevent precipitation of the polymer as it formed. In our case, we kept the concentration in the same range, and despite the fact that the temperature for our polymerizations was significantly higher (70 vs 35 °C), we did not observe any precipitation of the polymer during the reaction.

The study of the polymerization kinetics performed by ^{1}H NMR spectroscopy gave a series of spectra showing the disappearance of the signals from the methacrylate double bond hydrogens as compared to those of the rest of the monomer. The relative integral of one of these signals was related to monomer concentration. Analyzing the semilogarithmic plot for monomer concentration vs time, we could conclude that the polymerization displayed pseudo-first-order kinetics, indicating that the propagating radical concentration was constant, which is typical for free radical polymerizations (Figure 1). Similar induction periods have already been described in the literature,[26] probably due to slow fragmentation of the intermediate RAFT radicals in the preequilibrium or to slow reinitiation of the leaving group radicals of the initial RAFT agent.[27]

Table 1 shows the results for the polymerization of MA-VPGVG using different ratios of RAFT agent **1** and radical initiator V-501. The SEC results for all polymerizations showed narrow molecular weight distributions, as expected for a "controlled" polymerization. Importantly, excellent control was displayed up to reasonably high molecular weights (entries C−E).

The M_n values obtained by SEC using a RALLS detector (see Experimental Section) should in theory be "absolute"; i.e., they are not obtained by comparison with a molecular weight calibration standard or from a universal calibration curve. Therefore, they should agree very well with the values calculated from the initial monomer-to-initiator ratio and taking the conversion into account. However, Table 1 indicates a significant difference between theoretical and experimental values. Such differences are known for polymers of complex architecture and structure. For instance, similar differences have been described previously for the polymerization of *N*-vinylbenzyl-*N*,*N*,*N*-trimethylammonium chloride (VBAC) via RAFT.[28] To explain these results, it was suggested that MALLS tends to

Scheme 1. End-Group Modification of a VPGVG Side-Chain Polymethacrylate (Poly(MA-VPGVG)) by Treatment with Azo Initiator

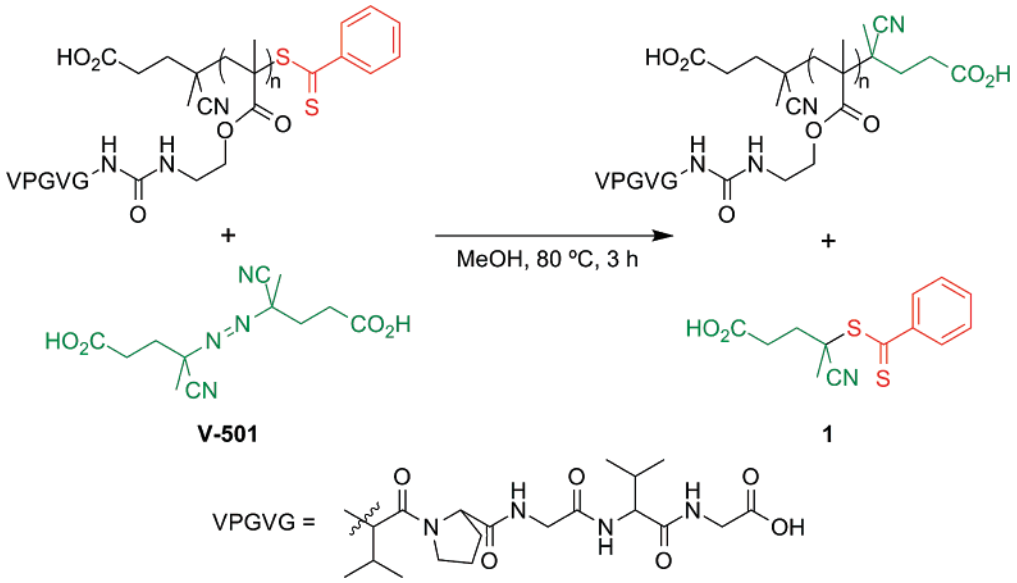

Table 2. Data for a VPGVG Side-Chain Polymethacrylate (Poly(MA-VPGVG)) after Treatment with Azo Initiator

polymer	$[V\text{-}501]_0/[EBP]_0$	$M_{n,DT}$ (kg mol^{-1})[a]	PDI_{DT}[b]	$M_{n,A}$ (kg mol^{-1})[c]	PDI_A[d]
F	28:1	25.9	1.06	24.3	1.05
G	26:1	25.1	1.03	23.5	1.05
H	25:1	57.9	1.19	57.0	1.20
I	22:1	62.5	1.23	61.6	1.25

[a] Number-average molecular weight of dithioester-terminated polymer. [b] Polydispersity index of dithioester-terminated polymer. [c] Number-average molecular weight of dithioester-terminated polymer after treatment with azo initiator. [d] Polydispersity index of dithioester-terminated polymer after treatment with azo initiator.

underestimate the contribution of the lower molecular weight fractions. This is also a possible explanation for the very low PDI values for the lowest molecular weight polymers (polymers A and B). An underestimation of M_w from the RALLS detector would consequently produce a narrower apparent PDI. The PDI values for polymers C−E are more realistic and are in line with values commonly found for RAFT polymers. Another possible explanation for the difference in experimental and theoretical M_n values could be that EBPs are effectively comb polymers and therefore may not fit the Rayleigh−Debye−Gans light scattering model for dilute polymers, which is used for the calculation of M_w. However, the experimental M_n values in Table 1 are in better agreement with the theoretical values than was the case in the previous work, and polydispersities are narrow. Therefore, we conclude that the RAFT polymerization of VPGVG-MA is a well-behaved and controlled process.

In order to avoid possible side reactions of the dithioester group upon heating during the turbidimetry experiments, this end group was modified using the methodology developed by Perrier et al.[22] that involves treatment of the polymer with an excess of initiator (Scheme 1). As may be seen in Table 2, both the M_n values and the polydispersities of the polymers were not affected by the reaction conditions. In all cases, an excess of at least 20 equiv of the radical initiator V-501 was used to ensure that no disproportionation occurred. The absence of double bonds from disproportionation in the final polymers was checked by NMR while the disappearance of the dithioester moiety can be followed by UV−vis spectroscopy. The SEC chromatogram of the polymer after treatment with the azo initiator (Figure 2, polymer F) does not indicate the presence of material from radical−radical coupling and shows little variation from that of the polymer before treatment (polymer B).

LCST Behavior. The thermal behavior of ELPs and EBPs can be characterized by a variety of techniques such as UV−vis turbidimetry[23] or DSC.[29] In our case, the transition temperature was taken as the midpoint of the cloud point curve determined by UV−vis spectroscopy, for solutions of polymers at different pH and concentrations. This procedure is in accord with that used previously for EBPs and allows a direct comparison between our results and those in the literature.[12]

pH Dependence. We decided to start our investigations into the LCST behavior of EBPs prepared by RAFT by determining the effect of pH, which would also provide the optimum value for further cloud point experiments. As reported by van Hest et al.,[12] the fact that our EBPs have a carboxylic acid group at the end of the peptide chain gives them the ability to respond to changes in the pH. It was found that a decrease in the pH causes the carboxylic acid moieties to become protonated, giving the polymer a more hydrophobic character, and therefore the phase transition temperature should be lower.

When we performed turbidimetry measurements on 0.22 mg mL^{-1} solutions of polymer H (M_n = 57.0 kg mol^{-1}), the same trend was observed, with no transition observed for the polymer solution at pH 5 (Figure 3). In our case, transition temperatures were considerably lower than those reported by van Hest et al. Two reasons are suggested for this: (i) our EBPs have a higher molecular weight (M_n = 57.0 kg mol^{-1} compared to 7−15 kg mol^{-1}), and (ii) the polymers described here are homopolymers whereas the previous materials were ABA triblock copolymers with a PEG middle block. It may be that the PEG blocks in some way affect the ability of the EBP blocks to phase separate, possibly by impeding the associated conformational rearrangements of the responsive units.[30] We decided to perform further turbidimetry experiments at a pH value of 3.2 since at this pH the transition temperature is near to physiological temperature, and this is also convenient from an experimental point of view (turbidimetry experiments can be carried out within the range 20−80 °C).

Concentration Dependence. Another physical parameter of interest when studying ELPs is polymer concentration. It has been described that the transition from a random coil to a more organized structure shows positive cooperativity, in which

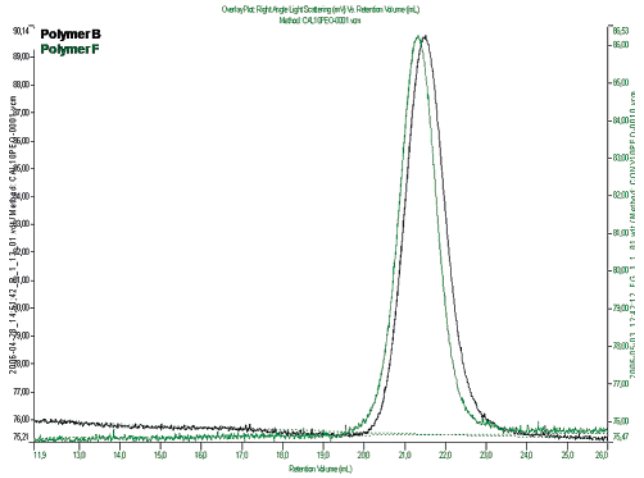

Figure 2. RALLS detector traces from SEC experiments for the transformation of polymer B into polymer F (see Tables 1 and 2 for data on polymers B and F).

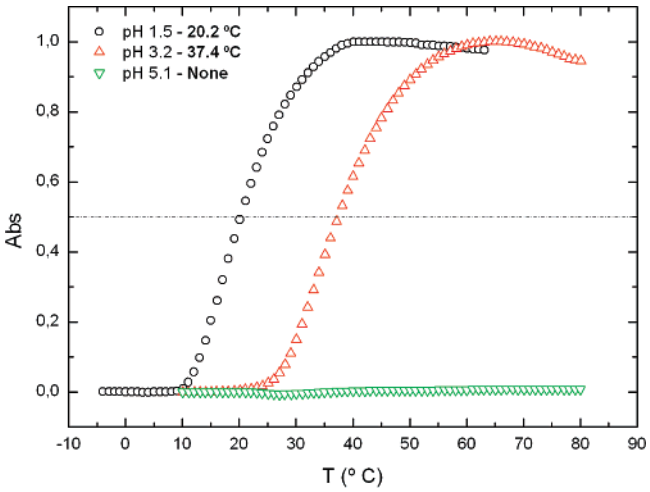

Figure 3. Turbidimetry measurement for polymer H (M_n = 57.0 kg mol^{-1}) at pH = 1.5, 3.2, and 5.1 perfomed by UV−vis spectroscopy (λ = 480 nm).

intermolecular interactions facilitate the structural change,[1] so as the concentration is increased, a decrease in the transition temperature should be observed. To investigate this effect, different solutions of polymer F (M_n = 24.3 kg mol^{-1}) and polymer H (M_n = 57.0 kg mol^{-1}) were prepared in a PBS buffered solution of pH 3.2 (Figure 4). In both cases a linear dependence of the transition temperature with the concentration is observed, which is in agreement with that reported for ELPs[23,31] and for the previously described EBPs.[12]

Molecular Weight Dependence. The effect of a change in the polymer chain length on the phase transition temperature was investigated next. It has been suggested that, as the linear VPGVG polymers become shorter, they become more ordered, increasing the energy required to change from one conformation to the other.[23,31] These hypotheses are in good agreement with the experimental data for the EBPs prepared by van Hest et al.[12] In order to determine whether our polymers behave similarly and whether the change in transition temperature is similar to that reported previously, we prepared 0.65 mg mL^{-1} solutions of polymers in a pH 3.2 phosphate buffer (Figure 5). These were then investigated as before.

When we compared this behavior with that reported for ELPs[23,31] and the EBPs prepared by van Hest's group,[12] the same general tendency was observed, but surprisingly, the

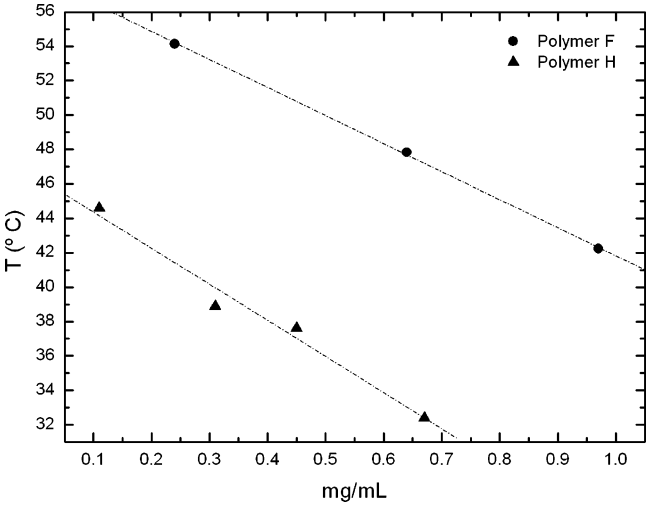

Figure 4. Transition temperature for polymer F (M_n = 24.3 kg mol^{-1}) and polymer H (M_n = 57.0 kg mol^{-1}) at different concentrations, as determined by UV−vis turbidimetry experiments at pH 3.2.

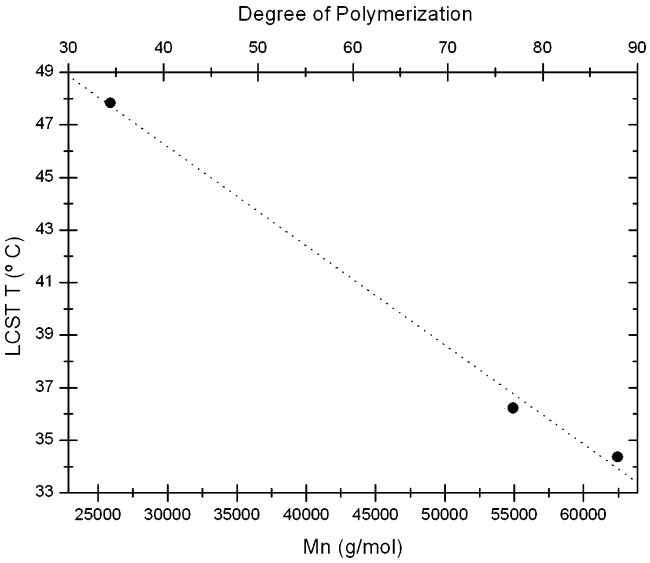

Figure 5. Transition temperature for EBPs with different degrees of polymerization, as determined by UV−vis turbidimetry experiments at pH 3.2 and a solution concentration of 0.65 mg mL^{-1}.

magnitude of this effect in the case of our polymers was closer to that described for linear polymers. Meyer et al.[23] reported that an increase of 30 units was needed to obtain a change in transition temperature of around 15 °C in linear polymers, while in the case of the EBPs prepared by Van Hest et al.[12] an increase of 12 units is sufficient to obtain the same change in the transition temperature. The explanation offered for this difference in behavior is that the polymethacrylate segments make an increasing contribution to the overall properties of the A−B−A triblock copolymer as their molecular weight increases. Thus, as the DP of the EBP blocks increases, the VPGVG units experience a more hydrophobic environment, which will reduce dramatically the phase transition temperature. Our EBPs are more similar to the ELPs of Meyer et al., in that the hydrophilicity does not change markedly with increasing molecular weight.

Conclusion

Elastin-based side-chain polymers (EBPs) have been prepared in a controlled manner using RAFT as the polymerization technique. This allowed us to study thoroughly the LCST

behavior of the polymers thus prepared and compare it with previously reported data for similar EBPs as well as linear polypeptide derivatives (ELPs). In all cases, the LCST behavior of the EBPs was similar to that reported for linear ELPs. This, and the fact that RAFT allows for the introduction of functional groups such as thiols at the polymer chain end, suggest that EBPs prepared by RAFT could be used as readily available mimics of ELPs for applications such as protein purification,[8] nanopatterning,[32] or drug delivery.[6] Some differences were observed compared to previously reported EBPs containing PEG blocks, which were ascribed to the greater hydrophilicity of the latter materials due to the presence of the PEG block.

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