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Selective Tuning of Elastin-like Polypeptide Properties via Methionine Oxidation

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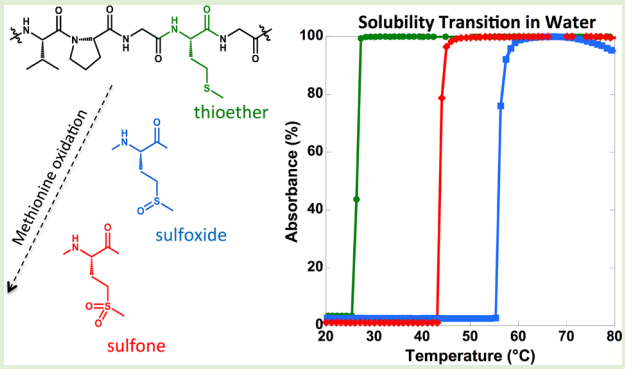
S *Supporting Information*

ABSTRACT: We have designed and prepared a recombinant elastin-like polypeptide (ELP) containing precisely positioned methionine residues, and performed the selective and complete oxidation of its methionine thioether groups to both sulfoxide and sulfone derivatives. Since these oxidation reactions substantially increase methionine residue polarity, they were found to be a useful means to precisely adjust the temperature responsive behavior of ELPs in aqueous solutions. In particular, lower critical solution temperatures were found to be elevated in oxidized sample solutions, but were not eliminated. These transition temperatures were found to be further tunable by the use of solvents containing different Hofmeister salts. Overall, the ability to selectively and fully oxidize methionine residues in ELPs proved to be a convenient postmodification strategy for tuning their transition temperatures in aqueous media.



INTRODUCTION

Along with their fundamental role as initiating molecules in protein synthesis, proteinaceous methionine residues also play an important role in biology as common substrates for redox reactions.[1] The thioether groups of methionine are readily oxidized into sulfoxides by a variety of reactive oxidative species (ROS), and can be reduced back to native methionine by endogenous methionine sulfoxide reductase (MSR) enzymes.[1−3] Deming and co-workers have taken advantage of these redox properties by developing enzyme-responsive vesicles from amphiphilic block copolypeptides containing poly(L-methionine sulfoxide) hydrophilic blocks.[4] In the case of recombinantly produced polypeptides, the use of redox chemistry on methionine residues has been reported in one instance for silks,[5,6] but not for elastin-like polypeptides (ELPs). Here, we report the design and preparation of ELPs containing precisely positioned methionine residues, and their controlled oxidation to sulfoxide and sulfone derivatives. We found that these oxidation reactions, since they substantially alter methionine residue polarity, are a convenient means to precisely adjust the temperature responsive behavior of ELPs in aqueous solutions.

Oxidation of methionine in naturally occurring proteins has been widely studied by the teams of Stadtman and Weissbach,[1,2,7,8] to better understand its mechanism and exact role in the biological activity of proteins. Methionine residues were found to act as ROS scavengers, thereby preventing irreversible oxidation of other amino acids such as those in active sites of proteins.[1,7] MSR enzymes can also reduce sulfoxide moieties to regenerate native methionine residues, thereby allowing the catalytic consumption of ROS.[8−10] In applications of methionine biochemistry to synthetic materials, the influence of reversible thioether group oxidations on the conformations and thermoresponsive properties of poly(L-methionine) and poly(*S*-alkyl-L-homocysteine) derivatives has been reported.[11−13] In these studies, reversible oxidation of thioether groups has been used as a conformational switch between α-helical and disordered conformations that depend on the oxidation state. However, in these examples, all of the oxidized sulfoxide-containing polypeptides were found to be fully water-soluble and did not exhibit any thermoresponsive properties in aqueous solution. Oxidation of thioether groups in synthetic polymers has also been used in order to

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modify their thermoresponsive properties.[14,15] By converting thioethers to sulfoxides, the solubility profile of polyether polyols has been modified,[15] while the cloud points of thioether containing oligoethylene glycol substituted poly(L-glutamates) have been shown to be readily tunable depending on the degree of polymerization, side-chain length, or degree of thioether oxidation.[14]

Recombinant ELPs are a special class of precision protein-like polymers with stimuli-responsive self-assembly properties, mainly developed for their potential use in biomedical applications.[16,17] ELPs are composed of repeating sequences of [-Val-Pro-Gly-*Xaa*-Gly-] pentapeptides, derived from the hydrophobic domain of tropoelastin, in which the guest residue *Xaa* can be any amino acid except proline.[18] ELPs with precisely controlled sequences and molecular weights can be produced by protein engineering techniques. In addition, ELPs with sufficient molecular weight and hydrophobicity have been efficiently produced at large scale using *Escherichia coli* as expression system.[19,20] The reversible aqueous solubility transition of ELPs, at a lower critical solution temperature (LCST), makes them particularly attractive candidates in biomaterials design. Soluble in aqueous media at low temperatures, ELPs phase separate as aggregates at an experimentally determined temperature called the inverse transition temperature (T_t).[21,22] The T_t can be tuned by modifying macromolecular parameters such as ELP molecular weight, the nature of the guest residue in the polypeptide repeats, and also by adjusting polypeptide concentration and composition of the aqueous medium.[23−25] In previous studies, we have shown that properties of ELPs can also be tuned by chemoselective modifications at the guest residue position, in particular by side chain alkylation of methionine-containing ELPs.[26] This current study was focused on examining the influence of oxidation on the temperature responsive properties of methionine-containing ELPs in aqueous solution. Oxidation of precisely positioned methionine residues in ELP chains was envisioned as a straightforward and convenient means to tune the hydrophilic character of these polypeptides. Such a method, that is simple and versatile, can be used to fine-tune the solubility behavior of genetically engineered biopolymers without requiring the design of new encoding genes or any additional molecular cloning steps. A specifically designed ELP containing periodically spaced methionine residues was produced recombinantly. This ELP was then selectively oxidized at low pH to form either sulfoxide- or sulfone-containing ELP derivatives whose properties in aqueous solution were then characterized and analyzed. The unique thermosensitive character of ELPs, combined with the selective oxidation of a few methionine residues precisely localized along the polymer chain, allows a large modulation of the *LCST* of the resultant polymer.

MATERIALS AND METHODS

Materials. LB medium was purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, FR). Bacto-tryptone, and yeast extract were purchased from Biokar Diagnostics (Allone, FR). Ampicillin was obtained from Eurobio (Courtaboeuf, FR). Glycerol and isopropyl β-D-thiogalactopyranoside (IPTG) were purchased from Euromedex (Souffelweyersheim, FR). Complete mini EDTA-free protease inhibitors were purchased from Roche Diagnostics (Mannheim, D). Hydrogen peroxide and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glacial acetic acid was obtained from Fisher Scientific (USA). Deionized water (18 MΩ·cm) was obtained by passing in-house deionized water through a Millipore Milli-Q Biocel A10 purification unit.

Construction of the Expression Vector. A synthetic gene corresponding to the MW[VPGVGVPGMG(VPGVG)$_2$]$_5$ sequence was designed. This gene sequence was selected according to *E. coli* codon usage while minimizing sequence repetition. The DNA fragment was extracted from the pEX-A plasmid by a double digestion with *Eco*RI and *Hind*III, and was ligated with the Quick ligation kit into similarly digested and dephosphorylated pUC19. After transformation into NEB 5a-F′Iq *E. coli* competent cells, a positive clone was selected by colony PCR with OneTaq hot start DNA polymerase and verified by DNA sequencing. The Sequence coding for MW[VPGVGVPGMG(VPGVG)$_2$]$_{10}$ was obtained by using a variation of the recursive directional ligation,[27] as previously described.[28] Cloning in the expression vector was as follows: The ELP sequence was extracted from pUC19- ELP-M-40 by a double digestion *Nde*I and *Bam*HI, and ligated with the Quick ligation kit into similarly digested and dephosphorylated pET-44a(+) plasmid. The different ligation products was then used to transform BLR(DE3)-competent cells for production. The sequence of the resultant plasmid was confirmed by DNA sequencing. The sequences of the ELP-M-40 gene and of the corresponding protein are shown below.

atgtgggttccaggcgttggagtgccaggcatgggcgtaccaggtgtgggagttccaggt
M W V P G V G V P G M G V P G V G V P G
gttggggtaccgggcgtcggagttcctgggatgggagttccgggagttggtgtgccgggt
V G V P G V G V P G M G V P G V G V P G
gtcggtgtgcctggggtgggtgttccaggtatgggggttccgggtgtcggcgttcccggc
V G V P G V G V P G M G V P G V G V P G
gttggtgttccaggcgtaggtgtgccgggaatgggggttccgggagttggtgtacctggc
V G V P G V G V P G M G V P G V G V P G
gtgggagtacctggagtcggcgtgcctggtatgggcgtgcctggcgtcggcgtacctggc
V G V P G V G V P G M G V P G V G V P G
gtaggtgttccaggcgttggagtgccaggcatgggcgtaccaggtgtgggagttccaggt
V G V P G V G V P G M G V P G V G V P G
gttggggtaccgggcgtcggagttcctgggatgggagttccgggagttggtgtgccgggt
V G V P G V G V P G M G V P G V G V P G
gtcggtgtgcctggggtgggtgttccaggtatgggggttccgggtgtcggcgttcccggc
V G V P G V G V P G M G V P G V G V P G
gttggtgttccaggcgtaggtgtgccgggaatgggggttccgggagttggtgtacctggc
V G V P G V G V P G M G V P G V G V P G
gtgggagtacctggagtcggcgtgcctggtatgggcgtgcctggcgtcggcgtacctggc
V G V P G V G V P G M G V P G V G V P G
gtaggttaa
V G -

Bioproduction of Recombinant ELP-M-40. A single bacterial colony was selected and cultured overnight at 37 °C on a rotary shaker at 200 rpm in 50 mL rich LB medium (1% bacto-tryptone, 0.5% NaCl, 1% yeast extract) containing 100 $\mu g \cdot mL^{-1}$ ampicillin. The seed culture was inoculated into 0.95 L rich LB medium supplemented with glycerol (1 $g \cdot L^{-1}$) and ampicillin (100 $\mu g \cdot mL^{-1}$), and bacteria were cultivated at 37 °C in 5 L flasks. When the optical density at 600 nm (OD_{600}) reached the value of 0.8, IPTG was added to a final concentration of 0.5 mM and the temperature of cultivation was decreased to 25 °C. Samples were then collected every hour for measurement of OD_{600} and SDS-PAGE analysis.

Isolation and Purification of Recombinant ELP-M-40. After 21 h IPTG-induction, the culture was harvested by centrifugation at 7500*g* and 4 °C for 15 min. The cell pellet was resuspended with 10 $mL \cdot g^{-1}$ (wet weight) phosphate buffer (PBS; NaCl 137 mM, KCl 2.7 mM, Na_2HPO_4 8 mM, KH_2PO_4 2 mM, pH 7.4) supplemented with one tablet/10 mL of Complete mini EDTA-free protease inhibitors. The mixture was incubated overnight at −80 °C and defrosted by incubation at 4 °C. Cell lysis was completed by sonication at 15 °C with sequential 4 s-pulses at 125 W separated by 9 s-resting time periods for a total duration of 15 min. Insoluble debris were removed by centrifugation at 16 000*g* and 4 °C for 30 min. The cleared lysate was thereafter subjected to three successive rounds of *Inverse Transition Cycling* (ITC).[29] ELP-M-40 polypeptide was precipitated with NaCl and retrieved by centrifugation at 16 000*g* and 25 °C for 30 min ("warm spin"). After removal of soluble proteins in the supernatant, ELP-M-40-containing pellet was resuspended in cold PBS. Insoluble, heat denatured proteins from *E. coli* were eliminated in the pellet after centrifugation at 16 000*g* and 4 °C for 15 min ("cold spin"), while the ELP-M-40 containing supernatant was subjected to

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an additional ITC round. Soluble ELP-M-40 was then extensively dialyzed against ultrapure water at 4 °C using 1 kDa MWCO-dialysis tubing (Spectra Por7) and lyophilized. The purity and average MW of ELP-M-40 were assessed by SDS-PAGE using 15% TRIS-glycine gels stained with colloidal blue G250.

Mass Spectrometry Analysis of ELP-M-40, ELP-M^{O}-40, and ELP-M^{O2}-40. Mass spectrometry analyses were performed on a MALDI-ToF-ToF (Ultraflex III, Bruker Daltonics, Bremen, Germany) equipped with a SmartBeam laser (Nd:YAG, 355 nm). Solutions of ELPs were prepared as follows: lyophilized ELPs were resuspended in water/acetonitrile (1/1, v/v) to obtain a final concentration lower than 100 μM. Samples were then mixed with the matrix solution of sinapinic acid prepared at the concentration of 10 mg/mL in water/acetonitrile (1/1, v/v). All MALDI-MS measurements were acquired in the linear positive mode and a mixture of standard proteins was used for external calibration in the suitable mass range (10−20 kDa).

NMR Spectrometry Analysis of ELP-M-40, ELP-M^{O}-40, and ELP-M^{O2}-40. NMR spectra were acquired in D_2O at 283 K (ELP-M-40) or 298 K (ELP-M^{O}-40 and ELP-M^{O2}-40) either on a Bruker AV400 NMR spectrometer (UCLA) operating at 400 mHz or a Bruker AV800 NMR spectrometer (NMR platform of Institut Européen de Chimie et Biologie) operating at 800 MHz. The solvent signal was used as the reference signal (δ = 4.70 ppm). Data processing was performed using Topspin software. Chemical shifts of amino acids are well-known in the literature.[30,31] We have identified the αCH protons of proline and the initial valine in each repeat (VPGXG) at 4.5−4.4 ppm (60.5−57 ppm for ^{13}C) and used these as reference for the calibration of integrations. Full assignment of ELP-M-40 (Figure 2A) was done with the help of the HSQC spectrum.

Oxidation of ELP-M-40 to Yield ELP-M^{O}-40. ELP-M-40 was dissolved in 30% H_2O_2 and 1% AcOH in water and stirred at 0 °C for 30 min.[4] After quenching with a few drops of 1 M sodium thiosulfate aqueous solution, the reaction mixture was transferred to a 2000 MWCO dialysis tubing and dialyzed against DI water for 48 h with water changes twice per day. The content of the dialysis bag was then lyophilized to yield ELP-M^{O}-40 (95% yield). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 4.5−4.4 (m, 80 H, αCH VPGXG and αCH Pro), 4.2−4.15 (d, 30 H, αCH VPGVG), 3.05−2.9 (m, 22 H, CH_2S Met), 2.76−2.73 (d, 33 H, SCH_3 Met), 1.05−0.9 (br m, 420 H, CH_3 Val). MS MALDI: Theoretical MW = 17 212.3 Da, experimental $[M + H]^+$ = 17 216.8 Da

Oxidation of ELP-M-40 to Yield ELP-M^{O2}-40. ELP-M-40 was dissolved in 30% H_2O_2 and 1% HCOOH in water and stirred at room temperature for 6 h.[4] After quenching with a few drops of 1 M sodium thiosulfate aqueous solution, the reaction mixture was transferred to a 2000 MWCO dialysis tubing and dialyzed against DI water for 48 h with water changes twice per day. The content of the dialysis bag was then lyophilized to yield ELP-M^{O2}-40 (94% yield). ^{1}H NMR (400 MHz, D_2O, 25 °C): δ 4.5−4.4 (m, 80 H, αCH VPGXG and αCH Pro), 4.2−4.15 (d, 30 H, αCH VPGVG), 3.4−3.35 (t, 22 H, CH_2S Met), 3.15−3.1 (br s, 33 H, SCH_3 Met), 1.05−0.9 (br m, 420 H, CH_3 Val). MS MALDI: Theoretical MW = 17 388.2 Da, Experimental $[M + H]^+$ = 17 383.1 Da.

Transition Temperature Measurements. Transition temperatures (T_t) were determined by measuring the turbidity at 600 nm between 20 and 80 °C at a 1 °C·min^{-1} scan rate at several concentrations in DI water for ELP-M-40 (25, 50, 100, 200, 750, 1000, and 1250 μM), ELP-M^{O}-40 (200, 300, 400, 500, 750, 1000, and 1250 μM) and ELP-M^{O2}-40 (25, 50, 75, 100, 200, 250, 500, 750, 1000, and 1250 μM). Additional measurements were carried out at a 1 mM concentration in $NaNO_3$ and NaI 0.1 M solutions. Data were collected on a Cary 100 UV−Vis spectrophotometer equipped with a multicell thermoelectric temperature controller from Agilent Technologies (Les Ulis, FR). The T_t is defined as the temperature corresponding to the point where the absorbance starts increasing on the absorbance versus temperature plot.

RESULTS AND DISCUSSION

ELP-M-40 (Figure 1A), containing a total of 40 ELP repeat units (n), was recombinantly produced in *E. coli* using protein engineering techniques. To create an ELP sequence with precise periodic placement of methionine residues, we constructed a gene that encodes the following amino acid sequence: MW[VPGVGVPGMG(VPGVG)$_2$]$_{10}$ (see SI). Methionine and valine were encoded at a 1:3 ratio, respectively, as guest residues in the VPGXG repeat units. Methionine residues were encoded as substrates for subsequent oxidation reactions, while valine residues were used as nonreactive hydrophobic residues to dilute the methionine content.[26] Additional methionine and tryptophan residues at the N-terminus of the ELP sequence were introduced for proper initiation of translation in *E. coli* and UV−vis detection purposes, respectively.

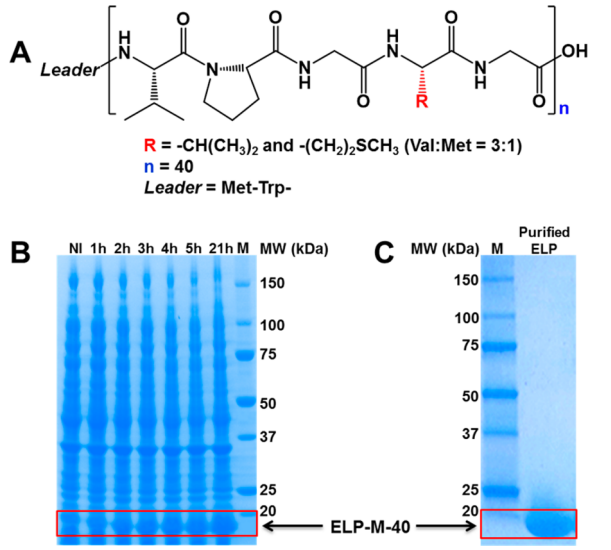

Figure 1. (A) Chemical structure of **ELP-M-40**. (B) Expression of recombinant **ELP-M-40** during bacterial fermentation as analyzed by SDS-PAGE. Lane NI = noninduced culture; lanes 1−21 h = time after IPTG induction in hours. M = molecular weight marker. (C) Purified **ELP-M-40** as analyzed by SDS-PAGE.

A clone expressing the **ELP-M-40** polypeptide was cultured in 5 L flasks. After induction of recombinant protein synthesis using isopropyl-β-D-thiogalactoside (IPTG), the culture was allowed to continue for 21 h (Figure 1B). Soluble proteins were then extracted from the cell lysates, and **ELP-M-40** was purified by inverse transition cycling (ITC),[29] extensively dialyzed against ultrapure water and lyophilized. The purity of **ELP-M-40** was assessed by SDS-PAGE (Figure 1C). The yield of the purified ELP was 150 mg/L culture. **ELP-M-40** was characterized by 1D and 2D NMR spectroscopy (Figure 2). Amino acids chemical shifts known from the literature[30,31] as well as the HSQC spectrum were used to fully assign I figured thall peaks in the ^{1}H NMR spectrum of **ELP-M-40**. For instance resonances for CH_2 groups bonded to sulfur and resonances for CH_3 groups bonded to sulfur were identified as the peaks centered at 2.56 and 2.08 ppm, respectively. In addition, the MALDI mass spectrum of **ELP-M-40** (Figure 3A) provided an experimental mass of 17,035.2 Da in excellent concordance with the expected mass (17,035.4 Da).

Samples of **ELP-M-40** were then subjected to different chemical oxidation conditions in order to obtain the sulfoxide-containing

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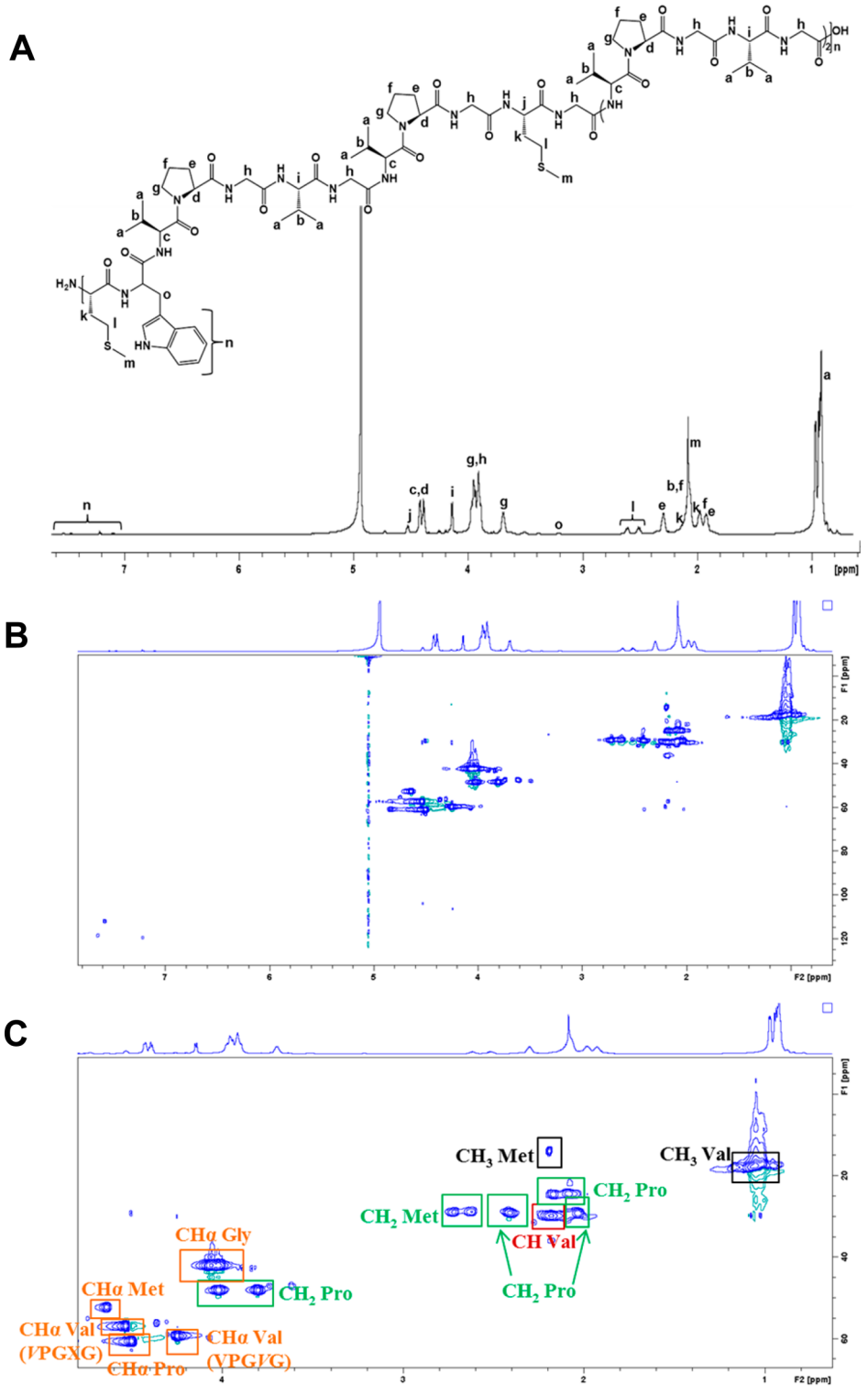
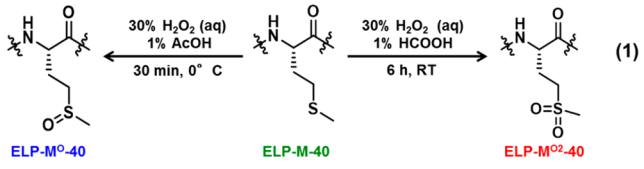


Figure 2. (A) ^{1}H NMR spectrum of **ELP-M-40** in D_2O at 10 °C. HSQC NMR spectra of **ELP-M-40** in D_2O at 10 °C, (B) full spectrum, (C) expanded region.

derivative (**ELP-M^{O}-40**) and the sulfone-containing derivative (**ELP-M^{O2}-40**) (eq 1).[4,32]



(1)

These derivatives were used to subsequently evaluate the effect of oxidation on the temperature-induced aggregation of the ELPs in aqueous solutions. **ELP-M^{O}-40** was obtained using mild oxidation conditions (hydrogen peroxide, acetic acid, 30 min, 0 °C), while stronger oxidizing conditions were necessary to obtain **ELP-M^{O2}-40** (hydrogen peroxide, formic acid, 6 h, 20 °C). Initial attempts to obtain **ELP-M^{O2}-40** using milder conditions (hydrogen peroxide, acetic acid, 3 h, 20 °C)

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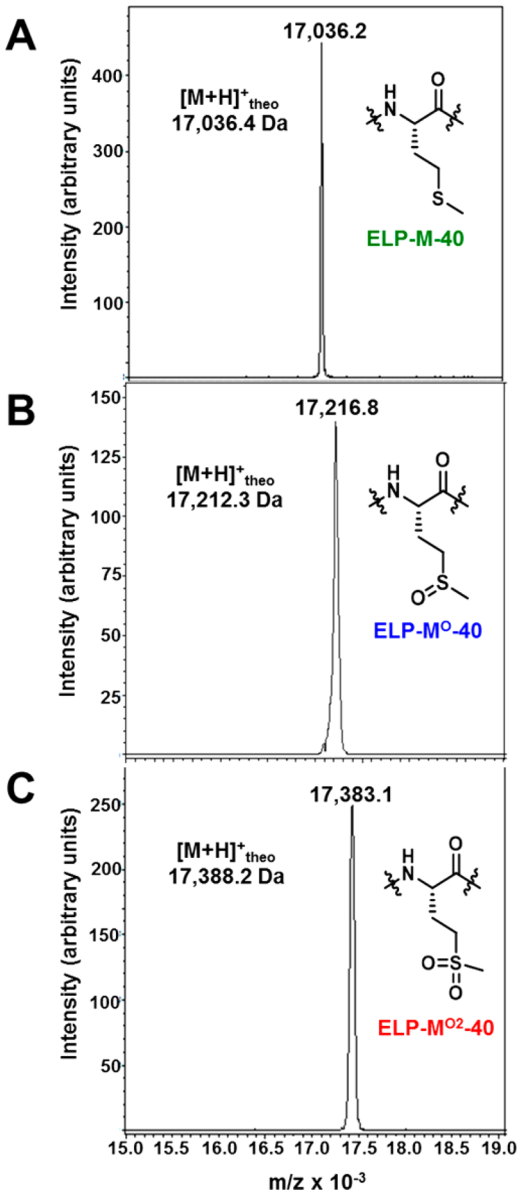

Figure 3. MALDI mass spectra of (A) **ELP-M-40**, (B) **ELP-M^{O}-40**, (C) **ELP-M^{O2}-40**. $[M + H]^{+}_{theo}$ = theoretical mass of monocharged species for each sample.

resulted in only 14% conversion to sulfone groups, with the remaining 86% thioether groups converted into sulfoxides. Acetic acid was therefore replaced for formic acid and the reaction time was increased to 6 h to fully oxidize all 11 thioether groups in **ELP-M^{O2}-40** into sulfones.

After isolation, **ELP-M^{O}-40** and **ELP-M^{O2}-40** were both subjected to MALDI mass spectrometry and ^{1}H NMR spectrometry analyses to confirm their molar masses and chemical structures, respectively. The MALDI mass spectra (Figure 3B,C) provided experimental masses of 17 215.8 Da for **ELP-M^{O}-40** and 17 382.1 Da for **ELP-M^{O2}-40** in concordance with the expected theoretical values. MS analysis also confirmed that monodispersity of **ELP-M-40** derivatives was retained after the oxidation reactions.

The complete conversion of all 11 thioether groups of **ELP-M-40** into sulfoxide and sulfone groups was also confirmed by ^{1}H NMR (Figure 4). The resonance for the protons of the methionine methyl group at δ = 2.12 ppm was shifted to 2.75 ppm in **ELP-M^{O}-40** (Figure 4B) and to 3.1 ppm in

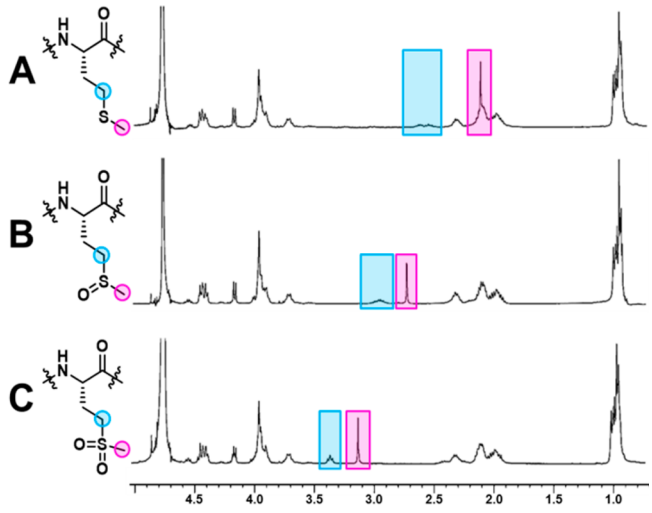

Figure 4. ^{1}H NMR spectra of (A) **ELP-M-40**, (B) **ELP-M^{O}-40**, (C) **ELP-M^{O2}-40**. Blue boxes highlight resonances for CH_2 groups bonded to sulfur, and red boxes highlight resonances for CH_3 groups bonded to sulfur.

ELP-M^{O2}-40 (Figure 4C), while the resonance of the methylene protons bonded to sulfur in methionine was shifted from 2.6 ppm in **ELP-M-40** to 3.0 ppm in **ELP-M^{O}-40** and to 3.4 ppm in **ELP-M^{O2}-40**. These shifts can be explained by the deshielding effect of the oxygen atom(s) present in **ELP-M^{O}-40** and **ELP-M^{O2}-40** compared to **ELP-M-40**, and are also consistent with NMR data on oxidized derivatives of poly(L-methionine).[4] Integrations confirming full conversions of **ELP-M-40** to sulfoxide and sulfone derivatives were accomplished by comparing the methyl and methylene resonances of all samples described above to the unchanging resonances centered at 4.45 ppm, which correspond to the αCH protons of proline and the initial valine in each repeat (*VP*GXG) (see SI, Figure S1).

The effect of methionine oxidation on temperature responsiveness of the ELP solutions was evaluated by carrying out cloud point measurements in DI water at different concentrations to determine T_t values (Figures 5A,B). Depending on sample concentration (25 to 1,250 μM), the *T*t of **ELP-M-40** ranged from 25 to 32 °C. After oxidation to give **ELP-M^{O}-40**, T_t's were shifted to higher values ranging from 54 to 75 °C depending on sample concentration (200 to 1,250 μM), and no measurable T_t could be determined below 200 μM concentration (i.e., T_t > 80 °C). The lack of T_t for low concentrations of **ELP-M^{O}-40** was likely due to increased water-solubility afforded by the sulfoxide groups as compared to thioether groups.[4,33,34] With T_t values greater than those of **ELP-M-40**, but lower than those of **ELP-M^{O}-40** at identical concentrations, the sulfone derivative **ELP-M^{O2}-40** (T_t = 42 to 69 °C, concentration = 25 to 1,250 μM) displayed intermediate temperature-dependent solubility compared to the other ELPs. Although sulfone groups have greater dipole moments compared to sulfoxides, the large dipoles of the sulfone groups can lead to sulfone−sulfone and sulfone−protein interactions, that can lead to decreased water solubility.[4,35]

The T_t values of the 3 ELPs were plotted as functions of concentration (Figure 5A) and the data were fitted using the empirical equation established by Chilkoti et al. (eq 2).[23]

$$T_t = T_{t,c} + \frac{k}{L}\ln\left(\frac{C_C}{C}\right) \quad (2)$$

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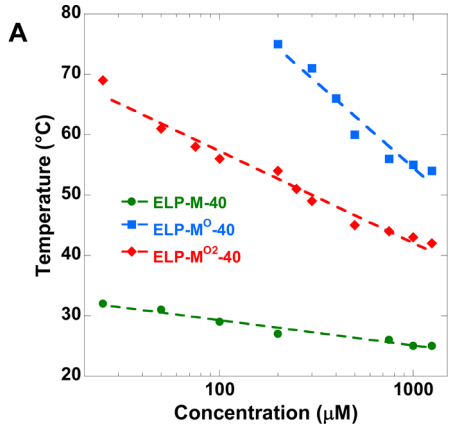

C

	Transition temperature (°C)		
	DI water	0.1 M $NaNO_3$	0.1 M NaI
ELP-M-40	25	26 (+1)[a]	26 (+1)[a]
ELP-M^{O}-40	55	54 (-1)[a]	59 (+4)[a]
ELP-M^{O2}-40	43	38 (-5)[a]	44 (+1)[a]

Figure 5. (A) T_t values of **ELP-M-40**, **ELP-M^{O}-40**, and **ELP-M^{O2}-40** plotted as functions of sample concentration in DI water; data fitted using eq 2. (B) Absorbance of solutions of **ELP-M-40**, **ELP-M^{O}-40**, and **ELP-M^{O2}-40** (1.0 mM in DI water) as functions of temperature. (C) Table showing T_t values of ELP samples at 1.0 mM in DI water or 0.1 M Hofmeister salt solutions. [a]Values in parentheses are the differences compared to the corresponding T_t in DI water.

This equation provided a satisfactory fits of all three data sets enabling accurate estimations of the *T*t of **ELP-M-40** as well as the oxidized **ELP-M^{O}-40** and **ELP-M^{O2}-40** at specific concentrations. We also noticed as hydrophilicity of the ELP increased, the slope of the fit became steeper. This result is comparable with observations by Chilkoti et al. regarding the increase of the slope with decreasing ELP chain length, which also correlates with increased hydrophilicity.

The impact of two different salts of the Hofmeister series on the thermoresponsive properties of the ELPs in aqueous solution was also studied (Figure 5C, see SI Figure S2).[36] Knowing that anions tend to have more influence on thermoresponsive properties of polymer solutions than cations,[37] we studied the effects of two anions, namely, NO_3^- and I^- (Na^+ counterions), on ELP solutions. The trends we noticed were similar to those observed with other biological and synthetic polymers.[11,12,37−40] In the presence of either 0.1 M NO_3^- or I^- salt, **ELP-M-40** was found to have only a minimal change in its T_t. In contrast, the T_t values of oxidized derivatives showed larger changes in the presence of salts. In particular, the T_t of **ELP-M^{O}-40** increased in the presence of I^-, while the T_t of **ELP-M^{O2}-40** decreased in the presence of NO_3^-. Although these two anions are both commonly considered to be chaotropic, they probably do not affect the T_t values of oxidized **ELP-M-40** derivatives in a similar manner. This can be explained by the interactions involved in the phase transition process where poorly hydrated anions, such as NO_3^- or I^-, can influence the phase transition by modifying the interfacial tension at the polymer/water boundary and by binding to hydrophobic surfaces of the biomacromolecules.[38]

CONCLUSION

The design and preparation of a new methionine-containing recombinant elastin-like polypeptide **ELP-M-40**, along with its oxidized sulfoxide **ELP-M^{O}-40** and sulfone **ELP-M^{O2}-40** derivatives, were reported. Complete and selective oxidization of all methionine thioether groups in **ELP-M-40** gave a total of three different ELP variants with significantly different T_t values in water. These T_t values were found to be further tunable by use of solvents containing different Hofmeister salts. Unlike homopolymers containing high densities of thioether groups where oxidation eliminates LCST behavior,[4,11,12] the precise spacing of methionine residues in the ELPs reported here allowed for the controlled tuning and retention of LCSTs in oxidized samples. Ultimately, the ability to selectively and fully oxidize methionine residues in ELPs proved to be a convenient postmodification strategy for tuning their transition temperatures in aqueous media, without requiring design of new encoding genes or additional molecular cloning steps. This combination of thermosensitive ELPs, selective oxidation of methionine residues to different states, and anion exchange capability can be viewed as a straightforward and versatile approach to predictably tune the LCST behavior of polymer chains.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.6b01696.

Experimental procedures and spectral data (PDF)

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Notes

The authors declare no competing financial interest.

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