JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Multimodal Switching of Conformation and Solubility in Homocysteine Derived Polypeptides

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Supporting Information

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.

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 differently and remained α-helical as sulfones.[6]

In related studies on oxidation of α-helical poly(L-methionine), we observed that the fully oxidized poly(L-methionine sulfone) also remains α-helical,[11] similar to the glycosylated poly(L-homocysteine)s.[6] However, limited oxidation to give poly(L-methionine sulfoxide) yielded a highly water-soluble disordered coil that can be reduced back to the

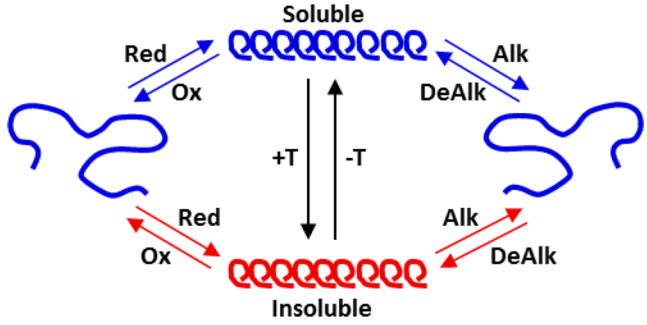


Figure 1. Schematic drawing showing multimodal environmentally responsive behavior of poly(homocysteine) derivatives, which are represented as coils and α-helices that are hydrophobic (red) or hydrophilic (blue). Red = reduction; Ox = oxidation; Alk = alkylation; DeAlk = dealkylation; T = temperature;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

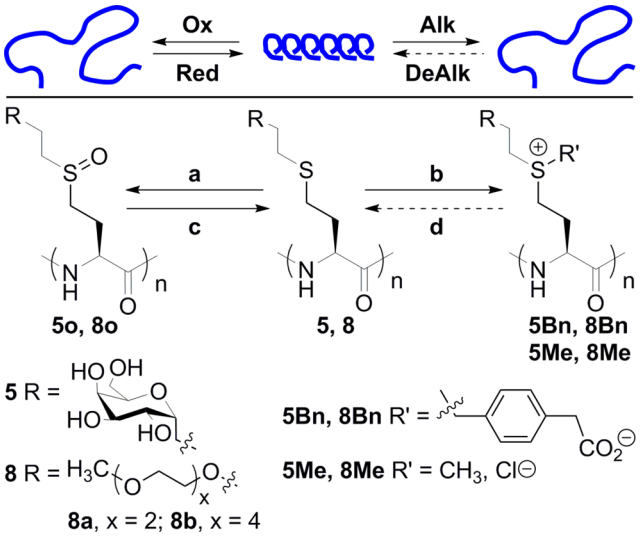
Received: January 13, 2014
Published: April 2, 2014

   dx.doi.org/10.1021/ja500372u | *J. Am. Chem. Soc.* 2014, 136, 5547−5550

parent polymer under mild conditions.[11] In a separate study, we found that poly(L-methionine) can also be alkylated in high yield to give water-soluble disordered coils in a fully reversible process.[12] While poly(L-methionine) readily undergoes these redox and chemically induced reversible α-helix to coil transitions, the poor water solubility of poly(L-methionine) limits its utility as a stimuli responsive polypeptide. Here, we sought to utilize the favorable molecular features found in methionine to design and prepare analogous *S*-alkyl-L-homocysteine based polypeptides, where water solubilizing alkyl functional groups are connected to the multiresponsive thioether linkages. The goal was to create water-soluble, multistimuli responsive polypeptides capable of responding reversibly and distinctly to temperature, alkylation, and oxidation (Figure 1).

We began by re-examining the glycopolypeptide, poly(α-gal-C^H), **5** (Scheme 1, see Scheme S1), which shares the same core

Scheme 1. Conformational Switching of Poly(homocysteine) Derivatives[a]



[a](a) H_2O_2, AcOH, H_2O; (b) 4-bromomethyl phenyl acetic acid or methyl iodide, H_2O; dialysis with NaCl; (c) thioglycolic acid, H_2O; (d) 2-mercaptopyridine, H_2O. Pathway d occurs for **5Bn** and **8Bn**, but not for **5Me** and **8Me**.

structure as poly(methionine) yet is water soluble. Like other glycosylated α-amino acid-*N*-carboxyanhydride (NCA) monomers we have reported,[6,13] α-gal-C^H NCA was found to polymerize efficiently using $(PMe_3)_4Co$ initiator in THF at room temperature (see Table S1).[14] Variation of α-gal-C^H NCA to initiator ratios gave glycopolypeptides whose lengths increased linearly with stoichiometry and which possessed narrow chain length distributions (M_w/M_n) (Figure 2A). After removal of protecting groups, water-soluble, α-helical **5** was obtained with reproducible and precisely controlled chain lengths up to over 300 residues long.

To study the ability of **5** to respond to stimuli, samples were subjected to either chemical alkylation[12] or mild oxidation[11] (Scheme 1). Unmodified **5** gave a circular dichroism (CD) spectrum with characteristic minima at 208 and 222 nm, indicating a greater than 95% α-helical conformation in DI water at 20 °C (Figure 2B).[15] Upon oxidation with 1% H_2O_2 to the corresponding sulfoxide, **5o**, the polypeptide remained water soluble yet its CD spectrum showed complete loss of the

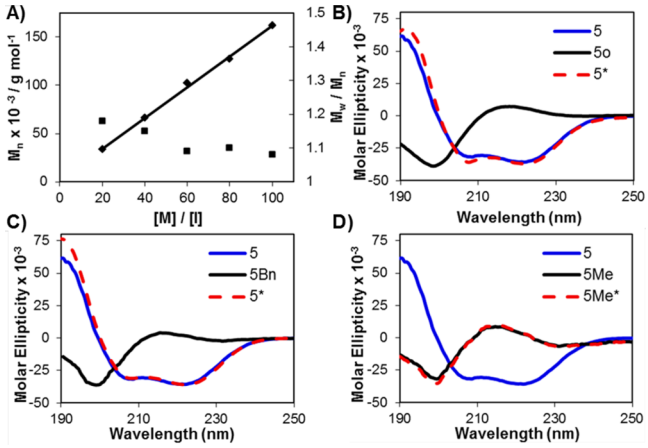


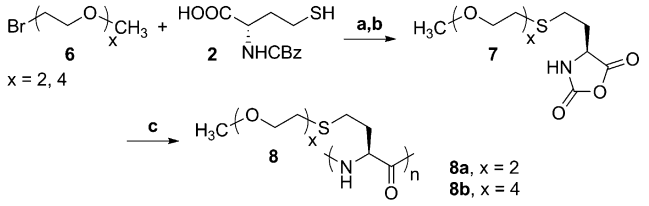
Figure 2. (A) Molecular weight (M_n, ♦) and polydispersity index (M_w/M_n, ■) of poly(α-gal-C^H), **5**, as functions of monomer to initiator ratio ([M]/[I]) using $(PMe_3)_4Co$ initiator in THF at 20 °C; (B–D) CD spectra, 0.1 mg/mL in DI water, 20 °C, molar ellipticity is reported in deg·cm^2·dmol^{-1}. (B) Reversible oxidization 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**.

α-helical signatures and instead was indicative of a disordered coil conformation (Figure 2B).[15] This conformational switch was completely reversible, since reduction of **5o** with thioglycolic acid regenerated unmodified, α-helical **5** (Figure 2B). Alkylation of **5** gave similar results, yet its chain conformation could be switched either reversibly or permanently, depending on the choice of alkylating agent. Alkylation of **5** with a benzylic halide yielded water-soluble polysulfonium **5Bn**, which adopted a disordered coil conformation (Figure 2C). This reaction was reversed to regenerate **5** by quantitative dealkylation using 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).

These initial results showed that the combination of optimally positioned reactive thioether groups and water solubilizing sugar functionality found in **5** gave a soluble polypeptide capable of reversibly switching between α-helix and coil conformations in response to different stimuli. To test the generality of this design and to add a thermoresponsive element, we prepared new *S*-alkyl-L-homocysteine based polypeptides containing water solubilizing ethylene glycol (EG) repeats. Since it is known that attachment of short EG segments onto polymer, and polypeptide, side-chains can impart thermoresponsive behavior in water,[5,10] we prepared alkylated poly(L-homocysteine) derivatives containing either 2 or 4 EG repeats (poly(EG_2-C^H), **8a**, and poly(EG_4-C^H), **8b**, respectively) (Scheme 2). Similar to α-gal-C^H NCA, EG_2-C^H NCA and EG_4-C^H NCA, obtained in high purity after chromatography,[16] were found to polymerize efficiently using $(PMe_3)_4Co$ initiator in THF at room temperature (see Tables S2 and S3).[14] Variation of NCA to initiator ratios gave polypeptides whose lengths increased linearly with stoichiometry and which possessed narrow chain length distributions (M_w/M_n) (Figure 3A, see Figure S1). While polypeptide **8b**

dx.doi.org/10.1021/ja500372u | J. Am. Chem. Soc. 2014, 136, 5547–5550

Scheme 2. Synthesis of Poly(EG_x-C^H)[a]



[a](a) 2M NaOH, H_2O (95-98% yield); (b) Cl_2CHOMe, DCM, 50 °C (64-69% yield); (c) $(PMe_3)_4Co$, THF (90-99% yield).

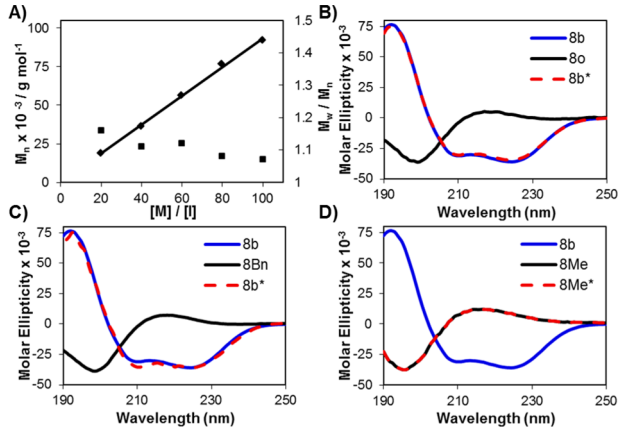


Figure 3. (A) Molecular weight (M_n, ♦) and polydispersity index (M_w/M_n, ■) of poly(EG_4-C^H), **8b**, as functions of monomer to initiator ratio ([M]/[I]) using $(PMe_3)_4Co$ in THF at 20 °C; (B–D) CD spectra, 0.1 mg/mL in DI water, 20 °C, molar ellipticity is reported in deg·cm^2·$dmol^{-1}$. (B) reversible oxidization 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**.

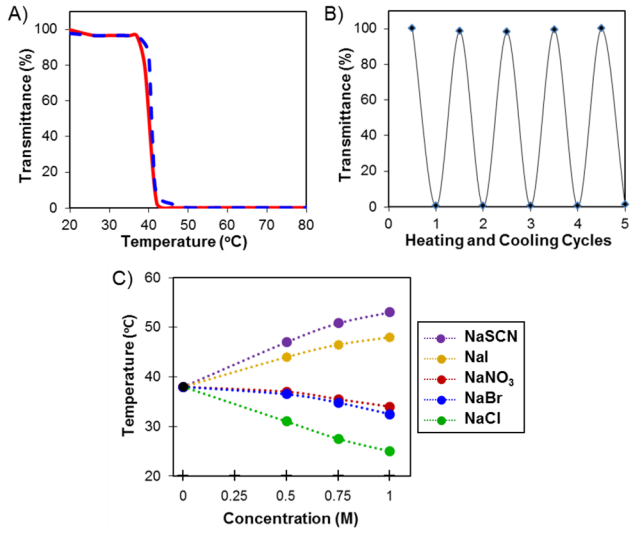


Figure 4. (A) Influence of temperature on light transmittance (500 nm) through a sample of aqueous poly(EG_4-C^H)$_{150}$, **8b**. Solid red line = heating; dashed blue line = cooling; 1 °C min^{-1}. (B) Reversible change in optical transmittance of aqueous **8b** when temperature was alternated between 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.

was very soluble in water at all chain lengths tested, polypeptide **8a** was only fully water soluble when <25 residues long and hence not a good candidate as a thermoresponsive polymer.

When oxidized or alkylated, **8a** was found to be water soluble and show reversible changes in chain conformation (see Figures S2–S4), yet these derivitatives were found to possess no thermoresponsive properties in water (see Figure S5). With excellent water solubility similar to glycopolypeptide **5**, **8b** was found to adopt a >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,C). 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.

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 Figures S6–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 Figure S9). The observed cloud point temperatures for **8b** were higher (~50 °C) at lower molecular weights but were found to plateau at ~40 °C for chains greater than 50 residues long (see Figure S10). In PBS buffer, the cloud point temperature of **8b** decreased from 40 to 37 °C due to slight salting out of the polymer (see 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 EG_4-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

dx.doi.org/10.1021/ja500372u | J. Am. Chem. Soc. 2014, 136, 5547–5550

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-homocysteine)s, combined with their exceptional multiresponsive properties, makes 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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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the IUPAC Transnational Call in Polymer Chemistry and the NSF under MSN 1057970.

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