Supporting Information

Title: Photoresponsive Retinal-Modified Silk-Elastin Copolymer

Authors: Zhongyuan Sun, Guokui Qin, Xiaoxia Xia, Mark Cronin-Golomb, Fiorenzo G. Omenetto, and David L. Kaplan

EXPERIMENTAL SECTION

Biosynthesis of silk-elastin-like polymer PS2E8K.

Construction of expression plasmids. DNA sequences were designed to encode the silk-elastin-like sequence: [$(GAGAGS)_2(GVGVP)_4(GKGVP)(GVGVP)_3$] using our previously reported procedures.[1] Monomer DNA sequences were purchased and cloned into the *Eco*RV site of the vector pUC57 from GenScript (GenScript USA Inc., Piscataway, NJ). The *Ban*II restriction sites were designed to flank the monomer DNA sequence. The monomer DNA sequence was liberated by digesting the pUC57 derivatives with *Ban*II, isolated by preparative gel electrophoresis, and purified using the QIAquick Gel Extraction kit (Qiagen, Valencia, CA). The purified monomer DNA was then self-ligated with T4 DNA ligase for 8 h at 16 °C to yield DNA multimers. Next, the *Ban*II and alkaline phosphatase treated pET-19b3 plasmid was added to the reaction mixture and incubated for an additional 16 h. The ligation mixture was then used to transform chemically competent cells of *Escherichia coli* DH5α (Invitrogen, Carlsbad, CA). The resulting transformants contained recombinant plasmids with repetitive genes of varying lengths. These expression plasmids were identified by restriction digests with *Nco*I and *Bam*HI and confirmed by dideoxy sequencing with both forward and reverse primers based on the T_7 promoter and terminator sequences (Tufts Core Facility, Boston, MA). The pET-19b3-derived expression plasmid also encodes an N-terminal hexahistidine tag that allows protein purification via metal chelating affinity chromatography.

Protein Expression and Purification. The plasmids PS2E8K were used to transform chemically competent cells of *E. coli* strain BL21Star (DE3) (Invitrogen, Carlsbad, CA). Protein expression and purification were performed as reported previously.[2-4] Briefly, the recombinant strains were grown in 1 L Luria–Bertani medium with 100 μg/ml ampicillin at 37 °C in a rotary shaker at 250 rpm. At $O.D._{600\ nm}$ of 0.6 to 0.8, protein expression was induced with 1 mM IPTG (isopropyl β-D-thiogalactoside) (Fisher Scientific, Hampton, NH). After induction for 6 h, cells were harvested by centrifugation at 9,000*g* for 20 min at 4 °C. Protein purification was performed under denaturing conditions on a Ni-NTA resin (Qiagen, Valencia, CA) using the manufacturer's guidelines. Briefly, the cell pellets were resuspended in lysis buffer (100 mM NaH_2PO_4, 10 mM Tris·HCl, 8 M urea, pH 8.0) overnight. The resuspension was centrifuged at 9,000*g* for 30 min and 15 °C. The resulting supernatant was loaded onto a column with Ni-NTA resin that had been equilibrated with the denaturing lysis buffer. The column was then washed and eluted with buffers (100 mM NaH_2PO_4, 10 mM Tris·HCl, 8 M urea) at pH 6.3, 5.9 and 4.5, respectively. The purified proteins were dialyzed (MWCO 3.5 kDa) against deionized water for 5 days. Protein concentrations were measured using a Pierce BCA Protein Assay kit (Thermo Scientific, Rockford, IL). Dialyzed proteins were lyophilized using a LabConco Lyophilizer. Purity and recovery rates were assessed by SDS-PAGE on 4-12% Bis-Tris precast gels (Invitrogen, Carlsbad, CA). The molecular weights of the purified proteins were confirmed via matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Tufts Core Facility, Boston, MA).

Chemical modification and determination of retinal-modified PS2E8K. Chemical modification of PS2E8K was conducted as a dehydration reaction between the aldehyde functional groups of all-*trans* retinal molecules and the amine side chains of the lysine residues.[5-7] The reaction was carried out by dissolving 5 mg (0.113 μmol) of silk-elastin polymer protein, PS2E8K, in 500 μl of anhydrous DMSO, and dissolving 1.61 mg (5.65 μmol) of all-*trans* retinal (Sigma-Aldrich, St. Louis, MO) in 500 μl of anhydrous DMSO. Three mg of triethylamine hydrochloride dissolved in 10 μl of ultrapure water was added into the PS2E8K/DMSO solution, and then the resulting PS2E8K/triethylamine solution was added to the retinal/DMSO solution, with an overnight chemical reaction in the dark at room temperature. The mixture was then freeze-dried to remove the DMSO using a LabConco Lyophilizer for 5 days. Excess all-*trans* retinal molecules were removed by dissolving 5 mg of product in 1 ml of ultrapure water, and centrifuging the solution to precipitate un-reacted retinal molecules. The concentration of resulting solution was determined by BCA assay.

^{1}H-NMR. ^{1}H-NMR of the silk-elastin polymer and retinal-modified silk-elastin polymers was analyzed with a 500 MHz Bruker Advance III NMR spectrometer.[5,8-10] All the chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO). The retinal-modified sample solutions were lyophilized and then re-dissolved to a concentration of 5 mg/ml in DMSO-d_6 and compared to silk-elastin polymers without retinal modification at the same concentration.

Biophysical Characterization of retinal-modified silk-elastin-like polymer PS2E8K.

Fourier transform infrared spectroscopy (FTIR). The PS2E8K biopolymers were freeze-dried and the structural characteristics were observed using FTIR as previously reported.[2-4] For each measurement, 128 scans were co-added and Fourier transformed employed a Genzel-Happ apodization function to yield spectra with a nominal resolution of 4 cm^{-1}. The frequency ranged from 400 to 4000 cm^{-1}. To identify structures of protein samples from the absorption spectra, we obtained the positions of

the absorption band maxima from Fourier self-deconvolution performed by using the Opus 5.0 software (Bruker) as described previously.[2-4] The fractions of secondary structural components were evaluated using Fourier self-deconvolution (FSD) of the infrared absorbance spectra, including random coil, alpha-helices, beta-sheets and turns. FSD of the infrared spectra covering the amide I region (1595 - 1705 cm^{-1}) was performed by Opus 5.0 software. The average percent composition of the secondary structures was assessed by integrating the area of each deconvoluted curve and then normalizing this value to the total area of the amide I region. The assignment of adsorption peaks in amide I band was as follows: broad peak centered at 1640–1660 cm^{-1} to random coil or helical conformation, or both; peak from 1615 to 1640 cm^{-1} to β-sheet conformation; the peak from 1660 to 1695 cm-1 to β-turn conformation. All figures presented in this work show only raw (unsmoothed) absorption data corrected by background subtraction.[2-4]

Circular dichroism (CD). CD spectra were also studied for the secondary structure of PS2E8K samples as previously described.[2,3] All spectra were recorded on an Aviv model 410 spectrophotometer equipped with a Peltier temperature controller (Aviv Biomedical, Lakewood, NJ) using a 0.5 mm path-length quartz cell. Spectra were obtained from 260 to 190 nm at a resolution of 0.5 nm for PS2E8K biopolymer samples with the concentration of 0.5 mg/ml. The CD spectra represented the average of three measurements and were finally smoothed using Aviv smoothing program (Aviv Biomedical, Lakewood, NJ). CD data are reported as mean residue ellipticity ([θ], deg cm^2 $dmol^{-1}$).

Optical studies and spectral measurements. Thin films of retinal-modified PS2E8K were prepared by dropping 1 ml of 5 mg/ml solution onto glass microscope slides and allowing them to air dry. Optical birefringence was assessed using a standard pump and probe setup with a 1 mm radius 488 nm pump polarized at 45° to the horizontal and a probe comprising a circularly polarized 632.8 nm He-Ne laser beam as reported previously.[11]

REFERENCES

(1) Xia, X. X.; Xu, Q.; Hu, X.; Qin, G.; Kaplan, D. L. *Biomacromolecules* **2011**, *12*, 3844.

(2) Qin, G.; Hu, X.; Cebe, P.; Kaplan, D. L. *Nat. Commun.* **2012**, *3*, 1003.

(3) Qin, G.; Lapidot, S.; Numata, K.; Hu, X.; Meirovitch, S.; Dekel, M.; Podoler, I.; Shoseyov, O.; Kaplan, D. L. *Biomacromolecules* **2009**, *10*, 3227.

(4) Qin, G.; Rivkin, A.; Lapidot, S.; Hu, X.; Preis, I.; Arinus, S. B.; Dgany, O.; Shoseyov, O.; Kaplan, D. L. *Biomaterials* **2011**, *32*, 9231.

(5) Murphy, A. R.; Kaplan, D. L. *J Mater. Chem.* **2009**, *19*, 6443.

(6) Serban, M. A.; Panilaitis, B.; Kaplan, D. L. *J. Biomed. Mater. Res. A* **2011**, *98*, 567.

(7) Serban, M. A.; Kaplan, D. L. *Biomacromolecules* **2010**, *11*, 3406.

(8) Murphy, A. R.; St John, P.; Kaplan, D. L. *Biomaterials* **2008**, *29*, 2829.

(9) Xu, L.; Tremblay, M. L.; Meng, Q.; Liu, X. Q.; Rainey, J. K. *Biomol. NMR Assign.* **2012**, *6*, 147.

(10) Yazawa, K.; Yamaguchi, E.; Knight, D.; Asakura, T. *Biopolymers* **2012**, *97*, 347.

(11) Cronin-Golomb, M.; Murphy, A. R.; Mondia, J. P.; Kaplan, D. L.; Omenetto, F. G. *Journal of Polymer Science Part B-Polymer Physics* **2012**, *50*, 257.