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Glycopolypeptides with a Redox-Triggered Helix-to-Coil Transition

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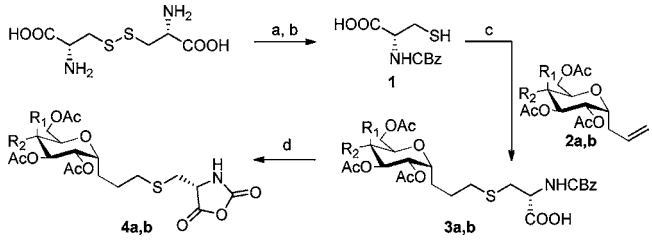
Supporting Information

ABSTRACT: Conformation-switchable glycopolypeptides were prepared by the living polymerization of glycosylated L-cysteine-*N*-carboxyanhydride (glyco-C NCA) monomers. These new monomers were prepared in high yield by coupling of alkene-terminated C-linked glycosides of D-galactose or D-glucose to L-cysteine using thiol−ene "click" chemistry, followed by their conversion to the corresponding glyco-C NCAs. The resulting glycopolypeptides were found to be water-soluble and α-helical in solution. Aqueous oxidation of the side-chain thioether linkages in these polymers to sulfone groups resulted in disruption of the α-helical conformations without loss of water solubility. The ability to switch chain conformation and remain water-soluble is unprecedented in synthetic polymers, and allows new capabilities to control presentation of sugar functionality in subtly different contexts.

The development of stimuli-responsive polymers has received much recent interest. These materials are promising since they aim to mimic adaptive biological systems, trigger release of therapeutics, or amplify signals in biosensors.[1] Polymers have been prepared that are responsive to light,[2] pH,[3] temperature,[4] or oxidation,[5] where the responsive component typically undergoes a transition between hydrophobic and hydrophilic states.[6] Polypeptide and protein materials offer an additional stimulus response mechanism since they adopt ordered chain conformations (e.g., α-helices) that can be disrupted under mild conditions. Thermal responsive elastin mimetics[7] and pH responsive synthetic polypeptides[8] have both taken advantage of ordered/disordered conformation transitions to drive the formation or disruption of aqueous self-assemblies such as vesicles and micelles. However, these conformational changes act primarily as a solubility switch, and thus do not replicate the more subtle structural changes found in biological systems, such as in motor proteins[9] and membrane proteins involved in signal transduction,[10] where bulk phase separation does not occur. Addressing this challenge, we have developed new glycopolypeptides that undergo α-helix-to-coil transitions upon oxidation and possess unprecedented good water solubility in both conformational states. A fine energetic balance was achieved by careful placement of oxidizable thioether groups combined with solubilizing and functional sugar moieties to give polymers with switchable, soluble conformations potentially useful for applications in sensing, diagnostics, and biomimetics.

The ability of simple synthetic homopolypeptides, such as poly-L-lysine or poly-L-glutamate, to undergo helix-to-coil transitions in aqueous solution is well known.[11] In these polymers, the transition occurs upon protonation or deprotonation of the side-chain functional groups where the charged polymers are disordered and the neutral forms adopt stable α-helices. The resulting uncharged, α-helical polypeptides are sparingly soluble in water and precipitate above micromolar concentrations, essentially making these helix-to-coil transitions a solubility switch.[11] Numerous conformation switchable synthetic polypeptides have been reported, including those that respond to pH,[12] light,[13] or sugar binding,[14] but none has good water solubility in both the ordered and disordered conformational states. Polypeptides that undergo helix-to-coil transitions and remain soluble in organic solvents are known,[15] but these are not amenable to biomimetic studies or biotechnological applications. To obtain polypeptides that can undergo a conformational change and remain water-soluble, we prepared glycopolypeptides based on L-cysteine residues (Schemes 1 and 2). The key design features in these polymers

Scheme 1. Synthesis of glyco-C NCA Monomers[a]



[a]Reagents and conditions: (a) CBz-Cl, THF:H_2O 1:9, NaOH (98% yield); (b) PPh_3, THF:H_2O 9:1 (98% yield); (c) **2a** or **2b**, 2,2-dimethoxy-2-phenylacetophenone, DMF, 365 nm (94% yield); (d) Cl_2CHOMe, DCM, 50 °C (63−67% yield). **4a** = α-gal-C NCA (R_1 = OAc; R_2 = H). **4b** = α-glc-C NCA (R_1 = H; R_2 = OAc).

are the incorporation of thioether linkages and stable 100% glycosylation via use of *C*-linked glycosides. The polar glycosides provide excellent nonionic, pH- and buffer-tolerant water solubility, and add the potential for selective binding to biological targets, such as sugar binding cell surface receptors. The thioether groups provide an oxidation sensitive switch from less-polar thioether to highly polar sulfone groups.

We had previously prepared stable glycopolypeptides based on L-lysine residues, using amide linkages to attach different

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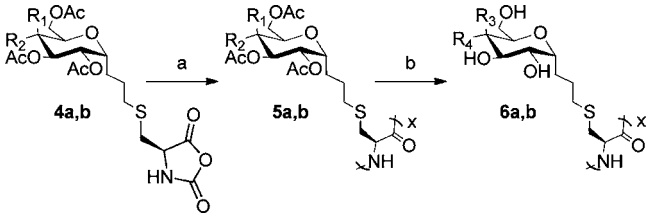
Scheme 2. Polymerization of glyco-C NCAs and Glycopolypeptide Deprotection[a]



[a]Reagents and conditions: (a) $(PMe_3)_4Co$, THF (98% yield); (b) $NH_2NH_2{\cdot}H_2O$, (95% yield). For **4a** and **5a**: R_1 = OAc; R_2 = H. For **4b** and **5b**: R_1 = H; R_2 = OAc. For **6a** (poly(α-gal-C)): R_3 = OH; R_4 = H. For **6b** (poly(α-glc-C)): R_3 = H; R_4 = OH.

monosaccharides.[16] These polymers displayed high α-helical contents in aqueous media and only became partially disordered when heated to ∼90 °C. L-Lysine is an inherently helicogenic residue,[11] which combined with the ability of side-chain amide groups to cooperatively H-bond favored formation of stable α-helices in this polymer. To obtain glycopolypeptides with lower helical stability, we chose to build off of L-cysteine, since the presence of the β-heteroatom weakens formation of α-helices.[11] Synthetic glycopolypeptides using *O*- and *S*-linked glycoside derivatives of L-serine and L-cysteine have been prepared,[17] yet this approach is not practical due to difficulties both in monomer synthesis and polymerization, and these materials also do not allow for conformational switching. In our design, we used the method of Dondoni to attach the alkene-terminated *C*-linked glycosides of D-galactose and D-glucose to L-cysteine using thiol−ene "click" chemistry,[18] followed by conversion to the corresponding new α-amino acid *N*-carboxyanhydride (glyco-C NCA) monomers (Scheme 1). Although these monomers use non-native linkages, it has been well demonstrated that *C*-linked glycopeptides can bind targets with nearly equal affinity and conformation as native *O*-linked analogues,[19] and have been widely utilized when stable glycoprotein mimetics are desired.[20] Our approach allows formation of the glycosylated amino acids in high yield, is amenable to a variety of sugars, and imparts these residues with many desirable features for polymer formation. The unbranched, thioether-containing side chains are long and hydrophobic and hence should favor α-helix formation in water, the spacing of sugars away from the backbone should relieve steric crowding and assist NCA polymerization, and the incorporation of thioether groups allows for facile postpolymerization stimulus response via oxidation.

The glyco-C NCA monomers were found to polymerize efficiently using $(PMe_3)_4Co$ initiator in THF at room temperature (Scheme 2), yielding side-chain-protected polymers in excellent yields (see Supporting Information (SI)).[21] Variation of monomer to initiator ratios for each glyco-C NCA gave glycopolypeptides whose lengths increased linearly with stoichiometry, and which possessed narrow chain length distributions (M_w/M_n). Data for the α-D-glucose-L-cysteine monomer (α-glc-C NCA) are shown in Figure 1, and data for the α-D-galactose-L-cysteine monomer (α-gal-C NCA) and statistical copolymers are given in the SI. Soluble, 100% glycosylated high-molecular-weight polypeptides were prepared with reproducible and precisely controlled chain lengths up to ca. 200 residues long, which is difficult to achieve using postpolymerization glycosylation strategies.[22] Chain extension experiments using sequential monomer additions to see if

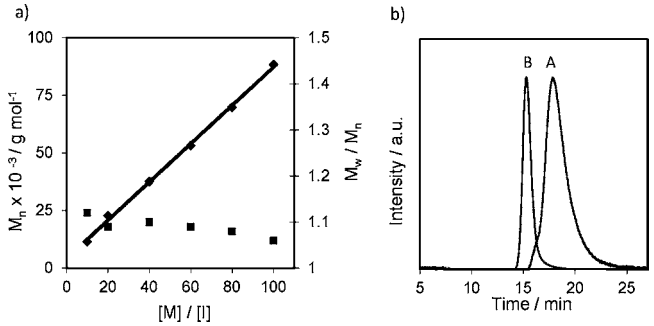


Figure 1. (a) Molecular weight (M_n, ◆) and polydispersity index (M_w/M_n, ■) of poly(α-glc-C) as functions of monomer to initiator ratio ([M]/[I]) using $(PMe_3)_4Co$ in THF at 20 °C. (b) GPC chromatograms (normalized LS intensity in arbitrary units (a.u.) versus elution time) of glycopolypeptides after initial polymerization of α-glc-C NCA to give a poly(α-glc-C)$_{58}$ homoglycopolypeptide (A) and after chain extension by polymerization of N_ε-CBz-L-lysine-*N*-carboxyanhydride to give a poly(α-glc-C)$_{58}$-*block*-poly(CBz-Lys)$_{290}$ diblock glycopolypeptide (B).

glyco-C NCAs can be incorporated into diblock copolymers (Table 1, see SI) all proceeded in high conversion to yield predictable compositions, and with no evidence of inactive chains by GPC analysis (Figure 1). Overall, these data show that the glyco-C NCAs were able to undergo living polymerization when initiated with $(PMe_3)_4Co$, similar to conventional NCAs.[21] Furthermore, both poly(α-glc-C) and poly(α-gal-C) were found to have good water solubility after removal of protecting groups (Scheme 2).

To investigate solution conformations, circular dichroism (CD) spectra of the poly(glyco-C)s were measured in PBS buffer (pH 7.4) after purification and removal of all residual cobalt ions by extensive dialysis against deionized water. Both poly(α-glc-C) and poly(α-gal-C) were found to be soluble in both DI water and PBS buffer at 20 °C (>50 mM), and gave CD spectra with characteristic minima at 208 and 222 nm indicating predominantly α-helical conformations (Figure 2, see SI).[23] CD analysis of a thin film of poly(α-gal-C) cast from a solution in DI water also showed that this polymer is predominantly α-helical in the solid state (see SI). The thioether groups in these polymers can be oxidized to either sulfoxide or sulfone functionalities, both of which have increased polarity. A similar change of polarity has also been used by Hubbell who oxidized thioether groups in synthetic copolymers to disrupt vesicular assemblies.[5b] Complete oxidation of our poly(glyco-C)s using hydrogen peroxide/acetic acid gave quantitative conversions to the sulfone derivatives, poly(glyco-C^{O2})s, by NMR analysis. To confirm these structures, we also prepared a fully characterized sulfone monomer (α-gal-C^{O2} NCA), and polymerized it to obtain a polymer that showed properties identical to oxidized poly(α-gal-C) samples (see SI). We also found no polymer backbone degradation after oxidation (see SI).

The oxidized poly(glyco-C^{O2})s also had good solubility in DI water and PBS buffer at 20 °C (>50 mM), yet their CD spectra showed loss of the α-helical minima at 208 and 222 nm. Instead, the spectra were characteristic of random coil conformations (Figure 2, see SI),[11,23] and showed that oxidation of the thioether groups to sulfones in poly(glyco-C)s was able to destabilize the α-helices and cause a transition to disordered conformations. Examination of the corresponding sulfoxide, i.e. poly(α-gal-C^{O}), prepared by milder oxidation of

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Table 1. Synthesis of Diblock Copolypeptides Using $(PMe_3)_4Co$ in THF at 20 °C

		first segment[b]			diblock copolymer[c]			
first monomer[a]	second monomer[a]	M_n	M_w/M_n	DP	M_n	M_w/M_n	DP[d]	yield (%)[e]
25 Lys NCA	100 α-glc-C NCA	15 980	1.15	61	131 880	1.01	305	97
25 α-glc-C NCA	100 Lys NCA	27 550	1.09	58	88 330	1.03	290	96

[a]First and second monomers added stepwise to the initiator; number indicates equivalents of monomer per $(PMe_3)_4Co$. Lys NCA = N_ε-CBz-L-lysine-N-carboxyanhydride. [b]Molecular weight and polydispersity index after polymerization of the first monomer (as determined by GPC/LS). [c]Molecular weight and polydispersity index after polymerization of the second monomer (as determined by GPC/LS and ^{1}H NMR). [d]Total degree of polymerization of diblock glycopolypeptide. [e]Total isolated yield of diblock glycopolypeptide.

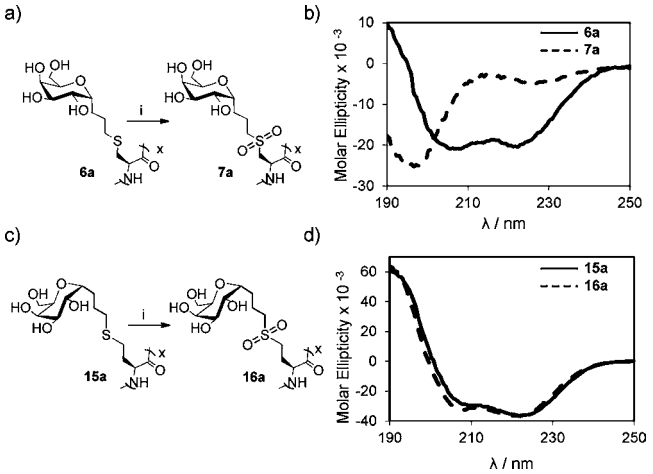

Figure 2. Circular dichroism spectra and corresponding structures of parent and oxidized glycopolypeptides. (a) Conversion of poly(α-gal-C) **6a** to poly(α-gal-C^{O2}) **7a**. (b) Circular dichroism spectra of poly(α-gal-C)$_{108}$ M_n = 33 200 Da (solid line), and poly(α-gal-C^{O2})$_{108}$ M_n = 36 650 Da (dashed line). (c) Conversion of poly(α-gal-C^{H}) **15a** to poly(α-gal-C^{HO2}) **16a**. d) Circular dichroism spectra of poly(α-gal-C^{H})$_{88}$ M_n = 28 280 Da (solid line) and poly(α-gal-C^{HO2})$_{88}$ M_n = 31 100 (dashed line). All samples analyzed at concentrations of 0.5 mg/mL in PBS buffer at 20 °C. Molar ellipticity is reported in deg·cm^2·dmol^{-1}. Conditions (i) = H_2O_2, H_2O–AcOH (98% yield).

poly(α-gal-C), revealed that this functionality with intermediate polarity is not sufficient to destabilize the α-helical conformation (see SI). The increased polarity of the sulfone groups likely drives the helix-to-coil transition by interacting strongly with solvent water molecules, which can disrupt hydrophobic packing of the polypeptide side chains and increase steric crowding around the backbone to destabilize the α-helical conformation. This substantial change in chain conformation from such small molecular changes in these samples suggests that even subtle shifting in the balance of forces that dictate chain conformation can have large effects. This hypothesis was further validated by preparation and study of the homologous sample poly(α-gal-C^{H}), based on L-homocysteine residues, where the thioether group is separated from the peptide backbone by one additional methylene unit (Figure 2, see SI). Poly(α-gal-C^{H}) is similar to poly(α-gal-C) in that both are water-soluble and α-helical; however, upon oxidation to the corresponding polysulfone, poly(α-gal-C^{HO2}) chains remain α-helical (Figure 2). The addition of methylene units that move the sulfur atoms just one bond further from the peptide backbone is enough to negate any conformation switching when the sulfur is oxidized. Hence, it seems the poly(glyco-C)s have an optimized structure that puts the α-helical and coil states close enough energetically to allow unprecedented switching from soluble α-helices to soluble disordered conformations.

While other synthetic homopolypeptides undergo triggered helix-to-coil transitions coupled with a change in water solubility, the incorporation of sugars in our polymers imparts good water solubility for both conformers. The solubility of both conformations is advantageous since it allows for the presentation of sugar functionalities from different polymer conformations in solution, which may be used to tune their interactions with biomolecules and biological surfaces in different applications.[24] Also, oxidative switching of the chain conformations occurs in aqueous solution, making the helix-to-coil transition amenable for use in devices in vitro, or in aqueous self-assembled materials, such as in glycosylated carriers for drug delivery. The oxidation of thioether groups in proteins is known to occur naturally, and the consequent perturbed folding can serve as a signal for the oxidized protein to be degraded by the proteasome.[25] In a similar manner, the poly(glyco-C)s may also be able to sense oxidative environments in biological systems and respond to them by changing conformation or morphology of self-assembled structures.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral data for all new compounds; polymerization data, M_n vs [M]/[I] plots, X-ray crystal structure of α-gal-C^{O2} NCA, 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.

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