Molecular Mechanisms of a Thermo- and Ion-responsive Silk-elastin-like Protein

Haoyuan Shi[1], Ting Ji[2], Chenxi Zhai[1], Junting Lu[2], Wenwen Huang[2,3,4], and Jingjie Yeo[1]
[1]Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, United States.
[2]The Zhejiang University–University of Edinburgh Institute, Zhejiang University School of Medicine, Zhejiang University, Hangzhou, China.
[3]Department of Orthopedics of the Second Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang University, Hangzhou, China.
[4]Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative Medicine, Zhejiang University School of Medicine, Zhejiang University, Hangzhou, China.

Statement of Purpose: Silk-elastin-like proteins (SELPs) exhibit excellent biocompatibility, degradability, and dynamically tunable properties. SELP-derived gels, fibers, and scaffolds are widely used in drug delivery, wound healing, and tissue repair.[1] SELPs are synthetic co-polymers of silk-like (GAGAGS) and elastin-like (GXGVP) domains. The silk-like domains mimic the *Bombyx mori* silkworm silk sequence that forms β-sheet crystallites to confer structural stability and mechanical stiffness. The elastin-like domains inherit the stimuli-responsiveness of elastin by exhibiting inverse temperature transitions as the temperature was raised through a lower critical solution temperature (LCST). Dynamic response towards other factors, such as ionic strength, pH, and light, can be tuned by altering the X residue.[2] By incorporating both domains, SELPs possess favorable mechanical properties and stimuli-response tunability. While numerous stimuli-responsive SELP hydrogels were synthesized experimentally and characterized by the deswelling processing after stimulation,[3] there remains nebulous understanding of SELP behavior at the molecular level in response to external stimuli. In particular, the molecular mechanisms are unknown for useful stimulating factors, such as ionic concentrations, acting on a single SELP or SELP cluster. Here, we combine molecular modeling with experiments to probe the structural transitions due to changes in temperature and ions for a new, recombinantly synthesized SELP with sequence $[(GAGAGS)_2(GVGVP)_4(RGYSLG)(GVGVP)_3]_{10}$.

Methods: SELP was synthesized using recombinant DNA technology and purified by inverse temperature transition cycling (ITC). The lyophilized SELP powder was dissolved in deionized water at 277 K for 4 h. Then, 3 μL of 40 mg/mL horseradish peroxidase (HRP) stock and 1 μL of 3 wt% H_2O_2 solutions were added to initiate the crosslinking reaction. For deswelling studies, the hydrogel was equilibrated in deionized water at 340 K and in 1M NaCl solution at 277 K, respectively. In simulations, the equilibrated SELP structure at 280 K was obtained through fully atomistic molecular dynamics (FAMD) simulations with CHARMM 36 forcefield and advanced methods of large-scale replica exchange (RE). Then, the representative SELP structures were coarse-grained using the scheme from Martini 3.0. Finally, a six-SELP crosslinked structure was built to explore the behavior of SELP clusters at 340 K and with 1M NaCl, respectively.

Results: The chain aggregation in the crosslinked CG model led to a structural shrinkage when increasing temperature or adding ions (Fig. 1), with the radius of gyration (R_g) decreasing by 12.76 % and 9.46 %, respectively. These observations were consistent with the noticeable shrinkage in both the hydrogel dimensions that were synthesized, as well as the pore sizes in the corresponding SEM images. Three direct effects in the SELP hydrogel explained the molecular mechanisms for its responses to temperature and ions: 1) the structural transition of SELP under high temperature, 2) the geometry restraints in hydrogel networks, and 3) the electrostatic interactions between molecules.

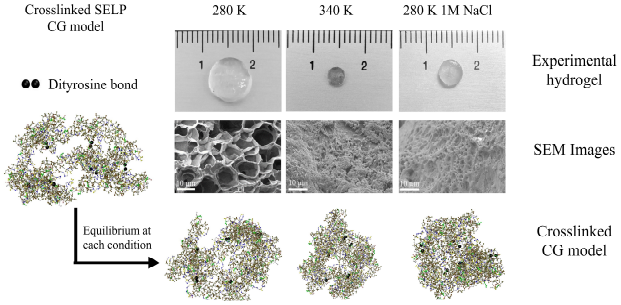

Fig. 1. The crosslinked CG models, SEM images, and SELP hydrogel showed the R_g, pore size, and volume size decrease, respectively, as the temperature increased from 280 K to 340 K or added 1M NaCl. Scale bars are 10 μm.

As the SELP hydrogel was exposed to a temperature above the LCST, the reversible transition in single molecules triggered a shrinkage of the hydrogel in volume. However, since the movement of individual SELP molecules is limited by the dense packing of the molecules and the intermolecular dityrosine bonds, the amount of structural transitions in a single SELP was inhibited. These two effects led to both simulations and experiments of the SELP responding to temperature. Different mechanisms were exhibited in the SELP hydrogels in response to changes in ionic concentrations. Positive charges almost entirely cover the solvent-exposed surface of SELP molecules due to the presence of positively charged arginine. Thus, ions can weaken and screen intermolecular electrostatic forces and induce molecular aggregation.

Conclusion: Reversible transition behavior and electrostatic potential govern the SELP response to temperature and ions, respectively. Understanding such interactions in the thermo- and ion-responsiveness of SELP hydrogels provided huge potential in designing, optimizing, and customizing SELP hydrogels for advanced biomaterials applications.

References:

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