Thermotropic Polypeptides Bearing Side-On Mesogens

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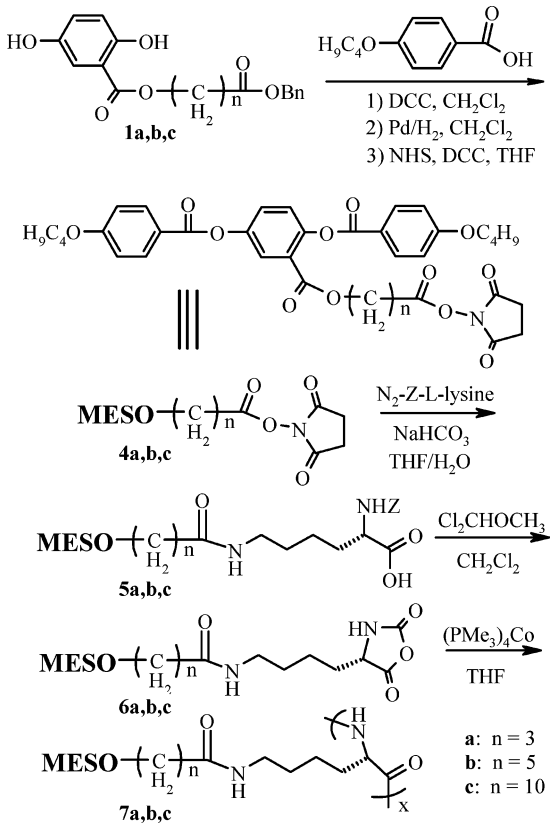
Received September 30, 2005
Revised Manuscript Received November 10, 2005

The polypeptide materials field has grown tremendously in recent years.[1] These polymers are of interest due to their abundant chemical functionality and since the chains can be designed to adopt ordered chain conformations. These features give materials useful in biomedical,[2] composite,[3] and sensor applications.[4] However, a major drawback of polypeptides has been the difficulty in using melt processing with these materials, since the abundant H-bonds and consequent poor chain flexibility prevent melting before decomposition. Although solution-based methods allow processing of these materials for most applications,[5] melt processing, or even capability for thermal annealing, would greatly expand the utility of polypeptides. Some thermotropic behavior has been demonstrated in long-chain alkyl esters of poly(glutamates), yet crystallization of these side chains disrupts the α-helix packing from hexagonal into layered structures upon solidification.[6] Alternatively, terminally attached (end-on) mesogenic groups have also been used to derivatize poly(glutamic acid)[7] and poly(lysine)[8] chains where ordering of the mesogens was observed, which also disrupted helix packing. Here, we have laterally coupled (side-on) thermotropic mesogenic groups to lysine residues to prepare polypeptides that form thermotropic liquid crystalline phases where packing requirements of both polypeptide and mesogenic groups are satisfied.

In the pioneering studies on thermotropic polypeptides by Watanabe's group, poly(glutamates) were derivatized either with long alkyl chains[6] or by end-on attachment of biphenyl mesogens.[7] Polypeptides with short alkyl side chains were not thermotropic, yet side chains greater than 10 carbons long gave melting transitions from −26 to 54 °C. These samples formed cholesteric liquid crystalline phases above the melting transition but formed layered structures at low temperatures driven by crystallization of the side chains. Furthermore, poly(γ-octadecyl-L-glutamate) was found to form a columnar hexagonal phase at temperatures above 200 °C, where the rodlike helices make up the 2D lattice.[6] When biphenyl mesogens were attached end-on to poly(glutamate) side chains via six carbon alkyl spacers, layered structures were observed in the crystalline and liquid crystalline states, followed by transition into a cholesteric structure at higher temperatures.[7] In these examples the liquid crystalline mesophases were dominated either by the side-chain group (layered structure) or the rodlike nature of the polypeptide backbone (hexagonal phase), but in no case was coexistence of both types of ordering observed.

Scheme 1. Synthesis of Mesogen-Modified Polypeptides 7a,b,c



We demonstrate here the first polypeptide system in which liquid crystalline ordering exists concurrently with backbone packing. To obtain this coexistence between mesogen and main-chain ordering, we utilized "side-on" mesogen[9] modification of the polypeptides as this method of attachment allows facile parallel orientation of mesogens and peptide backbones. Since it is known that varying the length of flexible spacers connecting polymer backbones and mesogens affects the mesophase behavior of side-chain liquid crystalline polymers,[10] we prepared amino acid monomers with spacers of 3, 5, and 10 methylene units between the lysine side chains and the mesogens.

The mesogen employed for our studies was a well-known three-ring aromatic ester molecule,[11] which was derivatized from a central carboxylic acid group via ester coupling to attach linkers of 3, 5, and 10 methylene units (**3a,b,c**; Scheme 1). The terminal acid groups of these mesogens were converted to *N*-hydroxylsuccinimide active esters (**4a,b,c**) that were subsequently coupled to the ϵ-amino groups of $N_α$-Z−L-lysine, yielding mesogen-derivatized lysines where the mesogen was coupled to the amino acid using a robust amide linkage (**5a,b,c**). These compounds were then cyclized using 1,1-dichloromethyl methyl ether to give the corresponding α-amino acid−*N*-carboxyanhydrides (NCAs, **6a,b,c**).[12] Mesogen-derivatized polypeptides (**7a,b,c**) were prepared by ring-opening polymerization of the NCAs using $(PMe_3)_4Co$ initiator in THF solvent.[13] The polypeptides were soluble in THF, and the polymerizations were all found to go to complete consumption of monomer giving high yields of polypeptide. Molecular weights were measured in THF using GPC. All three polymers were found

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10.1021/ma052127k CCC: $33.50
Published on Web 12/08/2005

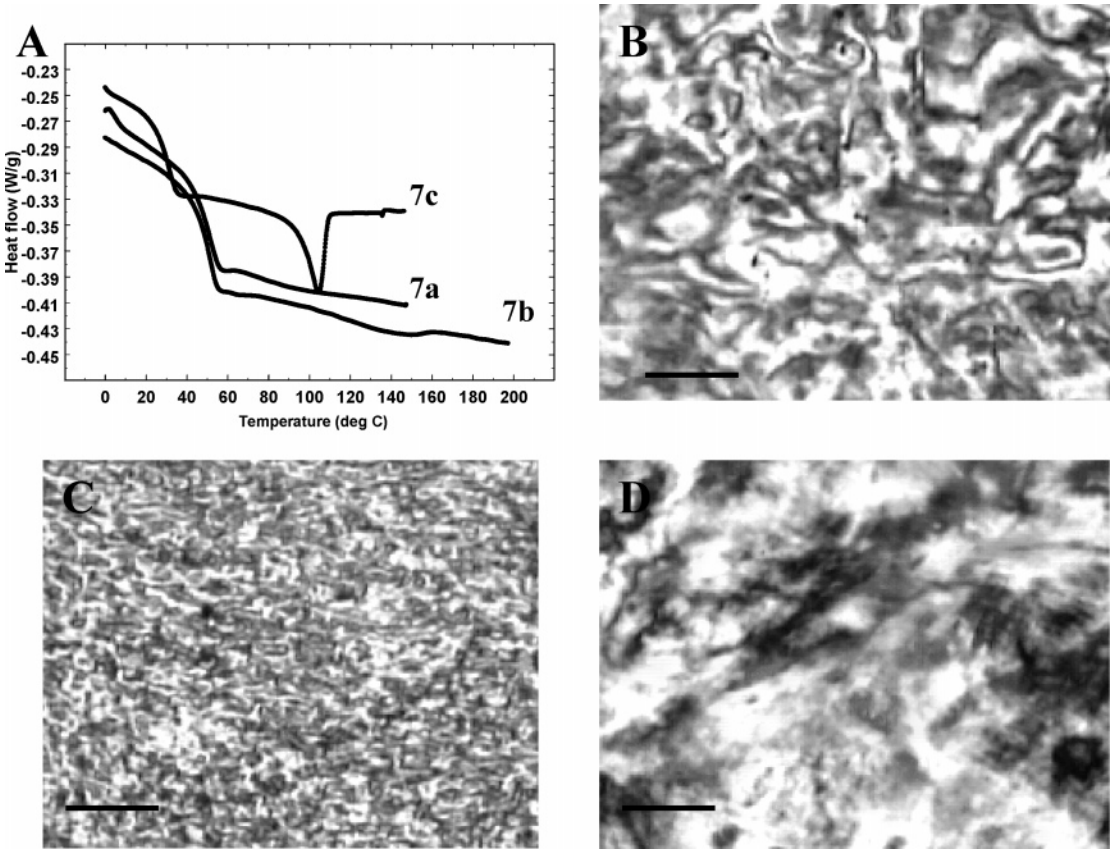

Figure 1. DSC traces and POM images of polypeptides **7a,b,c**. (A) DSC data for heating of the polypeptides at 10 °C/min. (B−D) POM images of polypeptides above the solid to nematic transition. Scale bar = 50 μm for all images. (B) Sample **7a** at 210 °C. (C) Sample **7b** at 180 °C. (D) Sample **7c** at 120 °C.

to adopt α-helical conformations in solution since CD spectra in THF showed double minima at ca. 208 and 222 nm.[14] However, absorption by the aromatic side chains prohibited quantitative calculation of molar ellipticity and helix content.[15] FTIR spectra of each polymer in THF contained amide I and amide II bands (1650 and 1540 cm^{-1}, respectively), also supporting the presence of α-helical conformations.[14]

Thermal properties were studied using differential scanning calorimetry (DSC, Figure 1a), and all three polypeptides exhibited glass transitions between 30 and 50 °C most likely due to side-chain motions since simple derivatives of polylysine show no transitions in this range. Polypeptide **7c** also displayed a distinct melting endotherm at 105 °C, and **7b** showed an endotherm at 150 °C although it was much broader and weaker. Other than the glass transition, **7a** showed no thermal transitions up to 250 °C, yet the sample was visually observed to soften around 200 °C. All of the thermal transitions were found to be reversible. TGA analysis of the polypeptides showed that they begin to lose mass around 300 °C. The intensities and temperature ranges of these endotherms were clearly correlated to the lengths of flexible linkers connecting the mesogens to the polypeptide backbones. With shorter linkers, the mesogens are closely crowded around the rigid helix and are immobilized, while longer tethers allow more mesogen mobility. As a result, **7c** can be melted at lower temperatures than **7b** and **7a** and will absorb more energy since the mesogen groups in this sample should have the most conformational freedom of the three polypeptides.

To see whether the endotherms were related to liquid crystalline phase transitions, the polymers were examined by polarized optical microscopy (POM). With all samples, the initially isotropic solids showed uniformly increasing birefringence with temperature until a critical temperature was reached, at which point the marbled textures characteristic of a nematic phase appeared (Figure 1b−d).[10] Upon further heating, the nematic phases gradually melted into isotropic phases. In samples **7c** and **7b**, the appearance of the nematic coincided with the endotherm observed by DSC, and the optical texture persisted to temperatures well above that transition (i.e., nematic phase exists from ca. 100 to 185 °C for **7c** and 150 to 240 °C for **7b**). Although **7a** exhibited no detectable endotherm by DSC, a broad nematic window was observed by POM at temperatures between ca. 190 and 270 °C.

Since optical microscopy textures of polymer liquid crystals can be ambiguous, we sought to confirm the mesophase structures using variable temperature wide-angle X-ray scattering (WAXS). The azimuthally averaged X-ray data for unoriented samples are shown in Figure 2. Two features of the scattering patterns were common to all three polypeptides: a set of sharp peaks at low q values that vary upon heating and a broad peak at higher q that remained relatively unchanged with temperature. The high q peak corresponds to a d spacing of ca. 5 Å, which is most likely due to the packing of side-chain mesogen groups and reflects the persistence of the nematic phase to high temperatures as observed by POM. The sharp peaks at low q were found to possess a periodicity of 1, $\sqrt{3}$, and $\sqrt{4}$ (as well as $\sqrt{7}$ for **7a**), indicative of hexagonal ordering of the rodlike α-helices. From these data, interhelical spacings were found to be 2.86, 3.45, and 3.57 nm for **7a**, **7b**, and **7c**, respectively, which are consistent with expected values for side-chain-substituted α-helical polylysines.[8,12] A distinct change in the WAXS patterns was seen when passing through the crystalline to nematic transitions of **7c** and **7b**. Below these temperatures, the first-order Bragg peaks and several higher-order peaks were

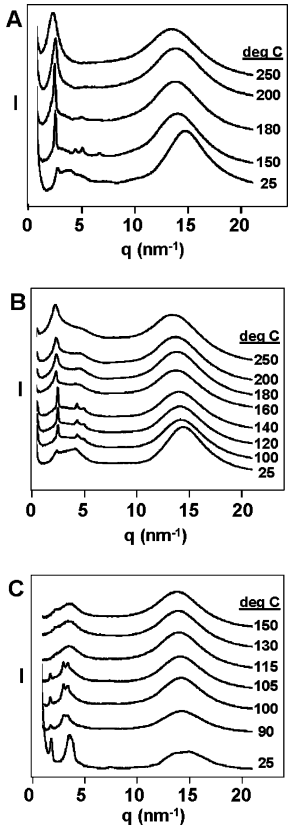

Figure 2. Azimuthally averaged WAXS data for unoriented samples of **7a,b,c** at different temperatures. I = intensity in arbitrary units. Numbers at the right of each scan (°C) are the sample temperatures at which the data were collected. (A) Sample **7a**; note higher-order reflections at 150 °C. (B) Sample **7b**; note loss of hexagonal order above 160 °C. (C) Sample **7c**; note loss of hexagonal order above 105 °C.

present, yet above the transitions the first-order peaks broadened and the higher-order peaks disappeared. This behavior indicated that the hexagonally packed helices had melted to a more disordered liquidlike state at higher temperatures where the helical rods were surrounded by a nematic solvent of the side chains.

Confirmation of this proposed nematic−hexagonal structure was obtained by pulling fibers of the polypeptides from samples in the nematic phase. X-ray diffraction from these oriented samples (Figure 3) gave 2D patterns showing characteristic reflections of both nematic and hexagonal morphologies aligned in the direction of the fiber axis. The reflections for hexagonal ordering were observed along the equatorial axis with a periodicity of 1, $\sqrt{3}$, and $\sqrt{4}$ (as well as $\sqrt{7}$ for **7a**). The four off-axis reflections (most easily seen in Figure 3a) as well as the wide-angle equatorial reflections are commensurate with nematic ordering of the mesogens. Thus, it appears that the DSC endotherms of **7b** and **7c** arise from a crystalline to nematic phase transition where the side chains gain mobility. Consequently, some of the long-range hexagonal order of the packed helices was lost, as evidenced by loss of higher order reflections, yet the rods were still present. The degree of hexagonal order remaining above this phase transition was found to correlate strongly with the length of the linker between the polymer backbone and the mesogens. With **7c**, the longest linker, hexagonal ordering was completely lost within the nematic phase. With this sample, it appears the tethers are long and flexible enough to act as a solvent for the rodlike chains at high temperature, allowing the chains to disorder. For the other samples, the first-order helix−helix Bragg peak was present well above the solid to nematic transition, indicating that the helices persist. The stability of the nematic phases in these polypeptides to very high temperatures also confirmed the stability of the helical backbone conformation. The anisotropy of the backbone constrains the side-on mesogens into a nematic arrangement (Figure 3b) and prevents their melting to an isotropic phase until the helices eventually disorder. Formation of such a nematic structure is not usually observed using conventional end-on linkage of mesogens where the side-chain groups are arranged perpendicular to the main chain.[7,8]

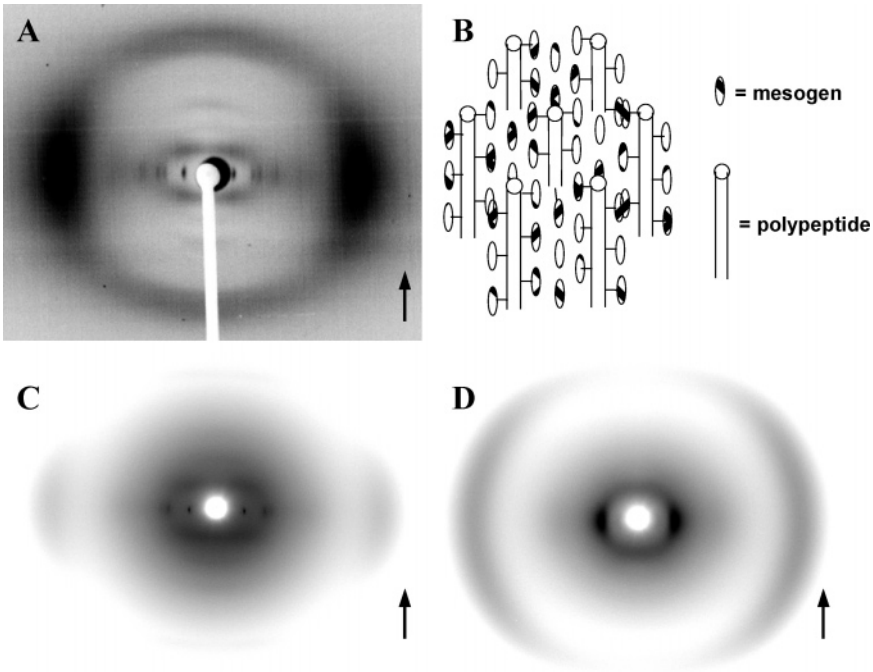

Figure 3. WAXS data for samples of **7a,b,c** oriented by drawing fibers from samples in the nematic phase. Fiber orientation is given by the arrows. (A) X-ray pattern from a fiber of **7a**. (B) Schematic drawing representing the ordering of polypeptide helices and mesogen units in the proposed nematic−hexagonal structure. (C) X-ray pattern from a fiber of **7b**. (D) X-ray pattern from a fiber of **7c**.

We have described the synthesis and thermotropic properties of the first polypeptides with side-on mesogenic groups. By varying the length of aliphatic linker between the polypeptide backbone and the mesogen, we were able to tune both the temperature of the melting transition and the degree of ordering of the rodlike polypeptides in the liquid crystalline nematic state. The intermediate sample, **7b**, possessed both a moderate phase transition temperature into the liquid crystal regime and good ordering of the polypeptide helices in this state. These materials display an unusual mesophase where both side-chain mesogens and polymer backbones are ordered and coexist in a nematic−hexagonal structure. Fibers of these polypeptides were readily drawn from the liquid crystal phase and demonstrate that melt-processable polypeptides with interesting structures are obtainable.

Acknowledgment. This work was supported by the MRSEC program of the National Science Foundation under Award DMR-0080034 as well as a Fellowship (to T.J.D.) from the Rothschild and Mayent Foundation, Institut Curie, Paris, France. The authors thank Professor Patrick Davidson (Orsay, France) for initial X-ray studies on polypeptide **7a**.

Supporting Information Available: Details of all polypeptide synthesis, DSC, TGA, and CD data. This material is available free of charge via the Internet at http://pubs.acs.org.

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MA052127K