Self-Assembly of a Polypeptide Multi-Block Copolymer Modeled on Dragline Silk Proteins

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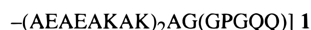
Arachnid dragline silk fibers are composed of naturally occurring protein polymers in which biological control of the polypeptide sequence affords a material with mechanical properties that exceed those of conventional synthetic fibers.[1] However, the sequences of silk proteins have been evolutionarily selected for in vivo formation of the dragline fiber, and are not necessarily amenable to conventional fabrication techniques.[2] The well-defined modular structure of these proteins[3] suggests potential opportunities for engineering of novel protein polymers based on dragline silk sequences that emulate the properties of natural materials while enhancing the in vitro processing characteristics. We report herein the self-assembly of a biosynthetic multi-block protein polymer **1** modeled on the sequences of dragline silk proteins, which spontaneously forms a self-supporting macroscopic film via rearrangement of segments within the polypeptide from α-helices to β-strands.[4]



$$[\{(\mathrm{AEAEAKAK})_2\mathrm{AG}(\mathrm{GPGQQ})_6\mathrm{GS}\}_9 - (\mathrm{AEAEAKAK})_2\mathrm{AG}(\mathrm{GPGQQ})]\ \mathbf{1}$$

Dragline silk proteins are composed of alternating sequences of conservatively substituted alanine-rich and glycine-rich oligopeptide segments.[5] Recent spectroscopic[6,7] and X-ray diffraction[8] studies of *N. clavipes* dragline fiber suggest that individual chain segments of the silk fibroins segregate into conformationally asymmetric domains,[1] and that this mosaic structure underlies the tensile properties of the fibers.[9] The design of protein polymer **1** mimics the modular arrangement and relative composition of alanine-rich and glyine-rich domains within dragline silk proteins. The sequence of the alanine-rich block is derived from a self-complementary, amphiphilic oligopeptide $(\mathrm{AEAEAKAK})_2$,[10] which adopts a β-strand conformation that persists in dilute aqueous solution over a wide range of pH and temperature. In contrast, most β-sheet forming oligopeptides, including the alanine-rich domains of dragline silk proteins, adopt random coil conformations under similar conditions.[12] The strong preference for β-sheet formation of this sequence should facilitate segregation between the two domains of **1**. The glycine-rich segments of **1** consist of six repeats of the pentapeptide sequence (GPGQQ) derived from *A. diadematus* dragline silk fibroin.[5b] The central Pro(2)-Gly(3) unit, which occurs in the consensus repeats of elastomeric polypeptides including elastin, glutenin, and flagelliform silk,[13−15] has a high propensity for the formation of type II β-turns.[16] Biophysical studies of synthetic elastin analogues indicate that the formation of β-turns within the repeats coincides with the development of elastomeric restoring force in the material.[13]

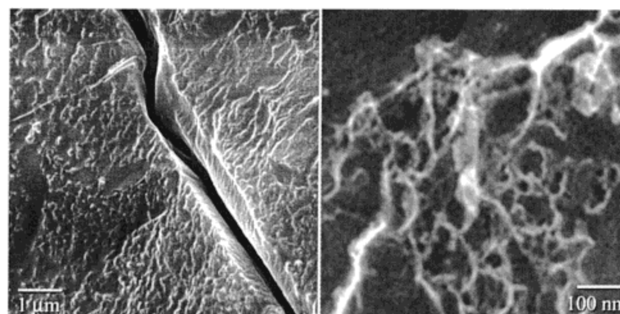


Figure 1. Cryo-HRSEM images of a water-swollen membrane derived from self-assembly of **1**.

The synthesis of **1** involved the construction and expression of a concatameric gene encoding a protein polymer based on a 150 base pair (50 amino acid) repeat sequence. A repetitive gene of 1350 bp was isolated from self-ligation[17] of an oligonucleotide cassette and inserted into a modified version of plasmid pET-19b. Expression of the synthetic gene from this recombinant plasmid in *E. coli* strain BLR(DE3) afforded **1** as a C-terminal fusion to a decahistidine tag. The fusion protein was isolated from the bacterial cell lysate (ca. 50 mg/L of culture) by immobilized metal affinity chromatography. Protein polymer **1** was liberated from the N-terminal leader sequence by cyanogen bromide cleavage.

Protein **1** spontaneously assembled into a transparent, self-supporting membrane upon concentration from aqueous solution. The FTIR spectra of lyophilized membranes displayed absorptions characteristic of antiparallel β-sheet (Amide I, 1693, 1624 cm^{-1}; Amide II, 1528 cm^{-1}; Amide III, 1225 cm^{-1}) and β-turn (Amide I, 1670, 1638 cm^{-1}; Amide II, 1665, 1648 cm^{-1}; Amide III, 1285 cm^{-1}) structures.[18] The spectroscopic evidence suggested that self-assembly of the polypeptide into a membrane occurred via formation of β-sheet segments between the alanine-rich domains of **1**. High resolution (field emission) scanning electron microscopy (HRSEM) of a cryo-immobilized, water-swollen membrane revealed a network of fibrils, approximately 10−20 nm in diameter, interspersed within a less structured matrix (Figure 1). The dimensions of the fibrils corresponded to those observed

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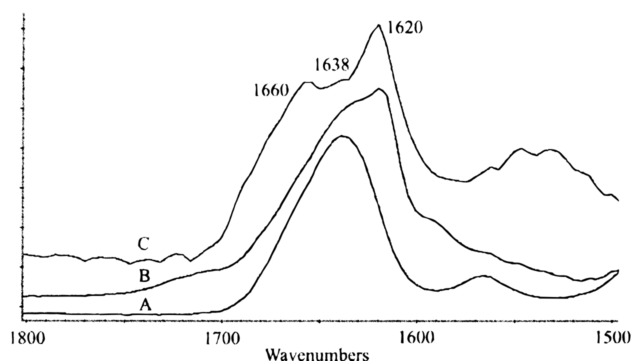

Figure 2. Attenuated total reflectance FTIR spectra of the Amide I region of **1** in D_2O during self-assembly: (A) initial specimen, (B) specimen after 30 min at 37 °C, and (C) the final membrane.

within films derived from self-assembly of $(AEAEAKAK)_2$,[11] which aggregated via the formation of β-sheet crystallites.

The conformational properties of **1** prior to self-assembly differed significantly from those of the membranes. The FTIR (Amide I, 1658 cm^{-1}; Amide II, 1548 cm^{-1}) and CD (minima at 206 and 221 nm in water) spectroscopic data indicated the presence of α-helix,[18,19] which was not detected in the corresponding spectra of the membranes. In situ attenuated total reflectance (ATR) FTIR spectroscopy of **1** in D_2O suggested that segments of the polypeptide undergo conformational rearrangement from α-helices to β-strands prior to self-assembly into the membrane (Figure 2). Protein **1** initially displayed an absorption maximum at 1638 cm^{-1}, which was separated into α-helix (1645 cm^{-1}) and turn (1663, 1632 cm^{-1}) contributions in the second derivative spectrum. These features slowly disappeared and were gradually replaced by an absorption at 1620 cm^{-1}, which was attributed to the formation of β-sheet. Formation of the membrane resulted in prominent IR absorptions at 1620 and 1660 cm^{-1} that were consistent with a high content of β-sheet and turn structures.

To confirm the identity of the segment undergoing the structural transition, the alanine residues within **1** were isotopically enriched at the structurally sensitive carbonyl positions. The synthetic gene was expressed in M9AA medium supplemented with 1-^{13}C-L-alanine to produce the labeled protein **1A**. Six resonances were observed in the solution ^{13}C NMR spectrum for the alanine carbonyl groups at 180.9, 180.8, 180.7, 180.5, 180.1, and 178.3 ppm.[20] These resonances were shifted downfield from the random coil chemical shift of the alanine carbonyl group (177.8 ppm),[21] which suggested that the alanine-rich segments formed α-helices in aqueous solution in agreement with the FTIR and CD data. The solid-state CP/MAS ^{13}C NMR spectrum of lyophilized specimens of **1A** displayed a composite resonance at 178.8 ppm for the labeled carbonyl groups (Figure 3). The chemical shift of this resonance was within the expected region for carbonyl groups of alanine residues in an α-helical conformation (cf. α-polyalanine, 179.4 ppm).[5a]

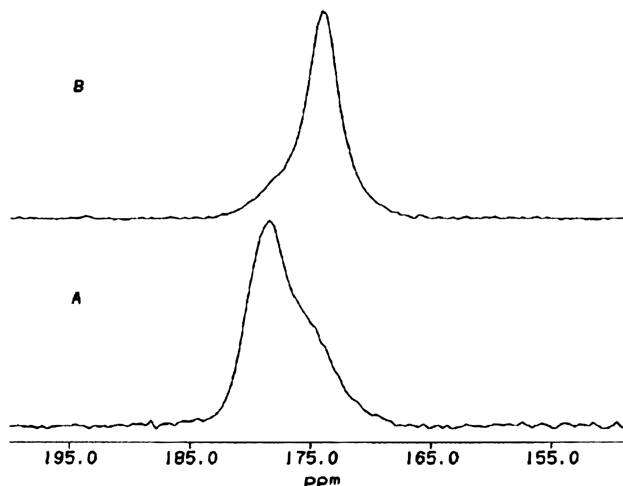

Figure 3. Solid state ^{13}C CP/MAS NMR spectra of lyophilized specimens of **1A** before (A) and after (B) formation of the membrane.

Membranes derived from **1A** displayed spectroscopic features consistent with rearrangement of the labeled alanine-rich segments during self-assembly. The Amide I absorption of the β-sheet in the labeled membrane shifted to 1610 cm^{-1} (vs 1624 cm^{-1} for **1**) due to isotopic substitution of the carbonyl groups of the alanine residues. In contrast, the Amide I absorptions of the turn sequences remained in the positions originally observed in the FTIR spectra of membranes derived from **1**. In addition, the solid-state ^{13}C CP/MAS NMR spectrum of the labeled membranes indicated a shift in the position of the alanine carbonyl resonance after self-assembly (Figure 3). The chemical shift of the ^{13}CO-labeled carbonyl group was observed at 174.9 ppm, which was within the expected range for a β-sheet conformation (cf. 174.5 ppm for β-polyalanine).[5a] These results clearly demonstrated that self-assembly of **1** proceeded through conformational rearrangement of the amphiphilic alanine-rich segments from α-helices to β-strands. Formation of β-sheet structure induced irreversible aggregation of the polypeptide into a hydrogel network through inter-strand hydrogen bonding interactions between chain segments. The alanine-rich segment acts as a conformational switch within the context of the protein polymer,[22] and its rearrangement provides a facile mechanism for fabrication of the polypeptide through manipulation of conditions that influence the relative stability of α-helix versus β-sheet.

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Supporting Information Available: Synthetic and spectroscopic information (CD, FTIR, ^{13}C NMR) for protein polymer **1** and **1A** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Solution ^{13}C NMR spectra were recorded in D_2O on a Varian INOVA 400 NMR spectrometer. Solid-state CP/MAS spectra were recorded on a Bruker DSX-400 spectrometer at a spinning speed of 10 kHz and a contact time of 1 ms. Chemical shift data are reported relative to the methyl carbons of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

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