Methylated Mono- and Diethyleneglycol Functionalized Polylysines: Nonionic, α-Helical, Water-Soluble Polypeptides

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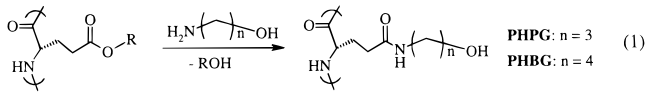
Polypeptides have been studied for use in biomedical applications for some time.[1] Applications such as drug delivery typically require water-soluble components to enhance their ability for circulation in vivo.[2] The problem with common water-soluble polypeptides (e.g. poly-L-lysine and poly-L-aspartate) is that they are polyelectrolytes that display pH-dependent solubility and limited circulation lifetime due to aggregation with oppositely charged biopolymers.[3] Nonionic, water-soluble polypeptides are desired for biomedical applications since they avoid these problems, and can also display the stable secondary structures of proteins that influence biological properties. However, in contrast to short peptides (<25 residues),[4] all nonionic homopolypeptides derived from naturally occurring amino acids are notoriously insoluble in water.[1,5] We have developed methylated mono- and diethyleneglycol-functionalized polylysines that are the first example of nonionic, water-soluble, high molecular weight polypeptides, which are completely α-helical in solution. These exceptionally stable helices are also resistant to proteases similar to pure poly(ethylene glycol), PEG. These polypeptides are new "rodlike PEG" building blocks that can be used to incorporate biochemical stability, self-assembly, and water solubility into polypeptides.

Once it was discovered that high molecular weight poly(L-serine) was insoluble in water,[6] there was considerable interest in development of nonionic water-soluble, conformationally regular polypeptides derived from chemically modified amino acids.[7,8] These materials were desired to allow fundamental studies on the α-helical structure in solution in the absence of electrostatic perturbations. The best materials resulting from this work are the poly(*N*-hydroxyalkyl-L-glutamines), derived from aminolysis of poly(L-glutamate esters) (eq 1).[8] Although this aminolysis reaction



(1)

resulted in significant polymer chain cleavage,[9] nearly all the side chains could be functionalized and the resulting polymers were found to be water soluble. However, studies on their helical structure were limited since these polymers contained substantial random coil content when dissolved in water. The best example, poly(*N*-hydroxybutyl-L-glutamine), PHBG, is ca. 65% helical in neutral water at 20 °C.[8b] To date, an entirely helical, nonionic water-soluble, high molecular weight polypeptide has not been developed.

There have been many attempts to use poly(*N*-hydroxyalkyl-L-glutamines) in biomedical applications; however, they are recognized as foreign and rapidly degraded in vivo.[10] Thus, these materials are useful only where fast erosion is desired and are ineffective at imparting protection from biological attack (i.e. biocompatability). Most biocompatability strategies employ PEG, which is typically grafted onto other polymers, including polypeptides, to improve their properties in vivo.[11] PEG is nonionic, water-soluble and, most importantly, not recognized by immune systems.[12] It is believed that PEG imparts biocompatability through formation of a hydrated "steric barrier" at the surface of a material that cannot be penetrated or recognized by biological molecules.[12] As such, block or graft copolymer drug carriers containing PEG are able to circulate for long periods in the bloodstream without degradation.[13]

We wanted to incorporate the attractive properties of PEG into polypeptides. Incorporation of short ethyleneglycol (EG) repeats[14] onto amino acid monomers was pursued as opposed to the well-documented approach of grafting PEG to the ends or side chains of polypeptides.[12] Our strategy avoids the need for expensive amino- or carboxylato-functionalized PEG molecules necessary for coupling, which typically must be short (less than 5000 Da) to ensure high functionalization. Furthermore, the presence of short EG repeats on every amino acid side chain should result in a high density of EG around the polymer chain. Whitesides et al. have shown that surfaces coated with a high density of short chains contaning as few as two EG repeats are as effective in passivating the surfaces as high molecular weight PEG.[15] In effect, our polypeptides would be surrounded by an EG sheath that should mimic the physical properties of PEG, yet not deleteriously affect the secondary structure of the polypeptide core. The molecular weights of these "PEG-mimic" polymers could also be easily adjusted by controlling the degree of polymerization of the amino acid.

EG-functionalized lysine monomers and polymers were prepared as shown in Scheme 1. Lysine was chosen as the amino acid component for the ease of coupling of the side-chain amine with inexpensive EG-containing carboxylates and for its propensity to form stable α-helical conformations. The formation of EG-

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Scheme 1. Synthesis of Polymers **1** and **2**

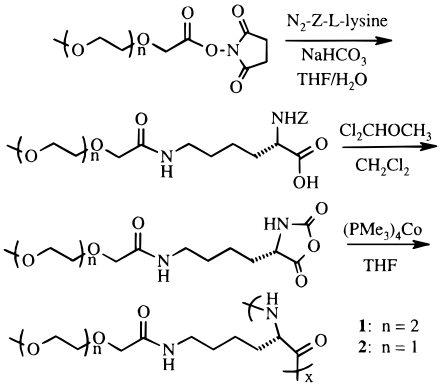

Table 1. Synthesis of Polypeptides and Block Copolypeptides Using $Co(PMe_3)_4$ in THF at 20 °C[e]

		1st segment[b]		diblock copolymer[c]		yield
1st monomer[a]	2nd monomer[a]	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	(%)[d]
50 EG-Lys NCA	none	58 200	1.16			93
100 EG-Lys NCA	none	115 800	1.08			99
33 EG_2-Lys NCA	none	45 300	1.15			94
66 EG_2-Lys NCA	none	93 100	1.10			99
33 EG_2-Lys NCA	108 Lys NCA	44 600	1.19	126 300	1.20	91
33 EG_2-Lys NCA	125 Glu NCA	45 200	1.15	166 250	1.17	90

[a] First and second monomers added stepwise to the initiator; number indicates equivalents of monomer per $Co(PMe_3)_4$. [b] Molecular weight and polydispersity index after polymerization of the first monomer (as determined by GPC). [c] Molecular weight and polydispersity index after polymerization of the second monomer. [d] Total isolated yield of polypeptide or block copolypeptide. [e] EG-Lys NCA = N_ϵ-2-(2-methoxyethoxy)acetyl-L-lysine-*N*-carboxyanhydride; EG_2-Lys NCA = N_ϵ-2-[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine-*N*-carboxyanhydride; Glu NCA = γ-benzyl-L-glutamate-*N*-carboxyanhydride; and Lys NCA = ϵ-Z-L-lysine-*N*-carboxyanhydride.

L-lysine-*N*-carboxyanhydrides allowed facile polymerization into high molecular weight polymers via transition metal catalysis.[16] Both **1** and **2** were found to be water soluble, although the greater solubility of **1**, which is completely miscible with water in all proportions, led to studies being focused on this polypeptide. Homopolymers of **1** and **2** were prepared with defined molecular weights and narrow molecular weight distributions (Table 1). Block copolymers could also be prepared and representitive examples are given in Table 1.

Circular dichroism analysis revealed that **1** and **2** are essentially 100% α-helical in pH 7 water at 25 °C ($[\Theta]_{222} = -40280$).[17] This conformation was unaffected by many environmental factors. The helical structure of **1** was stable in water over an examined pH range of 2−12. It was also stable in solutions containing up to 3 M NaCl, 1 M urea, or 1 M guanidium−HCl. Polymer **1** is soluble and helical in many organic solvents as well (e.g., THF, MeOH, and $CHCl_3$). The thermal stability of the helical conformation of **1** was also very high. It was found that **1** retains 75% of its helicity at 85 °C in water as compared to only 17% helicity for PHBG (Figure 1). Polymer **1** was not digested by hydrolytic enzymes that readily digest poly(L-lysine) (i.e. trypsin),[1] indicative of the PEG-like properties imparted by the EG sheath. Also similar to PEG,[12] **1** was found to display a lower critical solution temperature of ca. 102 °C, observed by reversible precipitation of the homopolypeptide from boiling aqueous solutions.

1 is a polymer with surface properties similar to unstructured PEG, but also possesses a rodlike backbone due to its α-helical character. As such, **1** is able to form aqueous liquid crystalline solutions at high concentrations. Examination of 50 wt % solutions of **1** under crossed polarizers showed birefringence indicative of liquid crystalline ordering, which is also commonly observed for other α-helical polypeptides in organic solvents, e.g. poly(γ-benzyl-L-glutamate) in $CHCl_3$.[18] Preliminary X-ray scattering experiments showed that films of **1** cast from water were highly ordered with well-defined packing of the chains (Figure 2). The 18 Å spacing corresponds roughly to the packing of the helical cylinders, while the spacing at 54 Å is indicative of higher level chain assembly (the 25 Å spacing is due to Kapton supporting film). Thus, by incorporation of EG units onto an amino acid backbone, polymers were prepared that have the ability to self-assemble in addition to possessing the water solubility and surface passivating properties of PEG.

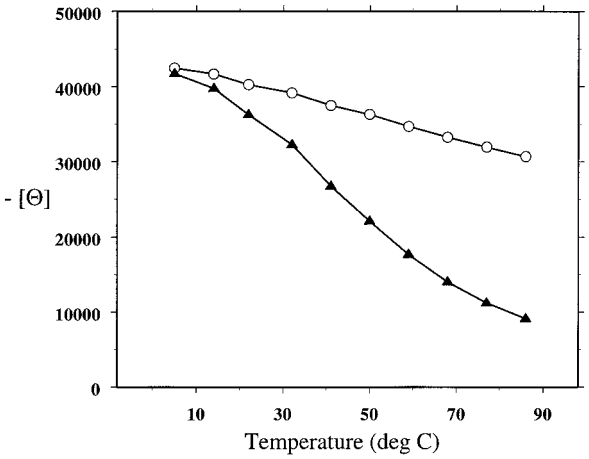

Figure 1. [Θ] at 222 nm as a function of temperature for **1** ($\bar{M}_n = 93100$) and PHBG ($\bar{M}_n = 105000$) in H_2O ([polymers] = 0.5 mg/mL, pH 7). Temperature was raised at a rate of 8 deg C/10 min. Open circles = **1**; black triangles = PHBG.

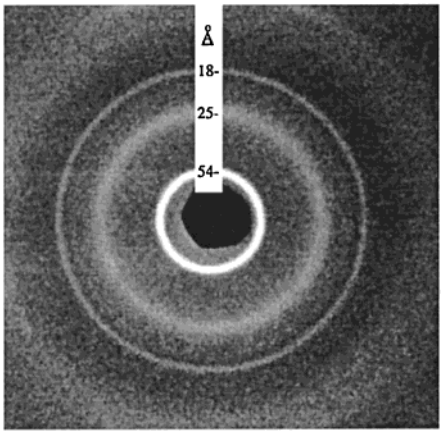

Figure 2. Small-angle X-ray pattern of a film of **1** cast from H_2O (pH 7). Spacings corresponding to major diffraction rings are indicated in the image.

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Supporting Information Available: Details of all experiments, synthesis of polymers, and physical measurements and CD spectrum of **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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