Self-assembly of the peptide amphiphile based on hydrolysed *Bombyx mori* silk fibroin

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**Experiments**

**Hydrolysis of the fibroin solution**

10 mL silk fibroin aqueous solution (4 wt %) was diluted to 100 mL using Tris-HCl buffer (pH 8.0). The silk fibroin was hydrolysed by adding 4 mg chymotrypsin into the diluted solution. The mixture was incubated at 37°C for 8 hours, followed by centrifugation to discard the precipitate. The supernatant was heated at 100°C for 5 minutes to inactivate the remaining enzyme, and then concentrated and lyophilized to obtain the silk peptide powder.

**Separation and purification of GAGAGAGY from hydrolysis product**

150 mg silk peptide powder was dissolved in 10 mL distilled water. Each time 600 µL peptide solution was injected for High performance Liquid Chromatography (HPLC) separation. Purification was performed with a Waters Autopurification System for liquid-phase chromatography-electrospray ionization mass spectrometry (LC/ESI-MS) Analyses (Waters 2545 Binary Gradient Module; 515 HPLC Pump; 2489 UV/Visible Detector; 3100 Mass Detector and 2767 Sample Manager; a 10 µm C18 250×19 mm stainless steel Waters XBridge™ BEH300 preparative reversed phase HPLC column). The mobile phase consisted of 0.1% TFA dissolved in acetonitrile/water and the gradient elution condition was listed in Table S1. The chromatograms were monitored concurrently at 214 nm and 254 nm, and the fraction eluted at 12.6 min (Figure S1) was collected, rotary evaporated, and lyophilized. The purity was checked on a Waters Autopurification System for Liquid-phase chromatography/Mass-Spectrum Analysis.
using a 5 µm C18 250×4.6 mm stainless steel Waters XBridgeTM BEH300 analytical reversed phase HPLC column (Figure S2). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrum analysis was carried out on Voyager DE-STR using α-Cyano-4-hydroxycinnamic acid (CHCA) as the matrix. The result indicated no significant impurities (Figure S3).

**Synthesis of C_{12}-GAGAGAGY**

0.24 mmol dodecanoic acid was dissolved in 20 mL DMF. After addition of 0.24 mmol TBTU (O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate) and 0.24 mmol DIEA (diisopropylethylamine), the solution was stirred at room temperature for 20 min to activate the carboxyl group of dodecanoic acid. Then 0.2 mmol GAGAGAGY was dissolved in 10 mL DMF and added to the solution dropwise. The reaction lasted for 3 h at 0 °C. The crude C_{12}-GAGAGAGY was loaded on the preparative RP-HPLC column and purified with a linear gradient of acetonitrile/water solution (55/45 to 45/55, containing 0.1% TFA). The purified peptide product was characterized by liquid chromatography-electrospray ionization mass spectrometry (LC/ESI-MS) and MALDI-TOF (Figure S4 and S5).

**Preparation of C_{12}-GAGAGAGY sample solution**

Stock peptide amphiphile aqueous solution (1 mg/mL) was prepared by dissolving the fibroin peptide amphiphile into 50 mM Tris-HCl buffer (pH 9.0). Then the stock solution was diluted to 0.1 mg/mL and 0.3 mg/mL using the same Tris-HCl buffer. The corresponding solution of pH 4 was adjusted by 10% HCl.

**Atomic Force Microscope (AFM)**

An aliquot (5 µL) of the sample solution (1 mg/mL at pH 9; 1 mg/mL, 0.3 mg/mL or 0.1 mg/mL at pH 4) was pipetted on to freshly cleaved mica and dried at room temperature before imaging. The AFM images were collected in tapping mode using a NanoscopeIV equipped with a 10 µm×10 µm scanner. The scanning speed was at a line frequency of 1 Hz. The images were saved at a resolution of 512×512 points.
Circular Dichroism (CD)

Measurements were performed on a JASCO J715 CD spectropolarimeter using a quartz cell with the 1 mm path length at 25 °C. The peptide amphiphile solutions at pH 4 and pH 9 were studied at a concentration of 1 mg/mL. Each spectrum was an average of ten scans from 190 nm to 260 nm at a bandwidth of 1 nm.

Transition Electron Microscopy (TEM)

Samples for TEM observation were prepared by pipetting one droplet of the peptide amphiphile solutions onto copper TEM grids (1 mg/mL, pH 4 and pH 9). Then the grids were stained with uranyl acetate for 1 min before excess stain was blotted. Prior to imaging, all the samples were dried for 2 hours in air. The samples were imaged under a Hitachi H-600 TEM operated at 75 kV.

Fourier Transform Infrared Spectroscopy (FTIR)

Transmission FTIR absorption spectra were collected on a Nicolet Nexus-470 spectrometer with a resolution of 4 cm\(^{-1}\). 20 μL of 1 mg/mL peptide amphiphile solutions at different pH values were spread on a CaF\(_2\) plate and vacuum dried. Each spectrum was an average of 64 consecutive scans.

Cryo-scanning electron microscopy (Cryo-SEM)

1 mg/mL peptide amphiphile solution at pH 9 was dipped on a concave copper sample holder and dropped into liquid nitrogen. The Cryo-SEM images were recorded by using a Hitachi S-4800 with 1 kV voltage after gold spray.

Zeta-potential measurements

The zeta-potential analysis of 1 mg/mL peptide amphiphile solution that containing 50mM Tris-HCl at pH 4 and pH 9 was carried out on a Zetasizer Nano from Malvern Instruments Ltd. The temperature was kept at 25 ± 1°C.
Figure S1. Preparative HPLC chromatogram of the silk fibroin hydrolysis product
a) Analytical LC chromatogram of GAGAGAGY

b) Electrospray ionization mass spectrum of GAGAGAGY

Figure S2. LC/ESI-MS analysis of GAGAGAGY
Figure S3. MALDI-TOF mass spectrum of GAGAGAGY
a) Analytical LC chromatogram of C_{12}-GAGAGAGY

b) Electrospray ionization spectrum of C_{12}-GAGAGAGY

Figure S4. LC/ESI-MS analysis of C_{12}-GAGAGAGY
Figure S5. MALDI-TOF mass spectrum of C$_{12}$-GAGAGAGY
Figure S6. Section analysis of the AFM image about the cylinder fibers at pH 9. The initial concentration was 1 mg/mL.
Figure S7. Cryo-SEM of the networks formed by $\text{C}_{12}$-GAGAGAGY at pH 9 (concentration=1 mg/mL)
Figure S8. TEM image of nanofibers formed at pH 6. The coexistence of cylindrical nanofibers (indicated by white arrow) and twisted ribbons (indicated by black arrow) was observed.
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