Synthesis and Solid-State Secondary Structure Investigation of Silk−Proteinlike Multiblock Polymers

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ABSTRACT: With the step-growth polymerization of bis(oligopeptides) and diisocyanates, two silk−proteinlike multiblock polymers (**P1**, **P2**), containing −$(Ala)_4$− and −(GlyAlaGlyAla)− sequence derived from the crystalline region of spider dragline silk and silkworm (*Bombyx mori)* silk respectively, has been synthesized successfully. The intrinsic viscosities of **P1** and **P2** measured in dichloroacetic acid at 25 °C were 0.31 and 0.26 dL/g. FT-IR, ^{13}C CP/MAS NMR and WAXD measurements revealed the oligopeptide segments in these multiblock polymers could aggregate spontaneously into β-sheet structure in solid state. In addition, it was also found there were non-β-sheet structures in the synthetic polymers, which indicated such polymers had a solid-state structure similar to that of natural silks.

Introduction

Silks (i.e., spider silk and silkworm silk) have been studied extensively for their excellent mechanical properties (e.g., on an equal weight basis, the major ampullate spider silk is stronger than steel and tougher than Kevlar).[1−2] They can be considered as a kind of composite materials, which contain a well-oriented crystalline region [which sequence is $(GlyAlaGlyAlaGlySer)_n$ for *Bombyx mori* silk, $(Ala)_n$ for *Nephila clavipes* dragline silk] and an amorphous region (composed of amino acids having bulky side groups).[1,3−4] To develop the relationship between structure and properties of such kind of fibrous proteins as well as the artificial production of high performance silklike fibers, a number of peptides and polymers based on both silk proteins have been prepared by genetic bioengineering or chemical method.[5−16] Unfortunately, those fibers that spun from the silk-based synthetic polymers, the regenerated silk proteins as well as the recombinant silk proteins were inferior to the native silks.[9,10,15−21]

Even though the recombinant DNA technology affords excellent control over both sequence and molecular weight of proteins based on silks, there are still two open questions need to be addressed.[2,22−23] One is how to artificially produce the proteins in large quantity, and another is how to spin them to the fiber with the appropriate three-dimensionally ordered silklike structure. The further insights into the mechanical properties of silk[24] and Sogah's works[13−16] showed that a certain amount of disorder domain or synthetic non-peptide segments could be introduced to form alternating block copolymers with β-sheet domain. That means it is not necessary or desirable to prepare purely β-sheet sequence, even purely peptide. Therefore, besides bioengineering method, chemical synthesis with the versatility in building multiblock copolymers could play another important role in improving processing of materials which are inspired from natural silks.

In our previous work,[25] we found that *B. mori* silks obtained from artificial reeling with a stable speed are much superior to those are spun naturally. This result reconfirms that the spinning technology may be a critical factor for manufacturing high-performance fibers.[23,26] It also implicates the possibility that the high performance fiber could be produced under an appropriate spinning condition with the materials contained the silklike structure. To obtain a kind of silk−proteinlike materials to explore the possibility of spinning them into silklike fiber and learn how the spinning condition influence the mechanical properties of silklike fibers, we developed a chemical method by introducing diisocyanates into the polymerization with bis(oligopeptides) based on silk proteins. This paper reports the synthesis of two well-defined structure multiblock polymers (**P1**, **P2**), containing −$(Ala)_4$− and −(GlyAlaGlyAla)− sequence derived from the crystalline region of spider dragline silk and silkworm (*B. mori)* silk, respectively. The secondary structure of the raw synthetic polymers was studied with FT-IR, ^{13}C cross-polarization magic angle spinning NMR (^{13}C CP/MAS NMR) and wide-angle X-ray diffraction (WAXD). All results revealed these synthetic polymers had solid-state structures similar to those of natural silks.

Experimental Section

Materials. Chloroform and *N*-methylmorpholine were distilled before use. *N*,*N*-Dimethylformamide (DMF) was distilled over calcium hydride. Dimethyl sulfoxide (DMSO) was used after dehydration with 4 Å molecular sieves for 2 days. Dicyclohexylcarbodiimide (DCC, from Shanghai Shesan Chemical Co., Ltd., China), *N*-*tert*-butoxycarboxyl-l-alanine (BocA-OH), *N*-*tert*-butoxycarboxylglycine (BocGOH), *N*-hydroxysuccinimide (HOSu) (from Yangzhou Baosheng Biochemical Co., Ltd., China), hexamethylene diisocyanate (HDI) (from Aldrich), hexafluoro-2-propanol (HFIP) (from Fluka), and other reagents were used as received.

Measurements. All measurements in this paper were based on the powder sample of our polymers, since the secondary structure of silk protein would be varied with its different solid states (e.g., powder, fiber or film) as well as the conditions of preparation.

FT-IR spectra were recorded on a Nicolet Magna-550 spectrometer, at 4 cm^{-1} resolution using 64 scans. ^1H NMR spectra were obtained on Bruker AVAVCE DMX500 spectrom-

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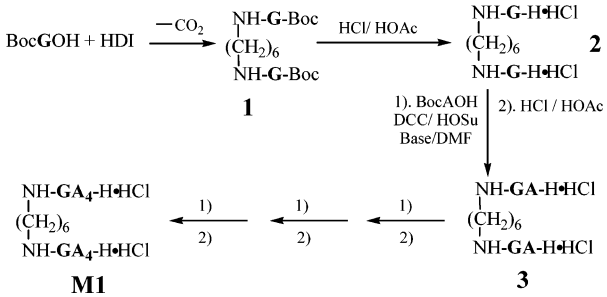
eter, using tertamethylsilane (TMS) as internal standard. The ^{13}C CP/MAS NMR experiments were performed at 25 °C with Bruker AVAVCE DSX 300 NMR spectrometer operating frequency of which was 75.47 MHz for ^{13}C nucleus. Samples were contained in a 7.5 mm cylindrical rotor and spun at 6.5 kHz. The number of acquisitions was 1K, and the pulse delay was 3 s. A 61.9 kHz radio frequency field strength was used for decoupling with a decoupling period of 15.5 ms and a 90° pulse width of 4.0 μs with 1 ms CP contact time was employed. ^{13}C chemical shifts were calibrated indirectly through the adamantane methenyl peak observed at 38.3 ppm relative to tetramethylsilane at 0 ppm. X-ray diffraction experiments of polymers and monomers (powders) were performed on Bruker D8 Advance Diffraction meter with Ni-filtered Cu K α radiation (λ = 1.5406 Å) in 2θ range from 10° to 55° at a scan rate 0.05°/s. The intrinsic viscosity of polymers were measured in dichloroacetic acid with a Ubbelohde capillary viscometer (i.d. = 0.85 mm) at 25.0 ± 0.1 °C. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) experiments were performed on the Perkin-Elmer pysis 1 TGA and DSC thermal analysis system under a heating rate of 10 °C/min, respectively. Glass transition temperature (T_g) of polymers was recorded from the second heating cycle.

Preparation. Synthesis of Bis(oligopeptide). Boc-Protected Bis(bipeptide) (1). [Boc—NHCH$_2$CO—NH-(CH$_2$)$_6$NH—COCH$_2$NH—Boc(1)]. BocGOH (11.200 g, 63.82 mmol), 50 mL of chloroform and 1.0 mL of *N*-methylmorpholine (as catalyst) were added into a 100 mL flask containing a magnetic stirring bar. With stirring, 15.0 mL of chloroform solution of HDI (5.360 g, 31.90 mmol) was added dropwise within 10 min. When the addition was completed, the mixture was allowed to stir at room temperature for 4 h and then refluxed for 14 h. After the flask was cooled to room temperature, the mixture was washed twice with 10% citric acid solution and salt-saturated solution, respectively, and dried with anhydrous sodium sulfate for 5 h. The drier was filtered off, and the solvent was removed under vacuum, and then a light yellow sticky residue (**1**) was obtained (12.930 g, yield: 94.4%). IR (casting chloroform solution on KBr plate, cm^{-1}): 3350, 2977, 1696, 1651, 1541, 1447, 1253, 1169: ^1H NMR (500 MHz, ppm, DCCl$_3$): 1.284 (s, 4H, C**H**$_2$ of (CH$_2$)$_2$**CH$_2$CH$_2$**-(CH$_2$)$_2$), 1.383 (s, 18H, C**H**$_3$ of Boc), 1.420 (s, 4H, C**H**$_2$ of CH$_2$**CH$_2$**(CH$_2$)$_2$**CH$_2$**CH$_2$), 3.223−3.233 (m, 4H, C**H**$_2$ of **CH$_2$**-(CH$_2$)$_4$**CH$_2$**), 3.787 (s, 4H, C**H**$_2$ of Gly), 5.45 (br, 2H, N**H** of Gly), 6.825 (s, 2H, N**H** of **NH**(CH$_2$)$_6$**NH**).

Bis(bipeptide) (HCl NH$_2$CH$_2$CO—NH(CH$_2$)$_6$NH—COCH$_2$NH$_2$ HCl) (2). Compound **1** (10.000 g, 23.23 mmol) was dissolved in 15.0 mL of ice−acetic acid, and then 60.0 mL of hydrochloride saturated acetic acid solution was added to remove the protective group (Boc). After stirring for 30 min, acetic acid was evaporated under vacuum, the obtained solid was triturated and suspended in chloroform with stirring overnight. The solid was collected, washed twice with chloroform and dried in vacuo at 60 °C for 24 h. Then bis(bipeptide) (**2**) was obtained (6.832 g, yield: 97.2%). IR (KBr, cm^{-1}): 3281, 3111−2865 (broad), 1658, 1567, 1446, 1280, 1126, 1112, 908; ^1H NMR (500 MHz, ppm, DMSO-d_6): 1.286 (s, 4H, C**H**$_2$ of (CH$_2$)$_2$**CH$_2$CH$_2$**(CH$_2$)$_2$), 1.410 (s, 4H, C**H**$_2$ of CH$_2$**CH$_2$**(CH$_2$)$_2$**CH$_2$**-CH$_2$), 3.084−3.121 (m, 4H, C**H**$_2$ of **CH$_2$**(CH$_2$)$_4$**CH$_2$**), 3.510−3.521 (d, 4H, C**H**$_2$ of Gly), 8.249 (br, 6H, N**H** of **NH**(CH$_2$)$_6$**NH** and N**H**$_3^+$ of end group), 8.606 (s, 2H, N**H** of Gly).

Bis(pentapeptide) [HCl H—A$_4$G—NH(CH$_2$)$_6$NH—GA$_4$—H·HCl] (M1). As described in Scheme 1, bis(pentapeptide) (**M1**) was synthesized step by step in DMF using traditional liquid-phase peptide synthesis method[27] with bis(bipeptide) (**2**) and BocAOH as raw materials and DCC and HOSu as complex condensation reagent. The Boc-protected intermediate products were purified by Silica gel column chromatography using the mixture of chloroform, methanol and acetic acid as eluent. The removal of protective group (Boc) with hydrochloride acetic acid solution and Soxhlet extraction by chloroform afforded bis(pentapeptide) (**M1**) (total yield was about 35%). FT-IR (KBr, cm^{-1}): 3330, 3120−2750 (broad), 1629, 1545, 1448, 1240, 1163. ^1H NMR (500 MHz, ppm, DMSO-d_6): 1.184−1.222 (m, 28H, C**H**$_2$ of (CH$_2$)$_2$**CH$_2$CH$_2$**(CH$_2$)$_2$ and C**H**$_3$ of Ala), 1.339 (s, 4H, C**H**$_2$ of CH$_2$**CH$_2$**(CH$_2$)$_2$**CH$_2$**CH$_2$), 3.030−3.040 (m, 4H, C**H**$_2$ of **CH$_2$**(CH$_2$)$_4$**CH$_2$**), 3.620−3.643 (m, 4H, C**H**$_2$ of Gla), 4.236−4.250 (m, 8H, C**H** of Ala), 7.921−8.185 (m, N**H** of Ala, N**H** of N**H**(CH$_2$)$_6$N**H** and N**H**$_3^+$ of end group), 8.280 (s, 2H, N**H** of Gla).

Scheme 1. Synthesis of Bis(pentapeptide) (M1)



Bis(tetrapeptide) (HCl·H—GAGA—NH(CH$_2$)$_6$NH—AGAG—H·HCl) (M2). Bis(tetrapeptide) (**M2**) was prepared with the similar synthesis procedure for bis(pentapeptide) (**M1**) by using BocAOH, BocGOH and HDI as raw materials. The total yield was about 43%. FT-IR (KBr, cm^{-1}): 3310, 3082−2670 (broad), 1628, 1547, 1448, 1245, 1166. ^1H NMR (500 MHz, ppm, DMSO-d_6): 1.158−1.172 (d, 6H, C**H**$_3$ of Ala^1, N → C terminus), 1.214−1.229 (d, 10H, C**H**$_3$ of Ala^2 and C**H**$_2$ of (CH$_2$)$_2$**CH$_2$CH$_2$**(CH$_2$)$_2$), 1.339 (s, 4H, C**H**$_2$ of CH$_2$**CH$_2$**(CH$_2$)$_2$**CH$_2$**-CH$_2$), 3.051−3.071 (m, 4H, C**H**$_2$ of **CH$_2$**(CH$_2$)$_4$**CH$_2$**), 3.585−3.762 (m, 4H, C**H**$_2$ of Gly), 4.017−4.111 (m, 2H, C**H** of Ala^1), 4.220−4.277 (m, 2H, C**H** of Ala^2), 8.020−8.158 (m, 10H, N**H** of N**H**(CH$_2$)$_6$N**H**, N**H**$^{3+}$ of end group and N**H** of Ala^2 and Gly^1), 8.310 (s, 2H, N**H** of Gly^2).

Polymerization. Polymer P1. As described in Scheme **2**, bis(pentapeptide) (**M1**, 1.500 g, 1.72 mmol) DMSO solution and *N*-methylmorpholine (0.55 mL, 5.01 mmol) were added to a two-necked flask equipped with a magnetic stirring bar and a gas-inlet adapter under nitrogen atmosphere. Afterward, 10.0 mL of DMSO solution of HDI (0.290 g, 1.72 mmol) was added dropwise into the flask under stirring within 3 min. The mixture was stirred at room temperature for 2 days under N_2. Then the temperature of the reactant mixture was raised to 60 °C and stirred for 3 h further in order to complete the polymerization. After the flask was cooled to room temperature, the product was poured into 200 mL of distilled water under stirring. The precipitate was collected, and washed with water, methanol, and chloroform sequentially and then dried under vacuum at 60 °C overnight. The polymer solid was redissolved in hexafluoro-2-propanol, the insolvable solid (cross-link fraction) was filtrated. After removal of the solvent, the solid was dried in a vacuum for 24 h to giving a light yellow solid (polymer **P1**, 1.302 g, yield: 78.5%). FT-IR (KBr, cm^{-1}): 3284, 2934, 2858, 1630, 1552, 1447, 1252, 1248, 963. ^1H NMR (500 MHz, CF$_3$CO$_2$D, ppm): 1.421 (s, 8H, C**H**$_2$ of (CH$_2$)$_2$**CH$_2$CH$_2$**-(CH$_2$)$_2$), 1.520−1.575 (m, 24H, C**H**$_3$ of Ala), 1.672 (s, 8H, C**H**$_2$ of CH$_2$**CH$_2$**(CH$_2$)$_2$**CH$_2$**CH$_2$), 3.336−3.347 (m, 8H, C**H**$_2$ of **CH$_2$**-(CH$_2$)$_4$**CH$_2$**), 4.231 (s, 4H, C**H**$_2$ of Gly), 4.334−4.376 (m, 2H, C**H** of Ala^4), 4.565−4.738 (br, 6H, C**H** of Ala$^{1-3}$). Solid-state ^{13}C NMR (300 MHz, ppm): 14.316 and 21.308 (β-C of Ala), 30.625 (C of CH$_2$(**CH$_2$**)$_4$CH$_2$), 42.837 (C of **CH$_2$**(CH$_2$)$_4$**CH$_2$** and α-C of Gly), 47.708 (α-C of Ala), 159.414 (C of urea carbonyl), 170.189 (shoulder, C of Gly carbonyl), 173.284 (C of Ala carbonyl).

Polymer P2. Polymer **P2** was obtained from the polymerization of bis(tetrapeptide) (**M2**) and HDI with the same procedure used for polymer **P1** (yield: 70.7%). FT-IR (KBr, cm^{-1}): 3284, 2932, 2866, 1630, 1561, 1448, 1249, 1216, 963.^1H NMR(500 MHz, CF$_3$CO$_2$D, ppm): 1.401 (s, 8H, C**H**$_2$ of (CH$_2$)$_2$**CH$_2$CH$_2$**(CH$_2$)$_2$), 1.525 (s, 12H, C**H**$_3$ of Ala), 1.650 (s, 8H, C**H**$_2$ of CH$_2$**CH$_2$**(CH$_2$)$_2$**CH$_2$**CH$_2$), 3.329−3.376(m, 8H, C**H**$_2$ of **CH$_2$**(CH$_2$)$_4$**CH$_2$**), 4.037 (s, 4H, C**H**$_2$ of Gly^2), 4.240 (s, 4H, C**H**$_2$ of Gly^1) 4.656 (m, 2H, C**H** of Ala^1), 4.709 (m, 2H, C**H** of Ala^2). Solid-state ^{13}C NMR (300 MHz, ppm): 20.674 (β-C of Ala), 30.583 (C of CH$_2$(**CH$_2$**)$_4$CH$_2$), 42.536 (C of **CH$_2$**(CH$_2$)$_4$**CH$_2$** and

Scheme 2. Polymerization of Bis(oligopeptides) (M1, M2) and HDI

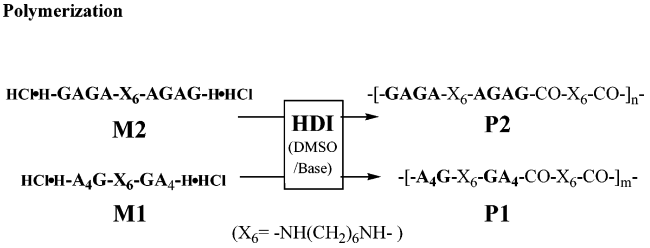


Table 1. Polymerization Results of Multiblock Polymers (P1, P2)

sample	polymn yield (%)	$[\eta]$ (dL/g)[a]	mol wt[b]	T_d (°C, TGA)	T_g (°C, DSC)
P1	78.5	0.31	44 900	219	*c*
P2	70.7	0.26	36 700	221	107
M1		0.07			
M2		0.06			

[a] Measured at 25.0 °C in dichloroacetic acid and concentration of 0.5 g/dL. [b] Calculated by $[\eta] = 2.78 \times 10^{-5}\ M^{0.8}$.[29] [c] Not observed.

α-C of Gly), 47.740 (α-C of Ala), 159.345 (C of urea carbonyl), 170.208 (C of Gly carbonyl), 173.260 (C of Ala carbonyl).

Results and Discussion

Synthesis. The reaction of amino-protected amino acid and aliphatic diisocyanate, of which mechanism had been described in details,[28] gave the formation of amide (bis(bipeptide)) via an unstable intermediate (*N*-carboxyanhydride). Bis(oligopeptides) (**M1**, **M2**), based on the amino acid sequences of the crystalline region of spider dragline silk and silkworm *(B. mori)* silk, were synthesized with traditional liquid-phase peptide synthesis method[27] (Secheme 1). Polymers **P1** and **P2** were prepared by step-growth polymerization of bis(oligopeptides) (**M1**, **M2**) and hexamethylene diisocyanate (**HDI**) in DMSO with a small amount *N*-methylmorpholine as catalyst (Scheme 2). A small amount of cross-link fraction (about 1%) in raw polymer, which due to the high reactive nature of isocyanate group of HDI, was separated based on its insolvability in hexafluoro-2-propanol. Hence the silk−proteinlike multiblock polymers (**P1**, **P2**), containing −(Ala)$_4$− or −(GlyAlaGlyAla)− sequence derived from the crystalline region of spider dragline silk and silkworm silk were obtained.

The silk−proteinlike polymers (**P1**, **P2**) could be dissolved in hexafluoro-2-propanol and strong organic acids (such as dichloroacetic acid, formic acid, and trifluoroacetic acid), while they showed a poor solubility in common solvents. Table 1 summarized the polymerization results and thermal properties of polymers, as well as the intrinsic viscosity of polymers and monomers measured in dichloroacetic acid at 25.0 °C. As showed in Table 1, both **P1** and **P2** exhibited the relative high intrinsic viscosity value (0.31 and 0.26 dL/g, respectively). From the comparison of the intrinsic viscosities of polymers and monomers, it could be drawn that the silk−proteinlike polymers were stable in dichloroacetic acid under the measurement condition. According to Doty's equation[29] ($[\eta] = 2.78 \times 10^{-5}\ M^{0.87}$), the viscosity average molecular weight (M_η) of polymer **P1** and **P2** were 44 900 and 36 700 respectively, which meant the synthetic multiblock polymers had a sufficiently high degree of polymerization (DP = 40−50) for their secondary structural analysis. Moreover, it could be seen in Table 1 that the polymers began to decompose around 220 °C (no melting point were observed before decomposition) and only P1 displayed an apparent glass transition at 107 °C. Comparing to that of natural silk[1], the lower thermostability of P1 and P2 could be due to the introduction of urea bond in molecular chain of polymers. Moreover, it is reasonable for the differences of thermal properties between the simulated polymers and animal silk proteins that could be attributed to their different primary structures.

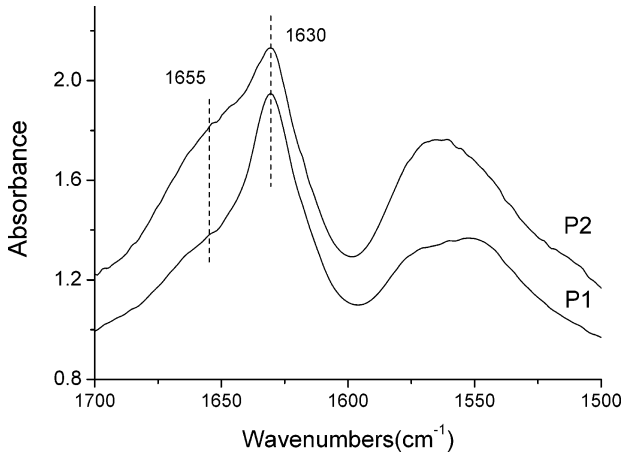


Figure 1. Amide I and amide II region of FT-IR spectra in KBr pellet of **P1** and **P2**.

Secondary Structural Analysis. FT-IR Measurements. In the vibrational spectroscopy, the carbonyl stretching frequencies of amide (amide I) are sensitive to the secondary structures of protein and polypeptide, both in solution and in solid state.[30−34] Thereby the secondary structure transition of silk proteins (*B. mori* fibroin and *N. seneglensis* spidroin) induced by solvents or ions had been monitored by time-resolved FT-IR spectroscopy in our previous works.[35−36] The amide I mode of β-sheet conformation in both fibroin and spidroin gives rise to a strong infrared band around 1630 cm^{-1} and a much weaker band at 1680−1690 cm^{-1}, while that of random coil/helical conformation exhibits a band at 1650−1660 cm^{-1}.

The amide I and amide II regions of FT-IR spectra of **P1** and **P2** are shown in Figure 1. An asymmetric peak at 1630 cm^{-1}, which attributed to the absorbance of β-sheet conformation in amide I region, was observed in FT-IR spectra of both **P1** and **P2**. This meant the oligopeptide segments in polymers have been aggregated spontaneously into the β-sheet conformation in the solid state. To examine the existence of other conformations, the second derivative techniques were employed to examine the spectra. Except the components around 1630 and 1686 cm^{-1} (which are characteristic of the β-sheet conformation), the components around 1655 and 1664 cm^{-1} (which attributed to random coil and other disorder conformations) were also observed in both second derivative spectra (figure not shown). Therefore, FT-IR spectra analysis revealed the coexistence of β-sheet conformation and non-β-sheet conformations in the solid-state synthetic polymers. Conclusively, the synthetic polymers had a secondary structure similar to that for natural silks.

^{13}C CP/MAS NMR. L. W. Jelinski[17−19] and T. Asakura[37−40] have done many works on the fibers and films originated from *B. mori* silk or spider silk by solid-state ^{13}C NMR measurement. The ^{13}C chemical shifts

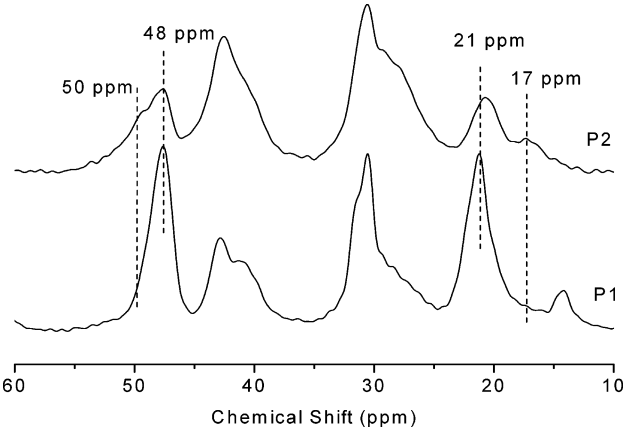


Figure 2. Expanded Ala C_α and C_β regions in solid-state ^{13}C CP/MAS NMR spectra of **P1** and **P2**.

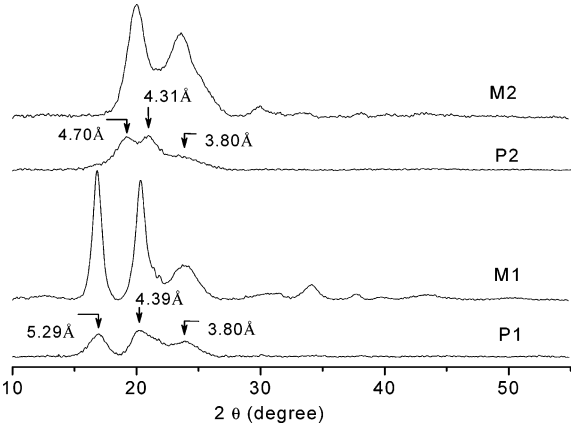


Figure 3. Wide-angle X-ray diffraction pattern of polymers (**P1**, **P2)** and monomers (**M1**, **M2**).

of Ala residue in silk are shown to be sensitive to the secondary structure environment. Generally, Ala−C_α and C_β resonances in β-sheet occur around 47−49 and 20−22 ppm, while those in random coil are at 50−52 and 14−19 ppm, respectively.

The expanded Ala C_α and C_β regions in solid-state ^{13}C CP/MAS NMR spectra of **P1** and **P2** were displayed in Figure 2. The predominant resonances were at 21 ppm in Ala C_β region (14−24 ppm) and 48 ppm in Ala C_α region, which could be assigned to β-sheet conformation. Meanwhile, the resonance peaks attributed to random coil conformation were observed at 14 ppm (Ala C_β) and 50 ppm (Ala C_α) as a shoulder in **P1**, at 17 and 50 ppm (shoulder) in **P2**, respectively. This confirmed that β-sheet conformation and non-β-sheet conformation coexisted in the solid-state synthetic polymers, but the predominant conformation was β-sheet.

Wide-Angle X-ray Diffraction. The WAXD patterns of **P1** and **P2** were shown in Figure 3. Three diffraction peaks with *d* spacings of 5.29, 4.39, and 3.80 Å could be observed in **P1**. These data were similar to that of the *d* spacings of antiparallel β-sheet reported for *N. clavipes* silk by Thiel et al.[41] and closely agreed with the X-ray diffraction data of silklike polymers contained oligomer segment of Alanine.[16] In the meantime, the X-ray diffraction of **P2** showed peaks with *d* spacings of 4.70, 4.31, and 3.80 Å (shoulder), which was in agreement with the antiparallel β-sheet reported for the unoriented fibroin film after treatment by methanol,[42−43] and was similar to that of the polymer $(AlaGly)_n$ reported by Fraser et al.[44] and Tirrell et al.[8] Thus, the powder X-ray diffraction analysis further confirmed the existence of β-sheet in our synthetic silk−proteinlike multiblock polymers. However, the WAXD pattern of monomers (Figure 3) displayed rather sharp diffraction peaks with smaller half-peak width relative to that of polymers. These meant the crystallizability of oligopeptide segment in polymers had been interrupted by non-peptide segment partially, namely the non-peptide segment played the role as a crystallinity adjuster in the multiblock polymers.

Conclusions

By taking advantage of the high reactivity of diisocyanates, two silk−proteinlike multiblock polymers (**P1**, **P2**), containing $-(Ala)_4-$ and the −(GlyAlaGlyAla)− sequence derived from the crystalline region of spider dragline silk and *B. mori* silk respectively, had been synthesized by the polymerization of bis(oligopeptides) (**M1**, **M2**) and hexamethylene diisocyanate (**HDI**). FT-IR and solid-state ^{13}C CP/MAS NMR spectra analyses of **P1** and **P2** revealed the coexistence of a major β-sheet conformation and a minor random coil/helical conformation in such synthetic polymers. The results of powder X-ray diffraction provided conclusive evidences for the formation of β-sheet by aggregation of oligopeptide segments in **P1** and **P2**. From these evidences, we concluded that our synthetic polymers had the similar solid-state structures with silk proteins (silkworm fibroin and spidroin). These synthetic silk−proteinlike polymers allows us to make film or fiber for the further investigation of the morphologies and mechanical properties with comparison to those of natural silks. Moreover, the introduction of diisocyanates into polymerization may lead a convenient way to achieve a series of silk−proteinlike multiblock polymers by changing either bis(oligopeptide) or diisocyanate.

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