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Multimodal switching of conformation and solubility in homocysteine derived polypeptides

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ABSTRACT: We report the design and synthesis of poly(S-alkyl-L-homocysteine)s, which were found to be a new class of readily prepared, multiresponsive polymers that possess the unprecedented ability to respond in different ways to different stimuli, either through a change in chain conformation or in water solubility. The responsive properties of these materials are also effected under mild conditions and are completely reversible for all pathways. The key components of these polymers are the incorporation of water solubilizing alkyl functional groups that are integrated with precisely positioned, multiresponsive thioether linkages. This promising system allows multimodal switching of polypeptide properties to obtain desirable features, such as coupled responses to multiple external inputs.

Stimuli responsive polymers are promising materials that aspire to mimic responsive and adaptive biological systems, such as proteins and protein complexes that can react to changes in pH, oxidation, and other stimuli.¹ Polypeptides offer advantages as protein mimics since they adopt ordered chain conformations (e.g. α -helices) that can also respond to stimuli and influence properties. Responsive polypeptides have practical applications in devices and therapeutic delivery, such as thermoresponsive hydrogel formation for cell scaffold formation *in vivo*,² or oxidation responsive nanocarrier disruption for triggered drug release.³ However, synthetic polypeptides are often only able to respond to a single stimulus, and sometimes only in an irreversible manner.⁴ The development of polypeptides that can reversibly respond under mild conditions to multiple stimuli, and in a distinct, predictable manner for each stimulus, is an important step toward realization of more sophisticated and useful materials.¹ For example, a polypeptide that undergoes a chemically induced conformational change that in turn switches on thermoresponsive behavior would be valuable for creation of triggered-responsive systems. Addressing this challenge, we have developed polypeptides capable of responding to

multiple external stimuli (chemical, redox, temperature) by fully reversible changes in their chain conformation or aqueous solubility (Figure 1). This unique system allows multimodal switching of polypeptide properties to obtain desirable features, such as coupled responses to multiple external inputs.

Figure 1. Schematic drawing showing multimodal environmentally responsive behavior of poly(homocysteine) derivatives, which are represented as coils and α -helices that are hydrophobic (red) or hydrophilic (blue). Red = reduction; Ox = oxidation; Alk = alkylation; DeAlk = dealkylation; T = temperature; red arrows = reaction above 40 °C; blue arrows = reaction below 35 °C.

Water soluble polypeptides are known that are responsive to temperature,⁵ oxidation,⁶ pH,⁷ light,⁸ or sugar binding,⁹ where the responsive component typically undergoes a transition between hydrophobic and hydrophilic states, or a change in chain conformation.⁴ While most of these materials respond only to a single type of stimulus, Hammond’s lab has reported remarkable copolypeptides that are able to respond to both temperature and pH.¹⁰ While these materials are sensitive to two different stimuli, the polypeptide chains have only a single response to both, a change in solubility. In effort to develop polypeptides that are able to function as true multiresponsive materials, i.e. able to react differently to different stimuli, we have explored the chemistry and properties of polypeptides containing side-chain thioether functional groups. We previously prepared thioether containing glycopolypeptides based on L-cysteine residues, which were found to undergo α -helix to coil transitions upon oxidation, while remaining water soluble.⁶ One limitation of this system was the irreversibility of this transition, since the thioether groups had to be fully oxidized to sulfones. Analogous α -helical glycopolypeptides based on L-homocysteine residues behaved diff

Figure 2. A) Molecular weight (M_n , $\blacklozenge$) and polydispersity index (M_w/M_n , $\blacksquare$) of poly(α -gal- C^H), **5**, as functions of monomer to initiator ratio ($[M]/[I]$) using $(PMe_3)_4Co$ initiator in THF at 20 °C; (B-D) Circular dichroism spectra, 0.1 mg/mL in DI water, 20 °C, molar ellipticity is reported in $\text{deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}$. B) Reversible oxidation of **5** (average degree of polymerization = 145); C) reversible alkylation of **5**; D) irreversible alkylation of **5**. * = alkylated or oxidized polypeptide after reduction using thioglycolic acid for **5o**, or 2-mercaptopyridine for **5Bn** and **5Me**.

2-mercaptopyridine, as described previously for poly(L-methionine) derivatives.¹² Alkylation of **5** with methyl iodide also yielded a conformationally disordered, water soluble polysulfonium, **5Me**, yet this is a one-way switch since this alkylation was irreversible (Figure 2D).¹² **5** and its derivatives all remained water soluble at elevated temperature (80 °C).

Reaction scheme showing the synthesis of poly(8) and its derivatives:

Reaction 1: $\text{Br}(\text{CH}_2)_x\text{CH}_3 + \text{HOOC}(\text{CH}_2)_4\text{SH} \xrightarrow{\text{a,b}}$ **7** (where $x = 2, 4$)

Reaction 2: **7** $\xrightarrow{\text{c}}$ **8** (where $x = 2, 4$)

Chemical structures of **7** and **8** are shown, including the side chain structure of **8** with n units.

Figure 10 displays the M_n and M_w/M_n of the polymers as a function of monomer concentration $[M]/[I]$ (C) and the molar ellipticity of the polymers as a function of wavelength (D).

(C) Plot of $M_n \times 10^{-3}/\epsilon$ versus $[M]/[I]$. The data points show a linear increase in M_n with increasing $[M]/[I]$.

(D) Plot of Molar Ellipticity ($\times 10^3$) versus Wavelength (nm) for polymers **8b**, **8Bn**, **8Me**, and their corresponding Me^+ derivatives. The curves show characteristic peaks and troughs, indicating the presence of specific secondary structures.

Figure 3. A) Molecular weight (M_n , $\blacklozenge$) and polydispersity index (M_w/M_n , $\blacksquare$) of poly(EG_4-C^H), **8b**, as functions of monomer to initiator ratio ($[M]/[I]$) using $(PMe_3)_4Co$ in THF at 20 °C; (B-D) Circular dichroism spectra, 0.1 mg/mL in DI water, 20 °C, molar ellipticity is reported in $\text{deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}$. B) reversible oxidation of poly(EG_4-C^H)₁₅₀, **8b**; C) reversible alkylation of **8b**; D) irreversible alkylation of **8b**. * = alkylated or oxidized polypeptide after reduction using thioglycolic acid for **8o**, or 2-mercaptopyridine for **8Bn** and **8Me**.

When oxidized or alkylated, **8a** was found to be water soluble and show reversible changes in chain conformation (see SI Figures S2, S3, and S4), yet these derivatives were

found to possess no thermoresponsive properties in water (see SI Figure S5). With excellent water solubility similar to glycopolypeptide **5**, **8b** was found to adopt a greater than 95% α -helical conformation in DI water at 20 °C (Figure 3B).¹⁵ Oxidation of **8b** to give **8o**, or alkylation with a benzylic halide to give **8Bn**, were also both found to reversibly switch the chain conformations from α -helices to coils without affecting solubility (Figure 3B and 3C). Irreversible alkylation of **8b** with methyl iodide also yielded a conformationally disordered, water soluble polysulfonium, **8Me** (Figure 3D). These results show that the water solubilizing EG repeats in **8b** are viable substitutes for the sugar residues in **5**, as both provide water solubility and confer similar responsiveness to oxidation and alkylation. Based on comparison to other EG containing polypeptides,⁵ **8b** was also anticipated to display thermoresponsive behavior in water.

Figure 4. A) Influence of temperature on light transmittance (500 nm) through a sample of aqueous poly(EG₄-C^H)₁₅₀, **8b**. Solid red line = heating; dashed blue line = cooling; 1 °C min⁻¹. B) Reversible change in optical transmittance of aqueous **8b** when temperature was alternated between 30 °C (high transmittance) and 45 °C (low transmittance); 5 min per each heating/cooling cycle. C) Cloud point temperatures of **8b** measured in different Hofmeister salts (Na⁺ counterion) at concentrations up to 1.0 M. All polypeptides were prepared at 3 mg/mL.

Upon heating aqueous samples of **8b**, sharp transitions from clear solutions to opaque suspensions were observed, indicative of the presence of a lower critical solution temperature for this polypeptide (Figure 4A).¹⁷ These transitions were completely reversible, and could be repeated multiple times with no observable persistent precipitation or other changes to the sample (Figure 4B). The chain conformations of **8b** were found to remain highly α -helical when samples were heated well above the cloud points, indicating that a change in chain conformation was not responsible for the observed thermoresponsive behavior (see SI Figures S6, S7, and S8). The more hydrophilic derivatives **8o** and **8Bn** were not thermoresponsive, and remained soluble in water or PBS buffer at elevated temperature (80 °C) (see SI Figure S9). The observed cloud point temperatures for **8b** were higher (ca. 50 °C) at lower molecular weights, but were found to plateau at ca. 40 °C for chains greater than 50 residues long (see SI Figure S10). In PBS buffer, the cloud point temperature of **8b** decreased from 40 °C to 37 °C due to slight salting out of the polymer (see SI Figure S11). To study this effect in more detail, we examined solutions of **8b** in the presence of different Hofmeister anions, since anions are known to affect thermoresponsive properties of polymers more than cations (Figure 4C).¹⁸ The effects of different salt concentrations on the cloud point temperatures of polymer **8b** followed trends similar to those seen with other thermoresponsive polymers, and allow some tuning of the transition temperature.^{18,19} Similar to other thermoresponsive polypeptides,^{5,10} it also is likely that the cloud point temperature could be varied by copolymerization of EG₄-C^H NCA with EG_x-C^H NCAs containing different EG repeat lengths. Overall, polypeptide **8b** was found to possess reversible thermoresponsive behavior in water by undergoing a transition between hydrophobic and hydrophilic states, with no change in chain conformation.

Poly(S-alkyl-L-homocysteine)s, as exemplified by polypeptide **8b**, are a new class of readily prepared, multiresponsive polymers that possess the unprecedented ability to respond differently to different stimuli, either through a change in conformation or in water solubility. Their responsive properties are also effected under mild conditions and are fully reversible for all pathways. The completely decoupled multiresponsive nature of these polypeptides makes them particularly attractive as components in molecular devices or nanoscale assemblies capable of sequential, or triggered, responses to different stimuli, akin to switches capable of performing Boolean-like operations.²⁰ For example, water soluble polypeptide **8Bn** will switch to a water insoluble state only when presented with a dealkylation trigger AND an elevated temperature trigger. Such triggers may also be provided biologically, and studies to use these polymers under biological conditions are underway.^{11,12} Overall, the potential for tunability of poly(S-alkyl-L-homo-cysteine)s, combined with their exceptional multiresponsive properties, make them promising candidates for a broad range of stimuli responsive material challenges.

ASSOCIATED CONTENT

Supporting Information. Experimental procedures and spectral data for all new compounds, as well as additional polymerization data, M_n vs. [M]/[I] plots, temperature response studies, and CD spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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