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# Synthesis and Characterization of Multiblock Copolymers Based on Spider Dragline Silk Proteins

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Spider dragline silk with its superlative tensile properties provides an ideal system to study the relationship between morphology and mechanical properties of a structural protein. Accordingly, we synthesized two hybrid multiblock copolymers by condensing poly(alanine) [(Ala)<sub>5</sub>] blocks of the structural proteins (spidroin MaSp1 and MaSp2) of spider dragline silk with different oligomers of isoprene (2200 and 5000 Da) having reactive end groups. The synthetic multiblock polymer displayed similar secondary structure to that of natural spidroin, the peptide segment forming a  $\beta$ -sheet structure. These multiblock polymers showed a significant solubility in the component solvents. Moreover, the copolymer which contains the short polyisoprene segment would aggregate into a micellar-like structure, as observed by TEM.

## Introduction

During the years of evolution, human beings have learned many invaluable lessons from nature, and there is currently considerable interest in the biomimicry of biopolymers.<sup>1–3</sup> Therefore, the natural silks, including spider silks and silkworm silk, have been extensively studied for many years. The results showed that some of them, especially spider dragline silk, are particularly promising materials with outstanding comprehensive mechanical properties superior to most of the artificial materials.<sup>4,5</sup> Moreover, spider silk provides an ideal system to study the relationship between protein structures and mechanical properties. The structure of silk can be described as a nanocomposite made up of a cross-linked, amorphous polymer network reinforced by well-orientated crystalline filler.<sup>6,7</sup> Both solid-state NMR<sup>8</sup> and wide-angle X-ray diffraction (WAXD)<sup>9</sup> have been used to study the crystalline region, which greatly contributes to the extraordinary strength of the silk fiber. The amorphous network provides structural continuity and thus plays a major role in determining mechanical properties.<sup>10</sup> A lot of evidence has shown that the structure of the silk proteins, as well as the processing conditions and consequent morphology of the silk, greatly contributes to the final properties of silk materials.<sup>11–13</sup> Therefore, understanding the molecular basis of silk proteins and the processing procedures involved in silk fiber formation will facilitate efforts to synthesis spider silk-like materials with predicted combinations of desirable characteristics.

The precise control of architecture exhibited by spider silk protein (spidroin) has inspired a variety of novel materials that combine elements of natural and synthetic polymers. Sogah's group synthesized silk-like copolymers in which the glycine-rich blocks (amorphous regions) were replaced by a synthetic oligomer such as PEG, while the peptide blocks (for example,

poly(alanine)) were derived from the crystalline regions of the silk.<sup>14–17</sup> The resulting polymers showed similar secondary structure to that of natural silk proteins. Our group has explored multiblock copolymers in which GAGA and GAAAA were introduced to form the crystal regions as in certain silk proteins.<sup>18</sup> However, most of the synthetic silk-like copolymers reported previously only dissolved in highly polar solvents such as hexafluoro-2-propanol (HFIP) or dichloroacetic acid, which would probably prevent further investigation and industrial development of these materials.

Here, we report the synthesis and characterization of segmented multiblock copolymers which exploit the tendency of poly(alanine) blocks to phase-separate and self-assemble into ordered  $\beta$ -sheet stacks in order to control nanostructure of the material. We replaced the glycine-rich blocks in spidroin with polyisoprene (PI) oligomer to improve the solubility of the synthetic copolymer. FT-IR, WAXD, and <sup>13</sup>C CP/MAS NMR demonstrated the existence of  $\beta$ -sheet formation in the (Ala)<sub>5</sub> blocks in our multiblock copolymer as has been shown in previous reports.<sup>15,18</sup> After casting in the general component solvent CHCl<sub>3</sub>/HOCH<sub>2</sub>CH<sub>2</sub>Cl (chloroform and 2-chloroethanol), the micellar-like aggregation of multiblock copolymer was observed by transmission electron microscopy (TEM).

## Experimental Section

**Materials.** Chloroform and *N*-methylmorpholine were distilled before use. *N,N*-Dimethylformamide (DMF) was used after dehydration with 4 Å molecular sieves for 2 days. Dicyclohexylcarbodiimide (DCC; from Shanghai Shensan Chemical Co., Ltd., China), *N*-*tert*-butoxycarbonyl-L-alanine (BocAOH), *N*-hydroxysuccinimide (HOSu) (both from Yangzhou Baosheng Biochemical Co., Ltd., China), hexamethylene diisocyanate (HDI) (from Aldrich), and other reagents were used as received.

**Measurements.** For FTIR analysis, the chloroform solution of the polymer was dropped onto a KBr window and allowed to dry. Spectra were obtained on a Nicolet Magna-550 spectrometer (4 cm<sup>-1</sup> resolution; 32 scans). <sup>1</sup>H NMR spectra were obtained using the Bruker AVANCE EM\*500 spectrometer, using tetramethylsilane (TMS) as an internal

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standard.  $^{13}\text{C}$  CP/MAS NMR spectra were obtained at 25 °C with a Bruker AVANCE DSX 300 NMR spectrometer operating at 75.47 MHz for the  $^{13}\text{C}$  nucleus. Samples were contained in a 7.5 mm cylindrical rotor and spun at 6.5 kHz. Wide-angle X-ray diffraction was performed on Bruker D8 Advance Diffractometer with Ni-filtered Cu K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) with  $2\theta$  angles between 10° and 55° at a scan rate 0.05°/s. The measurements of CP/MAS NMR and X-ray diffraction were carried out on powder samples of our copolymers, as the secondary structure of silk protein might vary with the nature of the solid state (powder, fiber, or film) and the conditions of preparation.<sup>19</sup> The intrinsic viscosities of copolymers were measured on an Ubbelohde capillary viscometer (i.d. = 0.85 mm) at 25.0 °C and at a concentration of 0.5 g/dL dissolved in a 1:1 (v/v) mixture of  $\text{CHCl}_3$  and  $\text{HOCH}_2\text{CH}_2\text{Cl}$ . Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were carried out (also with the powders) under nitrogen with a heating rate of 10 °C/min using a Perkin-Elmer Pysis 1 TGA and DSC thermal analysis system.

Solutions of 0.001 wt % copolymers in 2:1 v/v  $\text{CHCl}_3/\text{HOCH}_2\text{CH}_2\text{Cl}$  were spotted on carbon film-coated 300 mesh copper grids and dried for one week at room temperature for TEM observation. They were examined both unstained and after staining with 1% w/v Congo red in methanol for 15 min before washing in deionized water and air-drying. Congo red was used because this dye is capable of intercalating between  $\beta$ -strands in  $\beta$ -sheet crystallites in proteins.<sup>20</sup> A Hitachi H-600 TEM was operated at 75 kV.

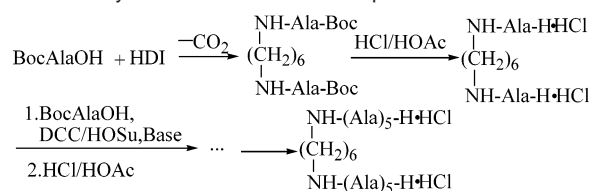
Gel permeation chromatography (GPC) was performed in tetrahydrofuran (THF) at 35 °C with an elution rate of 1.0 mL/min on an Agilent 1100 with a G1310A pump, a G1362A refractive index detector, and a G1314A variable wavelength detector. A 5  $\mu\text{m}$  LP gel column (500  $\text{\AA}$ , molecular weight range 500–20 000 g/mol) and a 5  $\mu\text{m}$  LP gel mixed bed column (molecular weight range 200–3 000 000 g/mol) were both calibrated using polystyrene standard samples.

**Polymer Synthesis.** *Boc-NHCH(CH<sub>3</sub>)CONH(CH<sub>2</sub>)<sub>6</sub>NHCOCH(CH<sub>3</sub>)-NH-Boc (I).* BocAOH (11.40 g), chloroform (50 mL), and *N*-methylmorpholine (0.67 mL) were added to a 100 mL flask. HDI (5.0 g) dissolved in 15 mL chloroform was added dropwise within 10 min with continuous stirring and left at room temperature for 4 h before refluxing for 10 h. After cooling to room temperature, the mixture was washed with aqueous 10 wt % citric acid followed by aqueous saturated NaCl solution and then dried with anhydrous sodium sulfate, which was then filtered off before removing the solvent under vacuum. The resulting light yellow sticky material, compound **I**, was obtained (12.9 g, yield 94.0%). FTIR spectra of the compound **I** gave peaks at 3344, 3317, 2983, 2937, 1683, 1658, 1525, 1448, 1253, and 1175  $\text{cm}^{-1}$ . Compound **I** dissolved in DMSO showed  $^1\text{H}$  NMR (500 MHz) chemical shifts (ppm) of 1.15 (m, 4H,  $\text{CH}_2$  of  $(\text{CH}_2)_2\text{CH}_2\text{CH}_2(\text{CH}_2)_2$ ), 1.23 (m, 4H,  $\text{CH}_2$  of  $\text{CH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_2\text{CH}_2$ ), 1.37 (s, 18H,  $\text{CH}_3$  of Boc), 3.90 (m, 2H, CH of Ala), 6.78 (m, 2H, NH of Ala), 7.67 (m, 2H, NH of  $\text{NH}(\text{CH}_2)_6\text{NH}$ ).

*HCl NH<sub>2</sub>CH(CH<sub>3</sub>)CO-NH(CH<sub>2</sub>)<sub>6</sub>NH-COCH(CH<sub>3</sub>)NH<sub>2</sub> HCl (II).* Compound **I** (10.0 g) was dissolved in 15 mL of chloroform, and then, 50 mL of hydrochloride-saturated acetic acid solution was added to remove the protective group (*N*-*tert*-butoxycarbonyl, 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 compound **II** was collected, washed three times with chloroform, and dried in a vacuum at 60 °C for 24 h (5.6 g, yield 92.4%). FTIR spectra showed peaks or shoulders ( $\text{cm}^{-1}$ ) at 3281, 3111–2865, 1658, 1567, 1446, and 1280, while  $^1\text{H}$  NMR (500 MHz, DMSO) gave resonances (ppm) at 1.20–1.29 (m, 10H,  $\text{CH}_2$  of  $(\text{CH}_2)_2\text{CH}_2\text{CH}_2(\text{CH}_2)_2$  and  $\text{CH}_3$  of Ala), 1.410 (m, 4H,  $\text{CH}_2$  of  $\text{CH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_2\text{CH}_2$ ), 3.10–3.15 (m, 4H,  $\text{CH}_2$  of  $\text{CH}_2(\text{CH}_2)_4\text{CH}_2$ ), 4.22–4.25 (m, 2H, CH of Ala), 7.90–8.20 (m, 8H, NH of Ala, NH of  $\text{NH}(\text{CH}_2)_6\text{NH}$  and  $\text{NH}_3^+$  of end group).

*HCl H-(Ala)<sub>5</sub>-NH(CH<sub>2</sub>)<sub>6</sub>NH-Ala<sub>5</sub>-H HCl (III).* Compound **III** was synthesized step by step in DMF, using traditional liquid-phase peptide synthesis,<sup>21</sup> from compound **II** and BocAOH as a raw material, using DCC and HOSu as the complex condensation reagents. The Boc-

**Scheme 1.** Synthesis Process of the Peptide



protected intermediate products were purified by column chromatography (silica gel (200–300 mesh)) eluted with a 1:1:1 (v/v/v) mixture of chloroform, methanol, and acetic acid. The removal of protective group (Boc) with hydrochloride acetic acid solution and purification by Soxhlet extraction with chloroform afforded compound **III**, a light yellow powder. The total yield was about 37%. FT-IR spectra showed peaks or shoulders at ( $\text{cm}^{-1}$ ) 3281, 3070, 2983, 2935, 1690, 1655, 1632, 1545, 1448, 1375, 1247, and 117, while  $^1\text{H}$  NMR (500 MHz, DMSO) gave resonances (ppm) at 1.16–1.37 (m, 38H,  $\text{CH}_2$  of  $\text{CH}_2(\text{CH}_2)_4\text{CH}_2$ ,  $\text{CH}_3$  of Ala), 2.99–3.05 (m, 4 H,  $\text{CH}_2$  of  $\text{CH}_2(\text{CH}_2)_4\text{CH}_2$ ), 3.85–4.40 (m, 10H, CH of Ala), 7.63–8.63 (m, NH of Ala, NH of  $\text{NH}(\text{CH}_2)_6\text{NH}$ , NH of  $\text{NH}_3^+$  of end group).

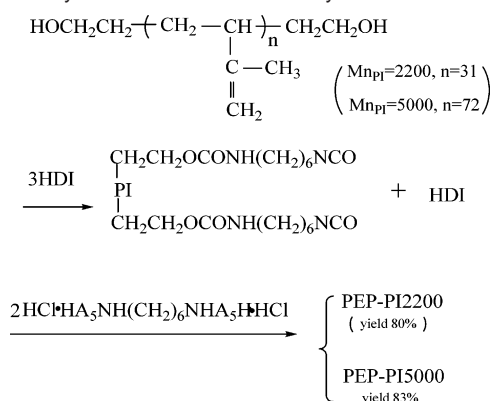
**Functional Isoprene Oligomers (HO-PI-OH).** While continuously stirring a mixture of 10 mL isoprene, 100 mL cyclohexane, and 5 mL tetrahydrofuran (THF) under dry argon, lithium naphthalene THF solution (initiator) was injected via syringe. The yellow solution was stirred for 1 h at room temperature. The solution lost its color immediately when excess dried ethylene oxide (4 times molar excess over the lithium naphthalene) was injected to add the functional group at both ends of the oligomer.<sup>22</sup> After stirring at room temperature for 4 h, 10 mL of methanol was added to terminate the reaction. The product was obtained by precipitation in methanol, washed with both water and methanol several times, and dried under vacuum for 2 days. The functional isoprene oligomers with hydroxyl at both ends (HO-PI-OH) were obtained. The molecular weight of the HO-PI-OH was determined by GPC as described above, e.g.,  $M_n = 2200$  ( $d = 1.11$ ) and  $M_n = 5000$  ( $d = 1.09$ ).

**OCN-PI-NCO (Scheme 2).** First, HO-PI-OH was dried by azeotropic distillation. Under anhydrous condition, a solution of 0.001 mol of this compound in 30 mL of chloroform was added slowly dropwise to 0.504 g of hexamethylene diisocyanate (HDI) (three times the molar quantity of the PI) and 0.05 mL of dibutyltin dilaurate over 2 h. The mixture was stirred for 10 h at room temperature. The final solution (H), should in theory contain equimolar quantities of OCN-PI-NCO and HDI. GPC results confirmed the absence of PI linked by oligomer themselves in the final product.

**Multiblock Copolymer (polypeptide-co-polyisoprene, PEP-PI).** The compound **III** (0.002 mol) was swollen in 15 mL of DMF for 10 h, and then, 0.023 mol *N*-methylmorpholine (excess) and 120 mL chloroform were added. The solution H was then slowly dripped in within 1 h under dry nitrogen. During the addition of H, the opaque solution gradually cleared. The mixture was allowed to react at room temperature for 48 h and then at 60 °C for 4 h. At the end of the reaction, the solvents were removed from the gel-like product. The crude product was washed successively with deionized water, methanol, and chloroform and vacuum-dried for 2 days at room temperature to give a faintly yellow powder.

## Results and Discussion

**Copolymerization and Physical Properties of the Silk-Like Polymers.** The compound **III**, based on the poly(alanine) blocks of the crystalline regions of spidroin I and II (MaSp1 and MaSp2) was synthesized by the traditional liquid method (Scheme 1),<sup>21</sup> while the isoprene oligomer containing two hydroxyl groups was synthesized by anionic polymerization.<sup>22</sup> The silk-like copolymer was prepared by step-growth polymerization of the compound **III** and HO-PI-OH with diisocyanate

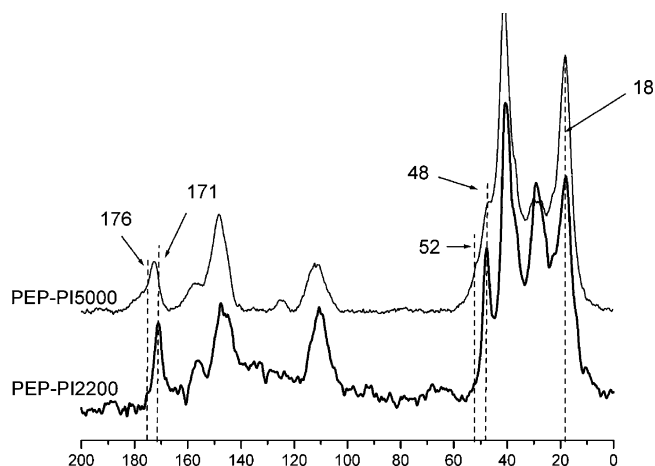
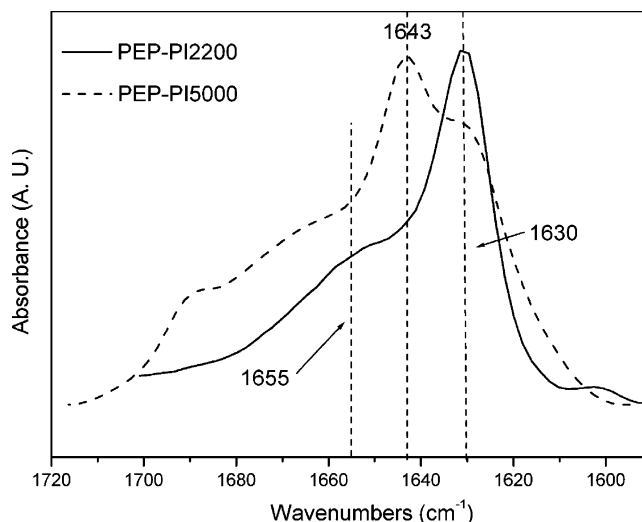
**Scheme 2.** Synthesis Process of the Polymers**Table 1.** Physical Properties of Compound **III**, Isoprene Oligomer (PI2200), and Multiblock Polymers (PEP-PI2200, PEP-PI5000)

sample	$\eta_{\text{inh}}$ (dL/g)	$T_d$ (°C, TGA)	$T_g$ (°C, DSC)
compound <b>III</b>	0.08 <sup>a</sup>		
PI2200	0.11 <sup>a</sup>	302	-15
PEP-PI2200	0.98 <sup>a</sup>	221	12
PEP-PI5000	0.76 <sup>b</sup>	210	15

<sup>a</sup> Measured at 25.0 °C in the mixture of  $\text{CHCl}_3/\text{ClCH}_2\text{CH}_2\text{OH}$  (v/v = 1:1) and the concentration of 0.5 g/dL. <sup>b</sup> Measured in the mixture of  $\text{CHCl}_3/\text{ClCH}_2\text{CH}_2\text{OH}$  (v/v = 2:1).

as the chain extender in  $\text{CHCl}_3/\text{DMF}$  and in the presence of *N*-methylmorpholine (Scheme 2). The silk-like copolymer we synthesized did not dissolve in general polar or nonpolar solvents, even in highly polar solvents including HFIP and HFA, probably as a result of the great contrast in the polarity between the (Ala)<sub>5</sub> block and PI block, as well as the strong hydrogen bonding between the (Ala)<sub>5</sub> blocks (see the discussion below). Instead, we were obliged to try complex solvent systems able to compete with the hydrogen bonds to break down the interactions between the (Ala)<sub>5</sub> blocks yet able to separate the aggregation of PI block. We were successful with a mixture of  $\text{CHCl}_3/\text{ClCH}_2\text{CH}_2\text{OH}$ . The inherent viscosity ( $\eta_{\text{inh}}$ ) measurements of the copolymers dissolved in  $\text{CHCl}_3/\text{ClCH}_2\text{CH}_2\text{OH}$  (1:1 or 2:1, depending on the molecular weight of the PI block) were used to estimate polymer molecular weights. To study the effect of the length of the oligomer on the structure of the poly(alanine) blocks, isoprene oligomers with molecular weights of 2200 and 5000 Da (whose molecular weight was confirmed by GPC) were used to synthesize PEP-PI 2200 and PEP-PI 5000, respectively. The  $\eta_{\text{inh}}$  values for PEP-PI2200 and PEP-PI5000 were 0.98 dL/g and 0.76 dL/g respectively, much larger than those of compound **III** (0.08 dL/g) and PI 2200 (0.11 dL/g). Moreover the copolymers began to decompose around 220 °C (PEP-PI2200) and 210 °C (PEP-PI5000) without melting (see Table 1). PEP-PI2200 and PEP-PI5000 displayed apparent glass transition temperatures ( $T_g$ ) at 12 °C and 15 °C respectively, probably arising from the mobile PI blocks. All of these results confirm that condensation between isoprene oligomer and the peptide had occurred as predicted. The higher  $T_g$  of the poly(alanine)-containing copolymer compared to that of isoprene oligomer (-15 °C) probably resulted from a reduction in the mobility of PI blocks in the copolymer caused by multiple hydrogen bonds between the (Ala)<sub>5</sub> blocks.

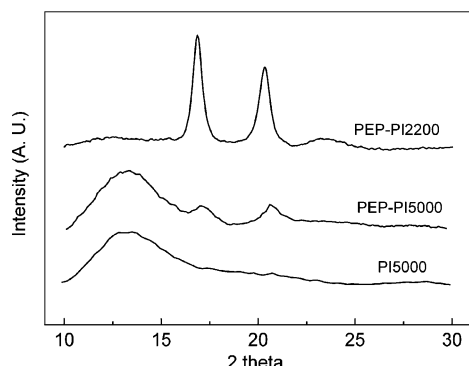
**Evidence for the Presence of  $\beta$ -Sheet Structure in the Synthetic Poly(Alanine)-Containing Copolymer.** *Solid-State  $^{13}\text{C}$  NMR.* Solid-state  $^{13}\text{C}$  NMR is widely used to demonstrate the formation of  $\beta$ -sheet in polypeptides and proteins.<sup>23,24</sup> Figure 1 shows  $^{13}\text{C}$  CP/MAS NMR spectra of the copolymers PEP-

**Figure 1.** Ala C $\alpha$ , C $\beta$ , and carbonyl regions in solid-state  $^{13}\text{C}$  CP/MAS NMR spectra of PEP-PI2200 and PEP-PI5000.**Figure 2.** FT-IR spectra (amide I) of PEP-PI2200 (solid line) and PEP-PI5000 (dash line).

PI2200 and PEP-PI5000. Because the resonance of Ala-C $\beta$  (at about 18 ppm) was overlapped by that of C<sub>5</sub> in the isoprene segment,<sup>25</sup> we only discuss the shifts of Ala-C $\alpha$  and Ala-C=O in the spectra. The resonance at 48 ppm (Ala-C $\alpha$ ) and 171 ppm (Ala-C=O) in both copolymers was attributed to  $\beta$ -sheet aggregates.<sup>26</sup> However, only in PEP-PI5000 was there a slight shoulder peak at 51–52 ppm and 176 ppm, suggesting the coexistence of other conformations for Ala-C $\alpha$  and Ala-C=O in this copolymer.

*FT-IR.* FT-IR has proven useful for analyzing the conformations of proteins and peptides and their secondary structural transitions, with the carbonyl stretching frequencies of amide (amide I) being the most sensitive to secondary structures in the solid state.<sup>27–29</sup> The antiparallel  $\beta$ -sheet conformation in both *B. mori* fibroin<sup>30</sup> and *Nephila* spidroin<sup>31</sup> gives rise to a strong infrared band around 1630 cm<sup>-1</sup> and a weaker band at 1680–1690 cm<sup>-1</sup>. We observed a peak in the amide I region at 1630 cm<sup>-1</sup> in the copolymer PEP-PI2200 (Figure 2) providing strong evidence for the antiparallel  $\beta$ -sheet conformation in the solid state. Except for the components around 1630 cm<sup>-1</sup> characteristic of the antiparallel  $\beta$ -sheet conformation, only a weak component around 1655 cm<sup>-1</sup> attributed to non- $\beta$ -sheet conformations was observed. Thus, FT-IR spectroscopy also indicated the predominance of the antiparallel  $\beta$ -sheet conformation in the copolymer PEP-PI2200; thus, the conformation





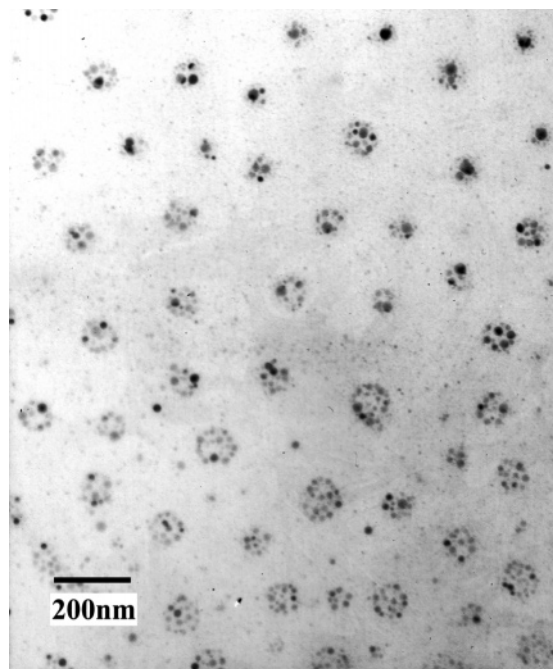
**Figure 3.** Wide-angle X-ray diffraction patterns of PEP-PI2200, PEP-PI5000, and PI5000.

of poly(alanine) blocks whether in our synthetic copolymer or spider dragline silk is similar.

The FT-IR spectrum of PEP-PI5000 (Figure 2) showed bands 1630 and 1689  $\text{cm}^{-1}$  attributable to antiparallel  $\beta$ -sheet conformation. However, other peaks, such as 1655, 1663, 1675, and 1643  $\text{cm}^{-1}$  indicate that other conformations may coexist in this copolymer. These observations suggest that the peptide in the copolymer with the smaller molecular weight PI showed much more selective assembly into the antiparallel  $\beta$ -sheet conformation than the peptide with the larger PI.

**Wide-Angle X-ray Diffraction.** Figure 3 shows the WAXD patterns of copolymers PEP-PI2200 and PEP-PI5000 and oligomer PI5000, respectively. Two diffraction peaks with  $d$  spacing of 5.29 Å ( $2\theta = 16.9^\circ$ ) and 4.39 Å ( $2\theta = 20.4^\circ$ ) could be detected in both multiblock copolymers, similar to the values reported for the antiparallel  $\beta$ -sheet of *N. clavipes* dragline silk (5.3 Å)<sup>32</sup> and other synthetic silk-like copolymers containing poly(alanine) (5.28 Å, 4.4 Å).<sup>15,18,33</sup> These peaks were particularly prominent in PEP-PI2200 but were undetectable in the oligomer PI5000. Thus, powder X-ray diffraction analysis confirmed the existence of the antiparallel  $\beta$ -sheet in both our synthetic silk-like copolymers. However, a larger and broader amorphous scattering peak with a maximum at about  $2\theta = 13^\circ$  was prominent in PEP-PI5000 and in the oligomer PI5000. This suggested that the poly(alanine) segments form much less crystalline domains in PEP-PI5000, probably as a result of the predominance of the longer PI block.

**TEM Observations.** The multiblock copolymer of PEP-PI has two kinds of segments with different polarity. Thus, it forms micelles in the selective solvent as common amphiphilic block copolymers and can be detected by TEM. Figure 4 is the TEM image of PEP-PI2200 that shows similar micellar-like structures as in poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide).<sup>34</sup> In the case of our experimental condition, electron-dense material was arranged in a remarkably regular pattern ("plum blossom") consisting of circular patches with a fairly constant diameter (80–110 nm) containing a variable number of dense granules (10–20 nm in diameter). The circular patches were rather uniformly distributed with a center-to-center separation of 10–20 nm. A few small, dense granules were also seen between the circular patches. We may suggest that they are micelles because of the circular profile and remarkably uniform diameter. On the other hand, such a pattern was not seen in PI-PEP5000 (image not shown), probably because of the long PI segments. Furthermore, Congo red was applied to stain the samples, as it was thought to be capable of intercalating between  $\beta$ -strands in  $\beta$ -sheet crystallites in proteins.<sup>20</sup> However, the image of Congo red stained samples did not have any difference



**Figure 4.** TEM image of unstained film prepared from 0.001% PEP-PI2200 copolymer in 2:1 v/v  $\text{CHCl}_3/\text{HOCH}_2\text{CH}_2\text{Cl}$ . This is thought to show a micellar-like aggregation.

from unstained one. This may imply that the electron-dense part in the images contains both (Ala)<sub>5</sub> and PI segments.

## Conclusions

By taking advantage of the high reactivity of diisocyanates and by copolymerizing bis(oligopeptides) and isoprene oligomers, we have synthesized spidroin-like multiblock copolymers containing (Ala)<sub>5</sub> blocks derived from the crystalline region of spider dragline silk. We have used FT-IR and solid-state <sup>13</sup>C CP/MAS NMR spectroscopy to demonstrate that the  $\beta$ -sheet conformation predominates in our synthetic polymers. Our X-ray data provides conclusive evidence for the formation of antiparallel  $\beta$ -sheet crystalline domains. Of the two copolymers we synthesized, the one with the shorter isoprene blocks formed micellar-like aggregation under certain conditions. Thus, our synthetic copolymers show not only similar structure and behavior to that of spider dragline silk protein, but also tunable assembly, for example, by varying the length of the soft block. Thus, it may eventually prove possible by conventional chemical routes to synthesize block copolymers capable of directed assembly into tough nanofibrillar composites resembling spider silk.

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