Strain energy and molecular potential energy in self-assembled collagen in response to water solvation.

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Statement of Purpose: Collagens are the most ubiquitous class of proteins in mammals comprising about 30% of all proteins. The most prevalent of these collagens is type I collagen, which is a fibril-forming collagen present in the extracellular matrix of tendon, ligament, bone, skin, and muscle. Collagen type I functions to provide tensile strength and viscoelasticity to these musculoskeletal tissues in the presence of other matrix proteins, proteoglycans, ions, and water. Viscoelasticity in collagen has been attributed to elastic energy storage (molecular and crosslink strain) and viscous energy loss (molecular rearrangement and slippage) (Silver FH. Connect Tissue Res. 2002; 43:569-580). The current motivation of this research is to better understand the molecular basis for energy storage, elasticity, and strength in collagen and how surrounding or incorporated factors such as hydration and crosslinking may influence these properties at the molecular level. In this study, mechanical testing and molecular modeling were used to investigate hydration effects on viscoelasticity in self-assembled collagen fibers and on molecular potential energy in a modeled collagen microfibril, respectively.

Methods: Self-assembled collagen fibers derived from rat tail tendon were tested under dry (air) and hydrated (deionized water) states at room temperature via **incremental stress-relaxation** using a strain rate of 10%/min. Initial and relaxed stresses were obtained and used to derive total, elastic, and viscous properties (Pins, GD. J Appl Pol Sci. 1997; 63(11):1429-1440; Freeman, JW. Mater Res Soc Symp Proc. 2005; 874). **Molecular modeling** with explicit water solvation was used to assess molecular potential energy of two collagen microfibril models representing *in vacuo* and water-solvated states. The molecular models were derived from a glycine-proline-proline collagen model and modified to glycine-proline-hydroxyproline templates with subsequent extensions and assembly with DS Visualizer 2.0 into five-segment compressed microfibril models with 67nm lengths (Berisio, R. Protein Sci. 2002; 11:262-270; Accelrys Software Inc., San Diego, CA, 2008; Wess, TJ. J Mol Biol. 1998; 275(2):255-67). Lateral intermolecular spacings of 12 and 17.6 angstroms were established to model microfibril swelling due to hydration (Katz EP. J Mol Biol. 1973; 73:351-369; Leikin S. Proc Natl Acad Sci. 1994; 91:276-280). Models were analyzed and energy minimized via molecular mechanics with SYBYL 8.0 using the Kollman United Atom force field with a non-bonded cutoff of 8.0 angstroms, constant dielectric function of 1.0, and 1-4 scaling factor of 0.5 (Tripos International, St. Louis, MO, 2007). The Powell gradient minmization method was used to refine the models towards a 0.01 kcal/(mol*angstrom) termination gradient. Amino acid sequences for rat tail tendon collagen type I were used to replace the template sequences (Chapman, GE. Proc R Soc Lond. 1971; 178(1053):465-476).

Results: From **incremental stress-relaxation** tests, dry collagen fibers exhibited total strain energy 25 times that of hydrated fibers, elastic energy 35 times greater, and viscous energy 14 times greater. Elastic mechanisms dominated viscoelastic behavior in dry fibers (74 to 26%), however viscous and elastic components contributed equally for hydrated fibers (55 to 45%). For **molecular modeling**, temporary extraction of water molecules from the solvated model allowed for separation of energies into intra-collagen, water-water, and collagen-water interactions. It was observed that total molecular energy was 1.8 times more negative for the solvated model, which was primarily due to attractive (opposite-charge) electrostatic interactions with additional van der Waals attraction and favorable hydrogen bonding.

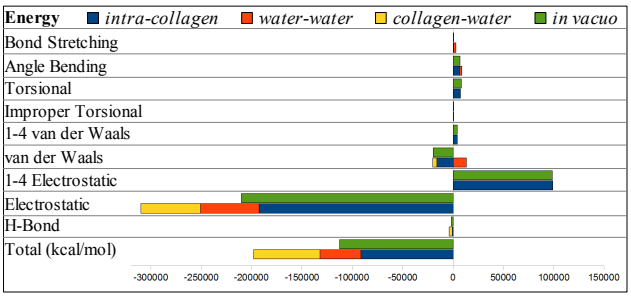

Figure: Molecular potential energy comparisons between the water-solvated and *in vacuo* molecular models.

Conclusions: Incremental stress-relaxation results showed that hydration drastically reduced the total strain energy and shifted the elastic fraction. Thus, the elastic energy storage exhibited the largest orders-of-magnitude difference between dry and hydrated states, which implies that hydration plays a strong role in modulating elastic energy storage. **Molecular modeling** suggests that the reduction in molecular potential energy in the solvated state is mainly due to attractive electrostatic interactions (collagen-water and water-water), with some further contribution by other favorable collagen-water interactions (van der Waals and hydrogen bonding). There was also a slight loss in attractive electrostatic and hydrogen bonding interactions when comparing intra-collagen (solvated) to *in vacuo*, which suggests that upon hydration electrostatic interactions and hydrogen bonds are lost between and/or within collagen molecules, but may be re-associated with the water molecules. This loss may be due to the increased intermolecular spacing established in the solvated model and/or more favorable collagen-water interactions that disrupt intra-collagen interactions. It is possible that these same energy reducing mechanisms translate to reducing strain energy storage in hydrated collagen. Future work will be to expand upon this work to investigate the combinatorial effects of solvation and crosslinking schemes on elastic strain energy storage and viscoelastic response in collagen.