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Elastin-Based Side-Chain Polymers Synthesized by ATRP

Lee Ayres,[†] Matthijn R. J. Vos,[†] P. J. Hans M. Adams,[†] Igor O. Shklyarevskiy,[‡] and Jan C. M. van Hest*,[†]

NSRIM Organic Chemistry Department, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands, and NSRIM High Field Magnet Laboratory, University of Nijmegen, Toernooiveld 7, 6525 ED Nijmegen, The Netherlands

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ABSTRACT: The synthesis of two monomers based on glutamic acid and the peptide VPGVG (V = valine, P = proline, and G = glycine), a commonly found sequence in tropoelastin, was investigated. The polymerizations of both monomers using atom transfer radical polymerization (ATRP) proceeded in a controlled manner and yielded polymers with narrow molecular weight distributions. The VPGVG sequence in the side chain of the polymer still maintained its characteristic LCST (lower critical solution temperature) behavior, according to CD spectroscopy. Triblock copolymers were prepared by polymerizing, using ATRP, the VPGVG monomer with a bifunctional poly(ethylene glycol) (PEG)-based initiator. This resulted in a material that upon heating in water clearly formed aggregates. These reversible aggregates were investigated using turbidity measurements and dynamic light scattering. A pH-dependent transition temperature of 40−70 °C was observed.

Introduction

Elastin is one of the most important classes of naturally occurring structural proteins.[1−5] It is commonly found in the ligaments, arteries, skin, and lung tissue of mammals where it functions as an elastomeric material.[6,7] There are many different types of elastin,[8] but tropoelastin (the precursor protein of mammalian elastin) is one of the most studied. VPGVG (V = valine, P = proline, and G = glycine) is its most prominent amino acid repeat.[9,10] The aggregation, conformational, and mechanical properties of both chemically synthesized[4,11,12] and recombinantly prepared poly(VPGVG)[13−16] have been extensively investigated. Poly(VPGVG) is soluble in water at room temperature, but as the temperature is increased the solubility of this polypeptide is decreased. This remarkable LCST behavior is a result of a conformational change in elastin from random coil to β spiral, which is caused by hydrophobic dehydration of the valine side chains.[17,18] Optimization of these hydrophobic interactions occurs when three of these long-chain β spirals twist together into a larger aggregate.[1] This behavior is completely reversible. The inverse transition temperature can also be fine-tuned by replacing the second valine with any other amino acid (VPGXG), except proline. By adding either acidic or basic amino acids in the fourth position, the LCST behavior becomes both pH- and temperature-dependent.[19,20]

This LCST behavior makes elastin-like peptides, or ELP's, of interest for a wide range of applications, for example, as thermally responsive hydrogels with an inverse temperature transition.[14,16,21] These peptide sequences are also biologically compatible,[22,23] opening up the possibility of medical applications for ELP's such as drug delivery.[14,24−27]

All of the previous investigations have focused on linear poly(VPGVG). However, it has been shown that this inverse temperature transition occurs not only in poly(VPGVG)$_n$ (where $n > 10$) but also in a single repeat of VPGVG.[28] This inspired us to investigate the possibility of synthesizing polymers with similar LCST behavior by introducing single repeats of VPGVG into the side chain. Connecting side-chain elastin oligomers to the ends of a polymer chain could result in a versatile approach to producing thermally responsive triblock copolymers.

To construct these peptide-based copolymers in a controlled way, we have used atom transfer radical polymerization (ATRP).[29,30] ATRP is a robust and versatile technique, capable of polymerizing a wide range of bioinspired materials.[31−34] Although oligopeptide-based monomers have been polymerized using a variety of techniques,[35,36] to the best of our knowledge, however, there is no example in which ATRP has been used.

This paper describes our successful synthesis of ABA triblock copolymers in which the A blocks are based on elastin oligopeptides. The syntheses of two peptide monomers are outlined as well as their successful polymerization via ATRP into triblock copolymers, using a poly(ethylene glycol) (PEG)-based bifunctional initiator. The thermally responsive behavior of these polymers was investigated using CD spectroscopy, light scattering, and turbidity measurements.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were measured on a 400 MHz Bruker Inova400 machine with a Varian probe.

IR spectra were measured on an ATI Mattson Genesis Series FTIR.

Elemental analysis was performed on a Carlo-Erba Instruments EA1180 CHNO/S elemental analyzer.

CD spectra were measured on a Jasco J-810 spectropolarimeter, with a temperature control unit. Samples were dissolved in Milli-Q water and sonicated for 1 h in an ice bath. A polymer solution was diluted until a maximum UV absorption of 1.5 was reached at 220 nm. The samples were measured using a 1 mm quartz cuvette at different temperatures. The same solution was also used for turbidity experiments using

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[†] NSRIM Organic Chemistry Department.
[‡] NSRIM High Field Magnet Laboratory.
* Corresponding author: e-mail vanhest@sci.kun.nl.

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the same CD spectropolarimeter. They were carried out at a fixed wavelength of 480 nm.

MALDI−TOF-MS spectra were measured on a Bruker Biflex III machine, with dihydroxybenzoic acid (DHB) as matrix. The sample was prepared by dissolving 2 mg of analyte in 1 mL of THF, after which this solution was mixed in a 1:1 ratio with a solution of 10 mg of DHB in 1 mL of H_2O containing 0.1% trifluoroacetic acid. This was then placed on a MALDI plate.

GPC measurements were performed using a Shimadzu GPC with Shimadzu RI and UV/vis detection, fitted with a Polymer Laboratories Plgel 5 μm mixed-D column and a PL 5 μm Guard column (separation range from 500 to 300 000 molecular weight) using THF or NMP as mobile phase at 35 and 70 °C, respectively. Polymer Laboratories polystyrene calibration kits were used.

Dynamic light scattering (DLS) measurements were carried out using an intensity-stabilized helium−neon laser (Spectra Physics, $\lambda = 632.8$ nm, 4.5 mW). The incident beam of the laser was focused in the center of the precision glass cylindrical cuvette (Helma), which was used as a scattering cell. The light, scattered at 90° with respect to the incident beam, was transferred to the single photon detector (ALV/SO-SIPD) through a single mode fiber to meet the spatial coherence conditions. The signals were processed with a 320-channel Multiple Tau Digital Correlator (ALV-5000/E). The measurements were carried out in a temperature range 20−90 °C (±0.05 °C) using a laboratory-made heating stage driven by an active feedback temperature controller (LakeShore 340).

Reagents. *p*-Alkoxybenzyl alcohol "Wang" resin (Bachem, 1.14 mmol/g), 9-fluorenylmethoxy carbamate protected valine (Fmoc Val-OH) (Bachem, >99%), Fmoc glycine (Fmoc Gly-OH) (Bachem, >99%), and Fmoc proline (Fmoc Pro-OH) (Bachem, >99%) were all used as received. CuCl (Aldrich, 97%) was purified by washing with glacial acetic acid three times and once with diethyl ether. PEG 1000 (Fluka), ethyl-2 bromo isobutyrate (EBIB; Aldrich, 98%), hydroxyl ethyl methacrylate (HEMA; Aldrich, 97%), 2-bromoisobutyric acid (Aldrich, 98%), 2-isocyanatoethyl methacrylate (Aldrich, 98%), 2,2′-bipiridyl (Bipy) (Aldrich, 99%), *N*,*N*-dicyclohexylcarbodiimide (DCC) (Fluka, 99%), 4-(dimethylamino)pyridine (DMAP) (Across, 99%), DMSO-d_6 (Aldrich, 99.9%), *N*,*N*′-diisopropylethylamine (DIPEA) (Fluka, 99%), 1-hydroxybenzotriazole hydrate (HOBt) (Fluka, ≥98%), *N*,*N*-diisopropylcarbodiimide (DIPCDI) (Fluka, ≥98%), trifluoroacetic acid (TFA) (Aldrich, 98%), and ethylenediaminetetraacetic acid tetrasodium salt hydrate (EDTA; Aldrich, 98%) were all used as received.

Dichloromethane (DCM) and ethyl acetate (EtOAc) were distilled from calcium hydride, and THF was distilled from sodium/benzophenone prior to use. Dimethylformamide (DMF) and isopropyl alcohol were used as received (J.T. Baker).

Monomer Synthesis. Synthesis of Methacrylate-Functionalized Glutamic Acid (Glu-EMA) 1. Boc-Glu-(OtBu)-OH (1.52 g, 5 mmol) was dissolved in 30 mL of EtOAc, and 650 mg (5 mmol) of hydroxyethyl methacrylate (HEMA) was added. While stirring vigorously, 60 mg (0.5 mmol) of DMAP and 1.03 g (5 mmol) of DCC were added in quick succession; a white precipitate was formed. The reaction mixture was stirred for 24 h at room temperature. Dicyclohexyurea (DCU) was filtered off and weighed to determine whether the reaction was complete. After evaporation of the solvent the crude product was obtained, which was purified by column chromatography using silica and 1% MeOH/$CHCl_3$ as mobile phase. Glu-EMA was obtained in 73% yield.

^{1}H NMR ($SO(CD_3)_2$): δ 1.4 ($C(CH_3)_3$, 18H, s); 1.7 ($NHCH(CH_2CH_2R)COOR$, 2H, m); 1.9 ($C{=}CCH_3$, 3H, s); 2.2 ($NHCH(CH_2CH_2R)COOR$, 2H, t); 3.8−4.3 (NHCHRCOOR, $O(CH_2)_2O$, 5H); 5,7 and 6.0 ($C{=}CH_2$, 2H, s); 7.2 (NH, 1H, d).

^{13}C NMR ($SO(CD_3)_2$): δ 17.73, 26.00, 27.53, 27.95, 30.98, 52.54, 62.31, 66.31, 78.02, 79.47, 125.75, 135.52, 155.34, 166.16, 171.21, 172.03.

MALDI−TOF-MS: *m*/*e* 437 ($M^+ - H + Na$); 438 ($M^+ + Na$).

Anal. Calcd for $C_{20}H_{33}NO_8$: C, 57.82; H, 8.01; N, 3.37. Found: C, 58.33; H, 8.15; N, 3.65.

Synthesis of Methacrylate-Functionalized VPGVG 2. Methacrylate-functionalized VPGVG **2** was synthesized by standard solid-phase methods using a "Wang" resin.[37,38] A suspension of Wang resin (30 g) in 300 mL of DMF was cooled in an ice bath, after which Fmoc Gly-OH (13.5 g, 45 mmol), 9.20 g (60 mmol) of HOBt, and 4.30 g (34.2 mmol) of DIPCDI were added. This mixture was shaken for 6 h. The functionalized resin was filtered and washed repeatedly with DCM, DMF, and isopropyl alcohol. Unfunctionalized groups on the resin were capped by adding 10.2 mL of benzoyl chloride and 8.4 mL of pyridine to a suspension of the resin in 300 mL of DCM at 0 °C. This mixture was shaken for 30 min, filtered, and washed repeatedly with DCM, DMF, and isopropyl alcohol. Then 10 g of the Fmoc Gly functionalized Wang resin (loading 0.9 mmol/g) was swollen and filtered three times in 90 mL of DMF. Next, 90 mL of DMF containing 20% v/v piperidine was added to remove the Fmoc group. A positive Kaiser test[39,40] indicated completeness of this reaction. The next amino acid was coupled by adding a mixture of 9.16 g (27 mmol) of Fmoc Val-OH, in 90 mL of DMF, with 4.08 g (32.4 mmol) of DIPCDI and 4.60 g (30 mmol) of HOBt. The mixture was shaken for 30 min, after which it was washed with DMF and twice with DCM and isopropyl alcohol. A negative Kaiser test indicated the completeness of the reaction. Free amines were capped by adding 90 mL of DMF containing 1 mL of Ac_2O and 2 mL of DIPEA. After 15 min this mixture was washed with DMF. This procedure was repeated with the following three amino acids: Fmoc Gly-OH (8.02 g, 32.4 mmol), Fmoc Pro-OH (9.11 g, 32.4 mmol), and Fmoc Val-OH (9.16 g, 32.4 mmol). While still on the resin the Fmoc protecting group on the terminal valine was removed, and the free amine was subsequently coupled with 3 equiv of 2-isocyanatoethyl methacrylate in DMF. The methacrylate-functionalized VPGVG was cleaved from the resin using 90% TFA/water solution and was concentrated after cleavage. The obtained monomer was first freeze-dried from acetic acid and subsequently from dioxane. The monomer was then purified by column chromatography using 60 H silica (Merck) and $CHCl_3$/MeOH/water (65:25:4) as eluent. From 10 g of Fmoc-Gly functionalized Wang resin, 3.5 g of peptide was obtained.

IR (KBr): ν 3318 (NH str); 2964 (CH str); 1718 (C═O str); 1644 (C═C str); 1555 (NH bend); 1173 (C−N str).

^{1}H NMR ($SO(CD_3)_2$): δ 0.5−1.2 ($NHC(C(CH_3)_2)COR$, 12H); 1.7−2.2 ($NHC(CH(CH_3)_2)COR$, $NHCH_2CH_2CH_2CHCOR$, 6H and $C{=}CCH_3$, 3H); 3.2−4.4 (NHCHRCOR, $NHCH_2COR$, $NHCH_2CH_2CH_2CHCOR$, $RO(CH_2)_2NHCONH$,13 H); 5.6 and 6.1 ($C{=}CH_2$, 2H, s); 6.7−8.5 (NH amide and NH urea, 5H).

^{13}C NMR ($SO(CD_3)_2$): δ 17.94, 18.30, 19.12, 24.52, 29.15, 30.77, 31.33, 55.92, 58.52, 59.67, 64.12, 125.81, 135.80, 157.83, 166.50, 168.46, 170.52, 171.27, 171.63, 171.91.

MALDI−TOF-MS: *m*/*e* 605 ($M^+ - H + Na$); 621 ($M^+ - H + K$); 627 ($M^+ - 2H + 2Na$).

Initiator Synthesis. Synthesis of Bifunctional PEG ATRP Macroinitiator (Di-α,ω-bromoisobutyrate-PEG) 3. PEG (M_n: 1000 g/mol, 5.00 g, 5 mmol) was melted and dissolved in 20 mL of benzene. The mixture was heated under vacuum until all benzene was evaporated. This procedure was repeated three times to remove all residual water from the PEG. Dried PEG was redissolved in 20−30 mL of dried THF, and 3.34 g (20 mmol) of 2-bromoisobutyric acid was added and stirred until dissolved. The reaction mixture was cooled to −20 °C, and 4.13 g (20 mmol) of DCC and 244.5 mg (2 mmol) of DMAP were added in quick succession. The solution was allowed to come to room temperature and stirred for 72 h. The progress of the reaction was monitored by TLC. After completion precipitated DCU was removed by filtration, and THF was removed by evaporation to obtain the crude product. The product was purified by column chromatography using 60 H silica, with 5% MeOH in $CHCl_3$ as a mobile phase. Bifunctional PEG initiator **3** was obtained in 91% yield.

^{1}H NMR ($SO(CD_3)_2$): δ 1.8 ($COC(CH_3)_2Br$, 12H, s); 3.5 ($O(CH_2)_2O$ backbone, mp); 3.6 ($ROCH_2CH_2OCO$, 4H, t); 4.3 ($ROCH_2CH_2OCO$, 4H, t).

^{13}C NMR ($SO(CD_3)_2$): δ 30.23, 57.06, 65.00, 67.90, 69.78, 69.90, 170.74.

MALDI−TOF-MS: *m*/*e* 1307 (M$^+$); 1351, 1395, 1439, 1483, 1527, 1571 (M$^+$ + O(CH$_2$)$_2$); 1263, 1219, 1175, 1139, 1095 (M$^+$ − O(CH$_2$)$_2$).

Anal. Calcd for $C_{54}H_{104}O_{26}Br_2$: C, 48.80; H, 7.89. Found: C, 48.20; H, 7.91.

ATRP Polymerization. Polymerization of Glu-EMA to Homopolymer. ATRP polymerization of Glu-EMA monomer **1** was carried out in solution using ethylbromo isobutyrylate (EBIB) as initiator. Glu-EMA (831 mg, 2 mmol), 10.1 mg (0.1 mmol) of CuCl, and 32.4 mg (0.2 mmol) of bipy were weighed into a Schlenk vessel. The vessel was evacuated and filled with N$_2$. This procedure was repeated three times. DMSO-d_6 (0.54 mL) was added to dissolve the reactants and then purged with N$_2$. The mixture was heated to 40 °C, and 1.46 mL (0.07 mmol of EBIB) of initiator stock solution (containing 10 mg/mL EBIB in DMSO-d_6) was added. During the reaction samples were taken, and conversion was determined by ^{1}H NMR spectroscopy (comparing the amide resonance at 7.2 ppm with the vinyl signal at 6.05 ppm). After polymerization, the mixture was poured in water/EDTA solution containing 2.5 g of EDTA/100 mL while stirring vigorously. When the precipitate was filtered, it was washed with cold water. The precipitate was then air-dried and characterized by GPC in THF and NMR in DMSO-d_6.

^{1}H NMR (SO(CD$_3$)$_2$): δ 1.4 (C(CH_3)$_3$, 18H, s); 1.7 (NHCH(CH_2CH$_2$R)COOR, 2H, m); 1.9 (C−CCH_3, 3H, s); 2.2 (NHCH(CH$_2$CH_2R)COOR, 2H, t); 3.8−4.3 (NHCHRCOOR, O(CH_2)$_2$O, 5H); 7.2 (NH, 1H, d).

GPC: M_n = 8.7 kg/mol, PDI = 1.11.

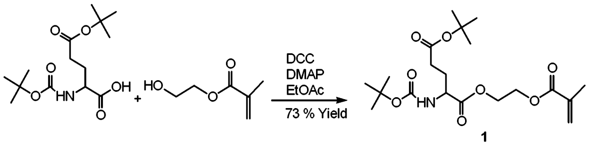
Polymerization of Glu-EMA to ABA Block Copolymer. ATRP polymerization of Glu-EMA monomer **1** was carried out in solution using α,ω-dibromoisobutyrate-PEG **3** as bifunctional initiator. Glu-EMA, (415 mg, 1 mmol), 10.0 mg (0.1 mmol) of CuCl, and 32.0 mg (0.2 mmol) of bipy were weighed into a Schlenk vessel. The vessel was evacuated and filled with N$_2$. This procedure was repeated three times. DMSO-d_6 (0.5 mL) was added to dissolve reactants, after which the vessel was purged again with N$_2$. Then 31 mg (0.025 mmol) of **3** was dissolved in 0.5 mL of DMSO-d_6 and added to the mixture. The reaction mixture was heated to 50 °C, and the initiator solution was added. During the reaction samples were taken, and conversion was determined by ^{1}H NMR spectroscopy (comparing the amide resonance at 7.2 ppm with the vinyl signal at 6.05 ppm). After polymerization, the mixture was poured in water/EDTA solution containing 2.5 g of EDTA/100 mL while stirring vigorously. When the precipitate was white it was filtered off and washed with cold water. The precipitate was air-dried and characterized by GPC in THF and NMR in DMSO-d_6.

^{1}H NMR (SO(CD$_3$)$_2$): δ 0.5−2.3 (−CH_2−CH(RCH$_3$)−, 4H, C(CH_3)$_3$, 18H, −CH$_2$−CH(RCH_3)−, 3H, NHCH(CH_2CH$_2$R)COOR, 2H and NHCH(CH$_2$CH_2R)COOR, 2H); 3.8−4.3 (NHCHRCOOR, O(CH_2)$_2$O, 5 H); 6.7−7.2 (NH, 1H).

GPC: M_n = 10.9 kg/mol, PDI = 1.22.

Polymerization of Methacrylate-Functionalized VPGVG to Homopolymer. ATRP polymerization of VPGVG-based monomer **2** was carried out in solution using EBIB as initiator. Monomer **2** (300 mg, 0.5 mmol), CuCl 10.1 mg (0.1 mmol), and bipy 34.0 mg (0.2 mmol) were weighed into a Schlenk vessel. The vessel was evacuated and filled with N$_2$. This procedure was repeated three times. DMSO-d_6 (1.57 mL) was added to dissolve reactants, and 45 μL (0.5 mmol) of xylene was introduced as an internal standard. The mixture was heated to 36 °C, and 0.43 mL (0.022 mmol of EBIB) of a 1 mL solution of DMSO-d_6 containing 10 mg of EBIB was added. During the reaction samples were taken, and conversion was determined by ^{1}H NMR spectroscopy based on the ratio of the vinyl signals (5.61 ppm) to the xylene aromatic signal (from 6.8 to 7.2 ppm). After polymerization, the mixture was precipitated in diethyl ether. The ether layer was decanted, and the brown precipitate was subsequently redissolved in water (resulting solution pH 6.0). A 1 M HCl solution was added until pH 1.0 was reached, causing the desired product to precipitate out as a white powder. The resulting polymer was filtered off and rinsed with ice-cold water. The product was then taken up in a 1:1 water/THF mixture. After evaporation of THF the turbid water mixture was freeze-dried. Characterization was performed by GPC with NMP as eluent and NMR in DMSO-d_6.

Scheme 1. Synthesis of Methacrylate-Functionalized Glutamic Acid 1



^{1}H NMR (SO(CD$_3$)$_2$): δ 0.5−2.1 (NHC(C(CH_3)$_2$)COR, 12H, NHC(CH(CH$_3$)$_2$)COR, NHCH$_2$CH_2CH_2CHCOR, 6H, −CH$_2$−CH(RCH_3)−, 3H and −CH_2−CH(RCH$_3$)−, 4H); 3.0−5.5 (NHCHRCOR, NHCH_2COR, NHCH_2CH$_2$CH$_2$CHCOR, RO(CH_2)$_2$NHCONH, 13H); 6.0−8.5 (NH amide and NH urea, 5H).

GPC: M_n = 53.0 kg/mol, PDI = 1.25.

Polymerization of Methacrylate-Functionalized VPGVG to an ABA Block Copolymer. ATRP polymerization of VPGVG-based monomer **2** was carried out in solution using α,ω-dibromoisobutyrate-PEG **3** as a bifunctional initiator. VPGVG monomer (154 mg, 0.25 mmol), 10.0 mg (0.1 mmol) of CuCl, and 34.0 mg (0.2 mmol) of bipy were weighed into a Schlenk vessel. The vessel was evacuated and filled with N$_2$. This procedure was repeated three times. DMSO-d_6 (0.5 mL) was added to dissolve reactants, and 45 μL (0.5 mmol) of xylene was introduced as internal standard. This was then purged with N$_2$. The bifunctional initiator (31 mg, 0.025 mmol) was dissolved in 0.5 mL of DMSO-d_6. The reaction mixture was heated to 35 °C, and the initiator solution was added. After polymerization, the mixture was poured in ether. After decantation of the ether layer, the brown polymer precipitate was redissolved in demineralized water (resulting solution pH 6.0). Acidification with a 1 M HCl solution to pH 1 resulted in precipitation of the polymer as a white powder. The polymer was filtered off and rinsed with ice cold water. The product was then redissolved in a 1:1 water/THF mixture. After evaporation of THF the turbid water mixture was freeze-dried, yielding the desired ABA block copolymer. Characterization was performed by GPC with NMP as eluent and NMR in DMSO-d_6.

^{1}H NMR (SO(CD$_3$)$_2$): δ 0.5−2.1 (NHC(C(CH_3)$_2$)COR, 168H, NHC(CH(CH$_3$)$_2$)COR, NHCH$_2$CH_2CH_2CHCOR, 84H, −CH$_2$−CH(RCH_3)−, 42H, and −CH_2−CH(RCH$_3$)−, 56H); 3.0−5.5 (NHCHRCOR, NHCH_2COR, NHCH_2CH$_2$CH$_2$CHCOR, RO(CH_2)$_2$NHCONH, 182H, and O−CH_2−CH_2−O, 84H); 6.0−8.5 (NH amide and NH urea, 70H).

M_n from NMR = 10.9 kg/mol.

GPC: M_n = 60.0 kg/mol, PDI = 1.24

Results and Discussion

Model Studies with Glutamic Acid-Based Monomer. To investigate the optimal conditions for ATRP of peptide-based monomers, first the polymerization of a single amino acid was explored. For this purpose glutamic acid-based monomer, **1**, was conveniently synthesized, using DCC to couple the free carboxylic acid of the glutamic acid with hydroxyl ethyl methacrylate (Scheme 1).

Polymerizations were performed using CuCl/Bipy as a catalytic system in DMSO-d_6 (1 M concentration). Two different initiators were used: a standard ATRP initiator, ethyl 2-bromoisobutyrate (EBIB), and a bifunctional macro-initiator **3** synthesized from poly(ethylene glycol) (PEG) (Scheme 2) giving a triblock copolymer. Table 1 shows the polymerization results for monomer **1**. Figures 1 and 2 show the semilogarithmic plots of monomer concentration vs time for both the EBIB and the PEG initiated polymerizations of **1**.

All polymerizations were carried out in DMSO-d_6, which is an unusual solvent for ATRP.[33] There were two

Table 1. Polymerization Results for Monomer 1. PDI and M_n Are Determined Using size exclusion chromatography

polymer	monomer	initiator	temp, °C	time, min	$[M]_0/[I]_0$	conv, %	M_n, kg/mol	$M_{n,th}$, kg/mol	PDI (GPC)
A	**1**	EBIB	40	120	20:1	60	8.7	7.5	1.11
B	**1**	EBIB	60	120	20:1	90	7.7	11.2	1.09
C	**1**	**3**	50	120	20:1	79	10.9	7.4	1.22

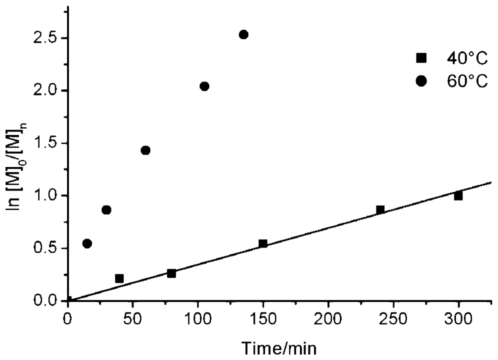


Figure 1. Linear plot of ln $[M]_0/[M]_n$ vs time for the polymerization of **1** with EBIB initiator. Polymerization was performed at 40 and 60 °C.

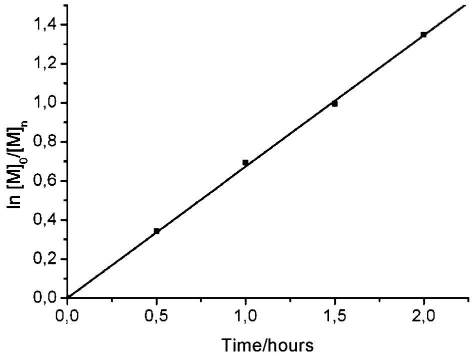
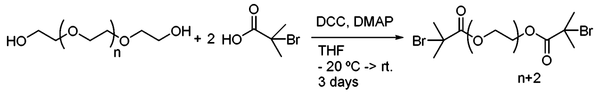


Figure 2. Linear plot of ln $[M]_0/[M]_n$ vs time for the polymerization of **1** with PEG initiator **3**. Polymerization was performed at 50 °C.

Scheme 2. Synthesis of PEG Based Bifunctional Initiator 3

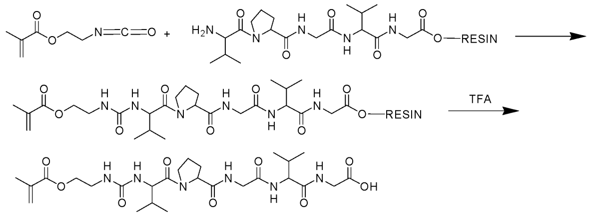


reasons for choosing DMSO as a solvent. The first was to solublize not only the peptide monomer but also the final polymer to prevent precipitation from occurring. Second, using DMSO-d_6 allows the conversion to be followed using NMR spectroscopy.

The gel permeation chromatography (GPC) results for polymers **A** and **B** (Table 1) show narrow molecular weight distributions for both polymers. In addition, the semilogarithmic plots of monomer concentration vs time are linear, indicating first-order kinetics at both temperatures, implying that we have a living/controlled polymerization.[30]

The GPC results for polymer **C**, the triblock copolymer, shows not only a narrow molecular weight distribution but also a complete shift from the M_n corresponding to the PEG initiator, indicating complete initiation. The plot of monomer concentration vs time is linear, which proves that also with the bifunctional initiator a high degree of control over polymerization is possible. As the GPC results are based on polystyrene calibration standards, only a relative estimate can be made about the M_n due to the difference in hydrodynamic volumes.

Scheme 3. Synthesis of Methacrylate-Functionalized VPGVG (2) on the Solid Phase



These model studies clearly show that ATRP is a suitable method for the polymerization of amino acid-containing monomers. DMSO proves to be a convenient solvent for this type of monomer, although it was expected that the polarity of DMSO would lead to an increase in the number of free radicals and thus a decrease in control over polymerization. However, there is no indication that this is the case.

Elastin-Based Monomers. On the basis of the encouraging results from our model studies, we started the polymerization of VPGVG side-chain polymers under similar ATRP conditions.

First, VPGVG-based monomer **2** was prepared using a modified version of standard solid-phase peptide synthesis techniques.[37,38] The peptide was synthesized on a Wang resin using standard Fmoc procedures.[39] Then, while the peptide was still on the resin, the final valine was deprotected and the methacrylate moiety was introduced, using 2-isocyanatoethyl methacrylate (Scheme 3). The peptide was then cleaved from the resin, resulting in a free carboxylic acid, and the pure product was obtained by column chromatography.

Table 2 shows the ATRP results of monomer **2** using ATRP. Again, both EBIB and bifunctional PEG **3** were used as initiators, resulting in both the homo-poly-VPGVG, **D**, and the block copolymer, **E**, respectively. To prevent precipitation of the polymer during polymerization, the polymerization was performed at a lower concentration (0.25 M) and a lower temperature than had been used previously for monomer **1**. The semilogarithmic plots of monomer concentration vs time were linear for both initiators, indicating that this was again a well-controlled polymerization (Figures 3 and 4).

The GPC results for the polymerization of monomer **2** showed a narrow molecular weight distribution, albeit slightly higher than for monomer **1**.

The M_n values obtained by GPC for these polymers differ significantly from the theoretical M_n calculated from the initiator-to-monomer ratio. This can be attributed to the difference in the hydrodynamic volume of the polystyrene calibration standards and our polymers. However, for polymer **E** it is possible to calculate the degree of polymerization, and thus M_n, from the NMR data by comparing the integral value of the peak at 3.7 ppm due to the initiator moiety with other peaks from the peptide block of the polymer, for example, the urea group at 6.2 ppm. This resulted in a value for M_n of 10.0 kg/mol ($DP_n = 14$), which is closer to what we expected and shows that the M_n obtained by GPC is anomalous.

Table 2. Polymerization Results for Monomer 2

polymer	monomer	initiator	temp, °C	time, min	$[M]_0/[I]_0$	conv, %	M_n(GPC), kg/mol	M_n(NMR), kg/mol	$M_{n,th}$, kg/mol	PDI (GPC)
D	2	EBIB	36	240	10:1	80	53.0	N/A	5.8	1.25
E	2	**3**	35	120	10:1	84	60.0	10.0	6.1	1.24

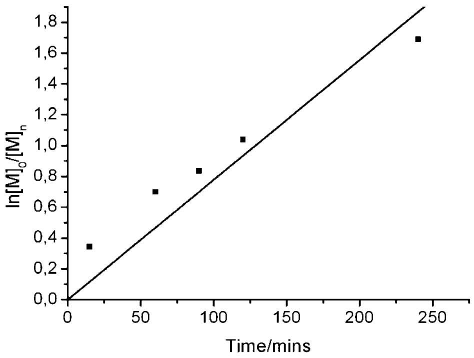


Figure 3. Linear plot of ln $[M]_0/[M]_n$ vs time for the polymerization of **2** with EBIB initiator. Polymerization was performed at 36 °C.

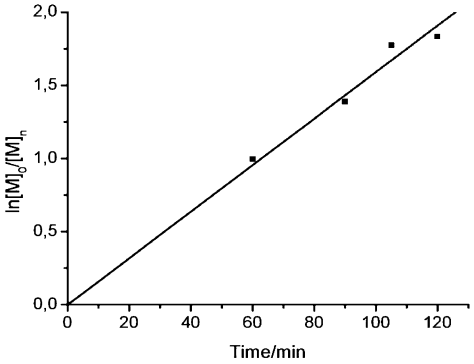


Figure 4. Linear plot of ln $[M]_0/[M]_n$ vs time for the polymerization of **2** with PEG initiator **3**. Polymerization was performed at 35 °C.

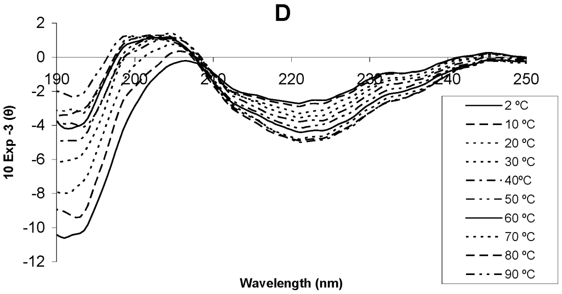


Figure 5. CD spectra of polymer **D**. CD spectra taken from 2 to 90 °C.

ATRP of these longer peptide monomers proceeds smoothly. The presence of many functional groups such as urea, amides, and even a free carboxylic acid end group does not interfere markedly with the polymerization. An explanation for this could be that the solvent, DMSO, breaks up complexes which these functional groups could form with the catalyst, allowing the polymerization to progress unimpeded.

Physical Properties of VPGVG-Based Polymers. CD spectroscopy was performed on polymers **D** and **E**, the results of which are depicted in Figures 5 and 6, to investigate the folding behavior of these two polymers at different temperatures.

The folding properties of single repeats of VPGVG have been extensively studied by Reiersen and Rees[28] using CD spectroscopy. They observed several changes in the spectra upon increasing temperature. Below the phase transition temperature the CD spectra have several characteristic features, a minimum at 200 nm, a maximum at 215 nm, and a shallow minimum at 220 nm. This is indicative of a random coil type of conformation. As the temperature is increased above the transition temperature, several changes occur. The minimum at 200 nm becomes shallower; at 220 nm the minimum deepens, while at 215 nm the maximum remains unchanged. This type of shift in ellipticity is known to be due to a change in structure from random coil to a type II β turn.[1]

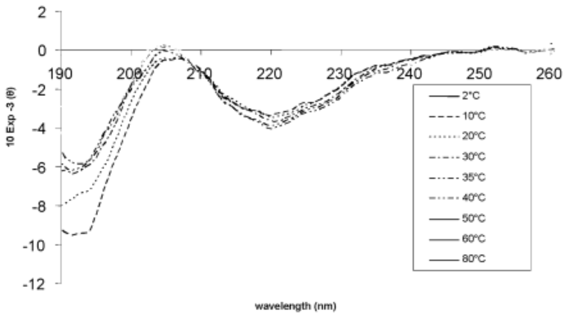


Figure 6. CD spectra of block copolymer **E**, CD spectra taken from 2 to 80 °C.

The spectrum of polymer **D**, the homopolymer, is very similar to that obtained by Reiersen and Rees (Figure 5), with only two minor differences. There is a slight shift in the wavelengths of the maxima and the minima, possibly due to the polymer backbone. The maximum at 200−210 nm is positive and not negative this time. However, the same characteristic increase at 195 nm and a decrease at 220 nm are observed.

The CD spectra of the triblock copolymer, **E**, at pH 1 (Figure 6) also shows this characteristic behavior, again with a slight shift to lower wavelength due to the polymer backbone, but this time the maximum at 200−210 nm is again negative. Therefore, it appears that the incorporation of the peptide into the backbone of the polymer has not affected its inverse phase transition behavior.

Turbidity Measurements. To determine whether the induced phase transition from random coil to β turn gives rise to aggregation, turbidity measurements were performed on polymers **D** and **E** at different temperatures and pH's.

Scattering was measured on an aqueous solution of each sample at 480 nm, while the temperature was increased from 2 to 80 °C. For the homopolymer, **D**, there was no increase in turbidity as the temperature was increased. For the block copolymer, **E**, the turbidity increased significantly with increasing temperature (Figure 7A). Also, a change in the transition temperature was observed for **E** when the pH was raised from 1 to 6 (Figure 7A,B). Furthermore, the heating and cooling cycle shown in Figure 7B demonstrates that this phase transition is reversible.

The transition temperature for polymer **E** at pH 1 is around 40 °C, which is slightly higher than observed

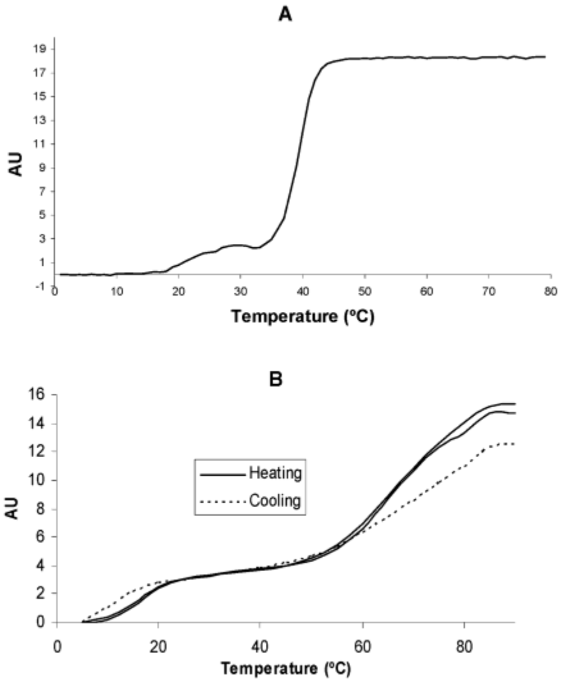


Figure 7. Turbidity measurements of polymer **E** taken at pH 1 (A) and pH 6 (B). Part B also shows a heating−cooling−heating cycle.

by Reiersen and Reese.[28] This can be attributed to the fact that the peptide is incorporated in a side chain of a polymer. Interestingly, this transition temperature can be increased to around 70 °C by raising the pH. This dependence on pH is probably due to the free acid end groups of the peptide side chain. Deprotonation of the carboxylic acid end groups at higher pH increases the hydrophilicity of the peptide side chain, making it necessary to go to a higher temperature to induce hydrophobic dehydration.

An interesting feature in both turbidity curves is the bump before the main transition. To investigate this feature, and to gain an insight into the aggregation that is occurring as the sample is heated, we performed light scattering measurements on polymer **E** (at pH 6). Preliminary results suggest that as we increase the temperature there is a shift from an open structure to a spherical structure of around 250 nm. This indicates that block copolymer assembly takes place, possibly as a result of the increased hydrophobicity of the peptide blocks, leading to an amphiphilic block copolymer structure. The formation of amphiphilic block copolymers also helps to explain why only the block copolymer **E** seems capable of a turbidity change. In the case of polymer **D,** there is no PEG chain present, and no pronounced amphiphilic behavior can therefore be introduced. A more detailed analysis of this temperature and pH-dependent aggregation behavior is underway and will be the subject of a further paper.

Conclusion

We have successfully shown that it is possible to prepare well-defined polymers from amino acid and peptide based monomers via ATRP. Both the homopolymer and the block copolymer could readily be synthesized from both monomers. ATRP in DMSO proves to be tolerant to a wide range of functional groups including free carboxylic acids.

The physical properties of the elastin-based polymers have been studied showing that the VPGVG side chains retain their characteristic transition from random coil to β-spiral behavior as shown by CD spectroscopy. Interestingly, when block copolymers were made from these materials, a turbidity change could be seen, which, according to light scattering results, was caused by block copolymer assembly. This can be explained by the formation upon heating of amphiphilic block copolymers due to the hydrophilic to hydrophobic transition of the peptide side chains. Our future work will be directed toward improving the understanding of the aggregation behavior of these block copolymers. This will be done through detailed light scattering studies combined with electron microscopy techniques. We also intend to substitute the fourth amino acid in VPGXG with different amino acids with the aim of developing a range of polymers suitable for a more accurate examination of the pH- and temperature-dependent aggregation phenomena of these elastin side-chain triblock copolymers.

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