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Silk–elastinlike protein polymer hydrogels: Influence of monomer sequence on physicochemical properties

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ABSTRACT

Silk–elastinlike protein polymer, SELP-815K, with *eight* silk and *fifteen* elastin units and a lysine (K) modified elastin, was genetically engineered with longer silk and elastin units compared to existing hydrogel forming analogs (SELP-415K and SELP-47K). Hydrogels of the three SELPs (with similar MWs) were investigated for their structure–function relationships. Results indicate that equilibrium swelling ratio in these hydrogels is a function of polymer structure, concentration, cure time and ionic strength of media. Swelling was not influenced by the changes in pH. Storage moduli observed by dynamic mechanical analysis and the Debye–Bueche correlation length obtained from small-angle neutron scattering provided structural insight that suggests the cross-linking densities in these hydrogels follow the order SELP-47K > SELP-815K > SELP-415K. These results allude to the importance of the length of elastin blocks in governing the spacing of the cross-linked hydrogel network and that of silk in governing the stiffness of their 3-dimensional structures.

1. Introduction

The majority of polymeric matrices used for biomedical applications are either naturally occurring or chemically synthesized. Natural polymers are generally biocompatible and have proven efficacies in certain applications including drug delivery and tissue engineering. However, they are not amenable to structural modifications of their architecture for specific needs. Chemical synthesis, on the other hand allows for customization of polymer structure. Chemical synthesis, however, often results in random copolymers with unspecified monomer sequences and statistical distribution of molecular weights. This limits the ability to correlate polymer structure with function and has prompted the need for exploration of novel synthetic strategies that allow exquisite control over monomer sequence and polymer length [1].

Advances in recombinant DNA technology have inspired the development of genetically engineered protein polymers that offer the advantages of biocompatibility of natural polymers on one hand and control over polymer architecture on the other hand [2–4]. These polymers are stereoregular, monodisperse and can be precisely engineered to have desired structure(s), sequence(s) and thereby envisioned functionalities [4,5]. Their unique structural features have been explored extensively and applied in the fields of drug and gene delivery and tissue engineering [6,7]. One unique

Abbreviations: A (Ala), Alanine (amino acid); ANOVA, Analysis of variance; bp, Base pairs; C (Cys), Cysteine (amino acid); CGC, Critical gel concentration; D (Asp), Aspartic acid; Da, Daltons; DNA, Deoxyribonucleic acid; E (Glu), Glutamic acid; *E. coli* HB101, *Escherichia coli* strain HB101; F (Phe), Phenylalanine; G (Gly), Glycine (amino acid); G^*, Complex shear modulus; GAGAGS, Silk-like repeat; GVGVP, Elastinlike repeat; H (His), Histidine; I (Ile), Isoleucine; K (Lys), Lysine; L (Leu), Leucine; M (Met), Methionine; MALDI-TOF-MS, Matrix-assisted laser desorption ionisation time-of-flight mass spectroscopy; MW, Molecular weight; N (Asn), Asparagine; P (Pro), Proline; Q (Gln), Glutamine; Q, Weight equilibrium swelling ratios of hydrogels (q); R (Arg), Arginine; S (Ser), Serine; S:E, Silk to elastin ratio; SAP, Shrimp alkaline phosphatase; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; SELP, Silk–elastinlike polymers; T, Thymine (deoxyribonucleic acid base); T (Thr), Threonine (amino acid); V (Val), Valine; W (Trp), Tryptophan; W_d, Dry hydrogel weight; W_s, Weight of swollen hydrogels; Y (Tyr), Tyrosine.

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class of genetically engineered biomaterials is the family of silk–elastinlike protein polymers (SELPs) [2]. SELPs are block copolymers composed of alternately repeated silk (GAGAGS) and elastinlike (GVGVP) units. Elastin units confer aqueous solubility and silk units provide crystallinity and mechanical strength for these polymers. By carefully controlling the sequence and length of the silk and elastin repeats it is possible to produce a variety of SELPs with diverse structural and material properties [8]. Two polymer analogs, SELP-47K and SELP-415K (Scheme 1A and B), with four silk and seven and fifteen elastin units respectively and an additional elastin with lysine (K) modification in each, are capable of self-assembly and irreversible sol-to-gel transition at 37 °C [9,10]. This allows their convenient mixing with biologicals such as naked DNA and viral vectors at room temperature minutes prior to injection in the body [11,12]. These analogs have been used for localized gene delivery [13], and tissue engineering [14]. Our previous observations indicate that increase in the length of elastin units in SELP-415K (compared to SELP-47K) resulted in hydrogels with larger swelling ratio and increased release of bioactive agents in vitro [10,13]. However the low silk to elastin ratio (S:E) in SELP-415K resulted in gel formation in a narrower polymer concentration (10–12 wt%) compared to that of SELP-47K (4–12 wt%) limiting the compositions available for biomedical applications including localized delivery needs. Design of a new hydrogel forming polymer with the same S:E ratio as SELP-47K but with longer elastin units than SELP-415K, namely SELP-815K (Scheme 1C), could facilitate enhanced bioactive agent release from the hydrogels while the longer silk block could maintain robust cross-linking.

Here we report on the biosynthesis of SELP-815K (Scheme 1C) with eight silk and fifteen elastin units and an extra elastin unit with a lysine (K) replacement in the monomer repeats. SELP-815K polymer with 6 repeats (SELP-815K-6mer) was selected to have similar molecular weight as that of SELP-415K-8mer and SELP-47K-13mer to allow a comparison of SELP analogs while maintaining the length of polymer chains approximately constant. Using an array of characterization tools we examined the influence of these structural changes on equilibrium swelling under various environmental conditions, mechanical properties and pore structure of the hydrogels.

2. Experimental section

2.1. Materials

Restriction endonuclease enzymes BanI, BanII, BamHI and EcoRV, and T4 DNA Ligase were purchased from New England Biolab (Beverly, MA). DNA ladder and Shrimp alkaline phosphatase (SAP) were purchased from Fermentas (Hanover, MD). QIAprep Spin Miniprep kits, QIAGEN Plasmid Maxi kits and QiaQuick Gel Extraction kits were obtained from Qiagen (Valencia, CA). Pro-Bond™ purification system was obtained from Invitrogen (Carlsbad, CA). *Escherichia coli* HB101 competent cells were purchased from Promega (Madison, WI). Bio-Spin®30 Tris columns, Precast Tris–HCl 4–15% linear gradient gels, Tris–glycine SDS buffer, Precision Plus Protein™ standards Bio-Safe™ Coomassie stain were obtained from Bio-Rad (Hercules, CA). pHydrion buffer capsules were obtained from Micro-Essential Laboratory (Brooklyn, NY).

A MDPVVLQRRDWENPGVTQLNRLAAHPPFASDPMG AGSGAGAGS [(GVGVP)$_4$ GKGVP (GVGVP)$_3$ (GAGAGS)$_4$]$_{12}$ (GVGVP)$_4$ GKGVP (GVGVP)$_3$ (GAGAGS)$_2$ GAGA MDPGRYQDLRSHHHHHH

B MDPVVLQRRDWENPGVTQLNRLAAHPPFASDPMG AGSGAGAGS [(GVGVP)$_4$ GKGVP (GVGVP)$_{11}$ (GAGAGS)$_4$]$_7$ (GVGVP)$_4$ GKGVP (GVGVP)$_{11}$ (GAGAGS)$_2$ GAGA MDPGRYQDLRSHHHHHH

C MDPVVLQRRDWENPGVTQLNRLAAHPPFASDPM [GAGS (GAGAGS)$_2$ (GVGVP)$_4$ GKGVP (GVGVP)$_{11}$ (GAGAGS)$_5$ GAGA]$_n$ MDPGRYQDLRSHHHHHH

Scheme 1. The amino acid sequence of SELPs. A. SELP-47K-13mer (MW – 69,814 Da). B. SELP-415K-8mer (MW – 71,500 Da). C. SELP-815K where $n = 3$, 4, 5, and 6 with MW – 35,638, 45,549, 55,461, 65,374 Da respectively. The properties of SELP-815K-6mer were compared to SELP-47K and SELP-415K. All polymers are composed of head and tail portions, and a series of silk-like (GAGAGS) and elastin-like (GVGVP) repeats (primary repetitive sequence in bold, number of repeats highlighted in gray). For amino acids see abbreviations.

2.2. Biosynthesis of SELP-815K

The cloning and expression vectors, pPT340, pSY1378 and pPT317 (Protein Polymer Technology, Inc., San Diego, CA) were propagated in *E. coli* HB101 and purified using a Qiagen Giga Kit according to the manufacturer's instructions. The concentration and purity of the plasmids were obtained using Ultrospec 4000 (Amersham Biosciences, Piscataway, NJ) at 260 and 280 nm. Plasmids with A_{260}/A_{280} ratio in the range of 1.8–2.0 were used. Plasmids were electrophoresed on a 0.9% agarose gel and stained with ethidium bromide to verify the absence of genomic DNA and the integrity of the plasmid. The general biosynthetic methodology is outlined in Scheme 2. The details of the methodology are described below.

2.3. Synthesis of the monomer gene segment. I

Plasmid pPT340 (containing SELP-415K monomer gene) was restriction digested with BanII to produce an SELP-215K oligonucleotide (184 bp), which was separated and purified from the reaction mixture by agarose gel electrophoresis followed by gel extraction using Qiagen's gel extraction kit (Qiagen). II. Plasmid pSY1378 (containing 6 silk units) was restriction digested with BanII for 1 h at 37 °C to linearize the plasmid. Enzyme was removed from the reaction using Micropure-EZ (Millipore) columns and the linearized plasmid was treated with shrimp alkaline phosphatase (NEB Labs) for 2 h at 37 °C. SAP was removed by agarose gel electrophoresis, and DNA was purified by gel extraction using Qiagen's gel extraction kit (Qiagen). III. The acceptor plasmid pSY1378 (from II) and the SELP-215K insert (from A) were ligated at a 1:1 molar ratio in the presence of T4 ligase for 16 h at 16 °C. The ligation mixture was used to transform chemical competent cells (Max efficiency Dh5α, Invitrogen) and plated on agar plate containing chloramphenicol. Colonies were picked, propagated and plasmid DNA was purified using Qiagen's miniprep (Qiagen). The presence of SELP-815K monomer in the purified pSY1378 clone was validated by restriction digestion, on either side of the cloning site (BanII), with BanI, followed by agarose gel electrophoresis to visualize a 384 bp fragment (SELP-815K) using ethidium bromide staining (Fig. 1A). The structure of the SELP-815K monomer gene segment was confirmed by fluorescence based automated DNA sequencing using appropriate sequencing primers (Fig. 1B).

2.4. Synthesis of the polymer gene segment. IV

A DNA fragment encoding the SELP-815K monomer was excised from a parental plasmid as a BanI fragment. SELP-815K monomers self-ligated in the presence of T4 DNA ligase to form 815K multimers followed by agarose gel electrophoresis and visualization using ethiduim bromide (Fig. 1C). V. Expression vector pPT317 was restriction digested with BanI for 1 h at 37 °C, enzyme was removed using Micropure-EZ columns and treated with shrimp alkaline phosphatase (NEB Labs) for 2 h at 37 °C, and DNA was purified by agarose gel electrophoresis followed by gel extraction. DNA