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Elastic energy storage in an unmineralized collagen type I molecular model with explicit solvation and water infiltration

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ABSTRACT

Collagen type I is a structural protein that provides tensile strength to tendons and ligaments. Type I collagen molecules form collagen fibers, which are viscoelastic and can therefore store energy elastically via molecular elongation and dissipate viscous energy through molecular rearrangement and fibrillar slippage. The ability to store elastic energy is important for the resiliency of tendons and ligaments, which must be able to deform and revert to their initial lengths with changes in load.

In an earlier paper by one of the present authors, molecular modeling was used to investigate the role of mineralization upon elastic energy storage in collagen type I. Their collagen model showed a similar trend to their experimental data but with an over-estimation of elastic energy storage. Their simulations were conducted in vacuum and employed a distance-dependent dielectric function. In this study, we performed a re-evaluation of Freeman and Silver's model data incorporating the effects of explicit solvation and water infiltration, in order to determine whether the model data could be improved with a more accurate representation of the solvent and osmotic effects. We observed an average decrease in the model's elastic energy storage of 45.1% ± 6.9% in closer proximity to Freeman and Silver's experimental data. This suggests that although the distance-dependent dielectric implicit solvation approach was favored for its increased speed and decreased computational requirements, an explicit representation of water may be necessary to more accurately model solvent interactions in this particular system. In this paper, we discuss the collagen model described by Freeman and Silver, the present model building approach, the application of the present model to that of Freeman and Silver, and additional assumptions and limitations.

1. Introduction

Collagens exist in approximately 20 distinct types and together constitute one-third of all mammalian proteins. Collagen type I is the most prevalent of these types and it functions to provide tensile strength to tendons and ligaments, which are crucial for proper locomotion and for the stability of the musculoskeletal system (Freeman and Silver, 2004). The collagen fibers within these tissues display viscoelastic behavior that accounts for elastic energy storage and viscous energy dissipation. Elastic energy storage has been attributed to molecular and crosslink strain, and viscous energy loss to molecular rearrangement and fibrillar slippage (Lodish et al., 2004; Silver et al., 2003). The collagen type I molecule is composed of two $\alpha1$ chains and one $\alpha2$ chain that each conform in left-handed minor helices and together establish a right-handed triple helix. The amino acid sequences of the collagen helix are described by a glycine–X–Y repeating pattern, where X and Y are often proline and hydroxyproline, respectively, but there can be a number of other residues in either position. This primary structure allows the collagen chains to form a stable triple helix, which affords strength to the collagen molecule (Lodish et al., 2004).

Fiber-forming collagens, such as type I collagen, can self-assemble into quarter-staggered arrays called microfibrils that extend laterally and axially to form larger structures such as fibrils and then fibers. In the axial or longitudinal direction, this staggered arrangement has been observed through electron microscopy and X-ray diffraction to be a characteristic length of 67 nm in type I collagen (Williams et al., 1978). In the lateral direction, a number of packing models have been proposed and investigated most notably the Smith 5-bundle and quasi-hexagonal models; the latter of which includes sheet structures and compressed microfibril structures. These models have been based primarily on X-ray diffraction experiments (Hulmes and Miller, 1979; Trus and Piez, 1980; Wess et al., 1998). These axial and lateral structural features have formed the basis for the supramolecular structure of the present collagen model, as depicted in Fig. 1.

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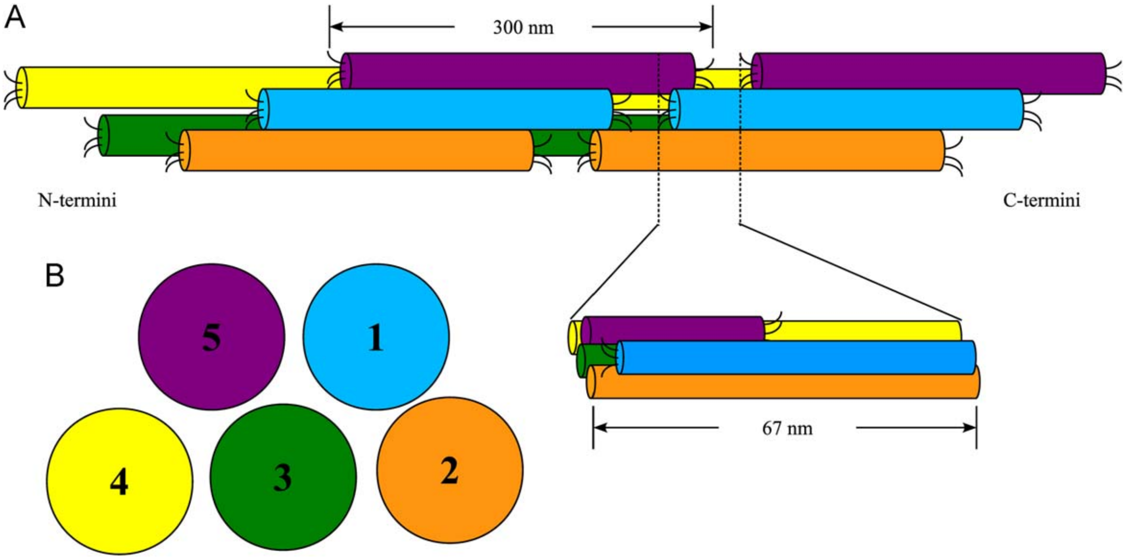

Fig. 1. Drawings of a quasi-hexagonal compressed microfibril model where each cylinder represents an individual collagen molecule with non-helical terminal extensions: (A) Two adjacent microfibrils, where each microfibril is composed of five segments with alternating gap and overlap regions that together make up the characteristic D repeat period of 67 nm. (B) A cross-section of the microfibril model looking down onto the N-terminal end to show the hexagonal packing arrangement.

The solvent surrounding a protein solute can impart significant influence over the folding behavior and the stability of the protein, and therefore, simulations involving proteins and other biomolecules are often performed using some form of solvation. In molecular mechanics and dynamics, solvents can be represented implicitly or explicitly, with each method presenting advantages and challenges. Implicit solvation is also referred to as continuum solvation and involves simulation in vacuum without solvent molecules; however adjustments or additions are made to the force field energy equations to account for the presence of a continuum solvent. The simplest implicit solvent method involves the use of a distance-dependent dielectric function incorporated into the calculation of electrostatic energy. However, limitations of the distance-dependent dielectric model as well as some other implicit solvent models include the absence of solvent viscosity effects that would normally slow down protein dynamics, the inability to account for a non-homogenous solvent environment (i.e., water with sodium and chloride ions), and the absence of directionality in hydrogen bonds (Hassan et al., 2005). Explicit solvation involves the actual presence of solvent molecules in the simulation, which often provides greater accuracy, but at the same time increases the computational burden of the simulation. Water in particular has been represented using three-site (i.e., TIP3P, SPC, SPC/E), four-site (i.e., TIP4P), and even five-site models (i.e., TIP5P). Each of these representations has been proposed in attempts to model the behavior of water more accurately with respect to some of the physical properties of water. The use of a particular water model is often based on first determining a force field appropriate for the solute of interest and then pursuing a water model that is consistent with that force field, since existing water models were developed with different force fields and under different assumptions (Mackerell, 2004).

There are studies described in the literature concerning the influence of environmental factors such as water and pH on the mechanical properties of collagen and collagen-like peptides; a few of these studies are mentioned below. Based on molecular modeling, Zhang et al. investigated the effects of crystallographic water (also referred to as tightly bound water or structural water) on a collagen-like microfibril simulated with stretching, sliding, or bending forces. Their models consisted of quasi-hexagonally arranged collagen-like helices with or without crystallographic water, which were simulated using a steered molecular dynamics approach. They proposed that structural water molecules may act as both a lubricant and as a cohesive element, depending on the type of mechanical loading (Zhang et al., 2007). From an experimental study, Seehra and Silver proposed that intramolecular and inter-molecular electrostatic interactions introduce attractive forces within and between collagen molecules, respectively, leading to the stiffening of collagen. They suggested that these interactions are dependent upon the pH environment and, therefore, the protonation states of basic and acidic residues (Seehra and Silver, 2006). Both of these studies provide important insights into the roles that intra- and inter-molecular interactions play in the mechanical properties of collagens and the influence of water molecules. Moreover, Chou has described low-frequency collective motions of alpha-helices (Chou, 1983a, b, 1984a), beta-sheet/barrel (Chou, 1985), DNA (Chou, 1984b; Chou et al., 1989), and further discussed their various interesting biological functions (Chou, 1987, 1989; Chou and Mao, 1988) as described in a comprehensive review (Chou, 1988). The presence of these motions localized to secondary structural motifs may lead to interesting mechanical implications for structural proteins such as collagens (Lazarev et al., 1999).

The basis of the present study was work performed by Freeman and Silver that involved the use of both experimental and molecular modeling techniques to investigate elastic energy storage in collagen type I. Freeman and Silver prepared their models in vacuum using a distance-dependent dielectric method (implicit solvent), and then they applied unidirectional strain to their models using constrained energy minimization. They observed that their model data produced an over-estimation of their experimental data, but that overall trends were promising. They also described simplifications and assumptions in their approach, most notably, the use of a distance-dependent dielectric function rather than the explicit representation of water molecules.

In this study, we proposed an extension of Freeman and Silver's work, but with the use of explicit solvation using the TIP3P water model and also attempting to account for osmotic swelling of collagen in aqueous solvent. We constructed both in vacuo and explicitly solvated molecular models representing a quasi-hexagonal compressed microfibrillar unit of type I collagen, and