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Hydration and distance dependence of intermolecular shearing between collagen molecules in a model microfibril

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ABSTRACT

In vertebrates, collagen tissues are the main component responsible for force transmission. In spite of the physiological importance of these phenomena, force transmission mechanisms are still not fully understood, especially at smaller scales, including in particular collagen molecules and fibrils. Here we investigate the mechanism of molecular sliding between collagen molecules within a fibril, by shearing a central molecule in a hexagonally packed bundle mimicking the collagen microfibril environment, using varied lateral distance between the molecules in both dry and solvated conditions. In vacuum, the central molecule slides under a stick–slip mechanism that is due to the characteristic surface profile of collagen molecules, enhanced by the breaking and reformation of H-bonds between neighboring collagen molecules. This mechanism is consistently observed for varied lateral separations between molecules. The high shearing force (>7 nN) found for the experimentally observed intermolecular distance (≈ 1.1 nm) suggests that in dry samples the fibril elongation mechanism relies almost exclusively on molecular stretching, which may explain the higher stiffnesses found in dry fibrils. When hydrated, the slip–stick behavior is observed only below 1.3 nm of lateral distance, whereas above 1.3 nm the molecule shears smoothly, showing that the water layer has a strong lubricating effect. Moreover, the average force required to shear is approximately the same in solvated as in dry conditions (≈ 2.5 nN), which suggests that the role of water at the intermolecular level includes the transfer of load between molecules.

1. Introduction

Motion in vertebrates is achieved through the generation of muscular forces that are then transmitted to joints by connective tissues such as ligaments and tendons (Alexander, 1983, 1984). Moreover, the mechanical behavior of these connective tissues is directly related to their complex hierarchical structure, to their specific macromolecular components and to the load transfer mechanisms acting at different hierarchical scales (Silver et al., 2001). The lowest hierarchical scale of several load-bearing collagenous tissues is represented by collagen type I molecules, which are the most abundant protein building blocks in vertebrates, and form the principal protein that provides mechanical stability, elasticity and strength to tendons and ligaments (Fratzl, 2008; Kadler et al., 2007). Electron microscopy analysis of stained collagen fibrils provided insight into the packing structure of collagen molecules. These micrographs display a pattern of alternating light and dark bands perpendicular to the axis of the collagen fibrils that repeat every 67 nm. This interval has been defined as the D-period. Light bands correspond to regions of more dense lateral packing, and dark bands correspond to 'gap' regions, domains of low density molecular packing first noted by Petruska and Hodge (1964). Various models for collagen have been proposed based on the observed staining pattern and on the length of a single collagen triple-helical molecule, which is ≈ 4.4 D (Fraser et al., 1983, 1974; Hofmann et al., 1978; Piez and Trus, 1977). It is generally agreed that groups of four to six triple helices are packed together to form microfibrils, which in turn aggregate to form fibrils. The microfibril model, proposed by Smith (1968) is able to explain much of the electron microscopy data. In this model the cross-section exhibits a regular hexagonal geometry, whereas the neighboring triple helices are longitudinally separated by a gap region, 0.6 D intervals in length.

From a mechanical standpoint, Sasaki and Odajma (1996b), assuming a two-dimensional quasi-hexagonal packing model as proposed by Lees et al. (1984), reported three molecular

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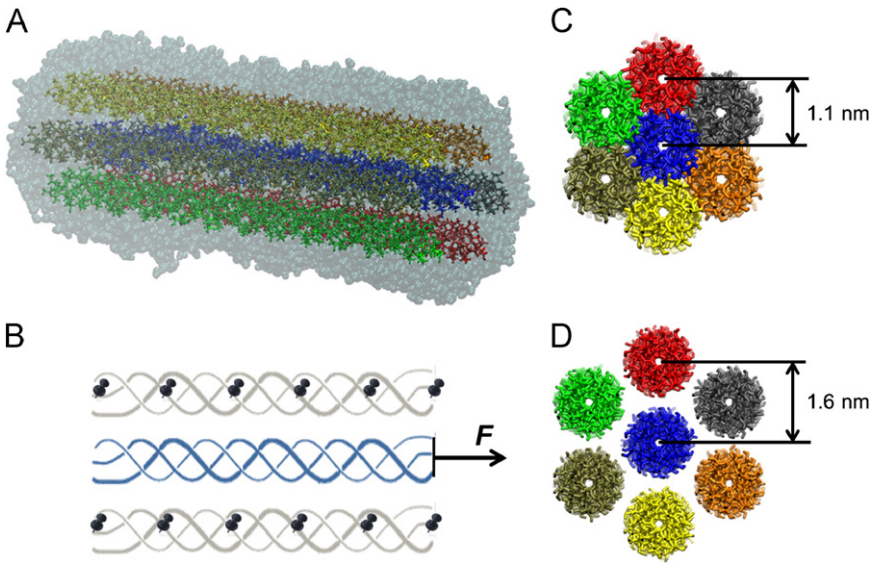

Fig. 1. Atomistic model of collagen microfibril. Molecular structure of the microfibril model used here, showing the seven collagen-like peptides solvated in a water layer. (A) Schematic of the loading conditions (B) the central molecule (blue) is pulled at one end by a virtual spring, while the surrounding molecules (gray) are held fixed by constraining the backbone atoms positions. (C) Cross-section of the microfibril in the case of an intermolecular distance of 1.1 nm, (D) the case of an intermolecular distance of 1.6 nm.

mechanisms during the elongation of collagen fibrils described as intra-molecular and inter-molecular rearrangements. The latter was divided into two possible mechanisms: the increase in the gap region from the longitudinal deformation of adjoining molecules along the fibril axis, and the relative slippage of laterally adjoining molecules along the fibril axis. Results showed that the mechanical resistance, in particular at small strains (up to 2%), is prevalently given by intramolecular phenomena (i.e. molecular elongation) which account for 1.7% strain (out of 2%), whereas intermolecular mechanisms such as molecular slippage and gap increase account for the remaining 0.3% strain. These results explain the wealth of data about the mechanical behavior of single collagen molecules (An et al., 2004; Cusack and Miller, 1979; Sun et al., 2002, 2004), which are the main determinants of the elastic properties of collagen tissue.

Indeed, in our recent work (Gautieri et al., 2012), by using molecular modeling to perform *in silico* creep tests of the single collagen molecule and by modeling the fibril as a system of viscoelastic elements, we showed that the elastic properties of the collagen fibril can be determined with good approximation based solely on the elastic properties of the single molecule and their geometrical arrangement. This suggests that the elastic component of fibril mechanical behavior is largely dictated by the stretching of triple helical molecules. On the other hand, and in addition to the mechanism of very large deformation of collagen fibrils, our model fails to capture the time-dependence of fibril mechanical response, suggesting that the viscous component does not primarily depend on single molecule relaxation but largely relies on other mechanisms, such as water-mediated sliding of adjacent molecules. Of particular interest is the role of water in collagen fibril mechanics. Water molecules may function as spacers between the molecules and may also bridge the connection between amide and carbonyl groups of two adjoining collagen molecules through water bridges (i.e. water-mediated hydrogen bonds) that are too far for a direct hydrogen bond (Leikin et al., 1997).

Despite the importance of these phenomena to the understanding of the mechanics of collagen tissue, there is limited knowledge on how water participates in load transfer when a collagen fibril is under mechanical stress, in particular on the contribution of water to the relative movement between neighbor molecules within the quarter–staggered lattice. In this work we focus on the sliding mechanism between collagen molecules within the fibril environment, using a simple microfibril model to assess the effect of lateral distance and hydration on the shearing of collagen molecules. The collagen molecules in our study are collagen-like peptides made of glycine–proline–hydroxyproline (GPO) triplets, a collagen model often used in experimental and modeling investigations as an archetype of the collagen molecule, since GPO is the most common and stabilizing triplet (Beck et al., 2000; Gautieri et al., 2008, 2009b; Persikov et al., 2000a, 2000b; Srinivasan et al., 2009; Veld and Stevens, 2008). Seven of these peptides are then arranged in a hexagonal pattern (in cross-section), mimicking the molecular arrangement in fibrils, and the lateral distance between the molecules is varied from 1.1 nm to 1.6 nm, according to the experimental evidence (Fratzl et al., 1993; see Fig. 1). This simplified microfibril setup is then used to slide the central molecule with respect to the surrounding molecules, testing the mechanical response of the system in different conditions.

2. Methods

2.1. Collagen microfibril model generation

Sliding within a model collagen microfibril is investigated using constant force Steered Molecular Dynamics (SMD) simulations. We consider the hexagonal packing of collagen microfibrils and we submit the central collagen molecule to pulling along its principal axis with constant velocity. We use the THeBuScr (Triple-Helical collagen Building Script) code (Rainey and Goh, 2004a, 2004b) to build a model of the collagen molecule, as done in earlier studies (Gautieri et al., 2009b; Srinivasan et al., 2009, 2010). We choose the simplest model of collagen, with only glycine–proline–hydroxyproline (GPO) triplets on each of the three chains. The collagen model we use, $[(GPO)_{21}]_3$, is truncated to 63 amino acids per chain due to computational limitations, since the full length collagen molecule (300 nm long) is too large for atomistic simulations. This leads to a collagen-like segment with a length of approximately 20 nm. Peptides of comparable length have been used both in earlier computational and experimental studies (Beck et al., 2000; Gautieri et al., 2008, 2009b; Persikov et al., 2000a, 2000b; Srinivasan et al., 2009; Veld and Stevens, 2008). Six peptides are then arranged in hexagonal packing surrounding a central molecule (resembling the arrangement found *in vivo*) (see Fig. 1a). The lateral distance between the central molecule and the external ones is varied from 1.1 nm to 1.6 nm in steps of 0.1 nm, for a total of six different lateral distances (see Fig. 1c–d). These lateral distances are chosen according to experimental evidence (Fratzl et al., 1993). We investigate the

shearing behavior of both dried and solvated collagen microfibrils. In the latter case the microfibril is solvated in a water layer using TIP3P water molecules as solvent. The total number of atoms of the solvated system ranges from 16,000 to 50,000 atoms. Thus, a total of twelve systems are considered (six systems in hydrated conditions and six systems in dry conditions).

2.2. Collagen model equilibration

Molecular dynamics simulations are performed using the NAMD code (Nelson et al., 1996; Phillips et al., 2005) and the CHARMM force field (MacKerell et al., 1998). However, collagen is the sole protein that features hydroxyproline (HYP), a non-standard amino acid resulting from hydroxylation of proline. Since it is rarely found, HYP is not parameterized in common biomolecular force fields (like CHARMM). However, a set for HYP has been developed by using quantum mechanical simulations and subsequently deriving the atomistic parameters that best match the quantum-mechanics calculation (Park et al., 2005), with particular focus on the correct modeling of the pucker of the HYP ring. Since the collagen triple helix considered here is truncated, the N-terminals are capped with ACE residues (acetylated N-termini), whereas C-terminals are capped with CT3 residues (amidated C-termini). Rigid bonds are applied to constrain covalent bond lengths, thus allowing a time step of 2 fs. Van der Waals interactions and electrostatic interactions are computed using a cutoff for neighbor list at 1.35 nm, with a switching function between 1.0 and 1.2 nm. During the simulations the C_α atoms of the six external molecules are fixed with position restraints in order to prevent unfolding of the microfibril system. The central molecule is instead unrestrained. The preliminary energy minimization is performed by using the steepest descent algorithm until convergence. The systems are then equilibrated at a temperature of 310 K (37 °C). We observe that the root mean square deviation (RMSD) of the protein backbone reaches a stable value within 500 ps of the simulation time, thus we assume that the collagen microfibril is equilibrated properly at the end of the molecular dynamics run.

2.3. In silico shearing tests

Shearing tests are performed pulling the center of mass of the three N-terminal C_α atoms of the central molecule with a virtual spring (elastic constant of 10 pN/Å) pulled at a velocity of 1 m/s (see Fig. 1b). We tested higher and lower pulling rates, and found that the force level does not change below 1 m/s, in agreement with previous studies on single collagen molecules (Gautieri et al., 2009a). The shearing test simulations are run for 20 ns, a time which is found to be long enough to fully extract the central molecule in all setups. The output of these simulations is the force applied by the virtual spring as a function of time.

2.4. H-bond analysis

We monitor the number of H-bonds between the central molecule and the surrounding proteins. We determine H-bonds using a geometric definition, i.e. an angle for donor–hydrogen–acceptor of 30° and a cutoff distance of 0.35 nm between the donor and the acceptor. H-bond analysis and general visualization and imaging of molecular models has been performed using VMD 1.9 (Humphrey et al., 1996). The H-bond analysis provides insight into the mechanical properties of protein molecules (Keten and Buehler, 2008; Keten et al., 2010).

3. Results

We perform *in silico* shearing tests of solvated (wet) and dry single collagen peptides within a microfibril model by applying a force with a virtual spring pulled at constant velocity. The force needed to slide the central collagen molecule over the surrounding peptides is monitored over time as a function of the lateral distance and hydration state. We observe that in dry conditions the forces needed to pull the central molecule show a characteristic sawtooth profile (see Fig. 2a), and that the average force is independent of the intermolecular lateral distance, except for the shortest distances (i.e., 1.1 nm and 1.2 nm) for which the force level is much higher (force >7 nN). This suggests that below the threshold of 1.3 nm lateral distance, the molecular packing is rather tight and that sliding between molecules is hampered. On the other hand, the average force for lateral distances ≥ 1.3 nm is unchanged with an average value of 2.56 ± 0.47 nN and a maximum value of 5.7 nN (see Fig. 2c). The forces between the central sliding triple helix and the surrounding molecules is mediated through H-bonds, whose number is found to be independent

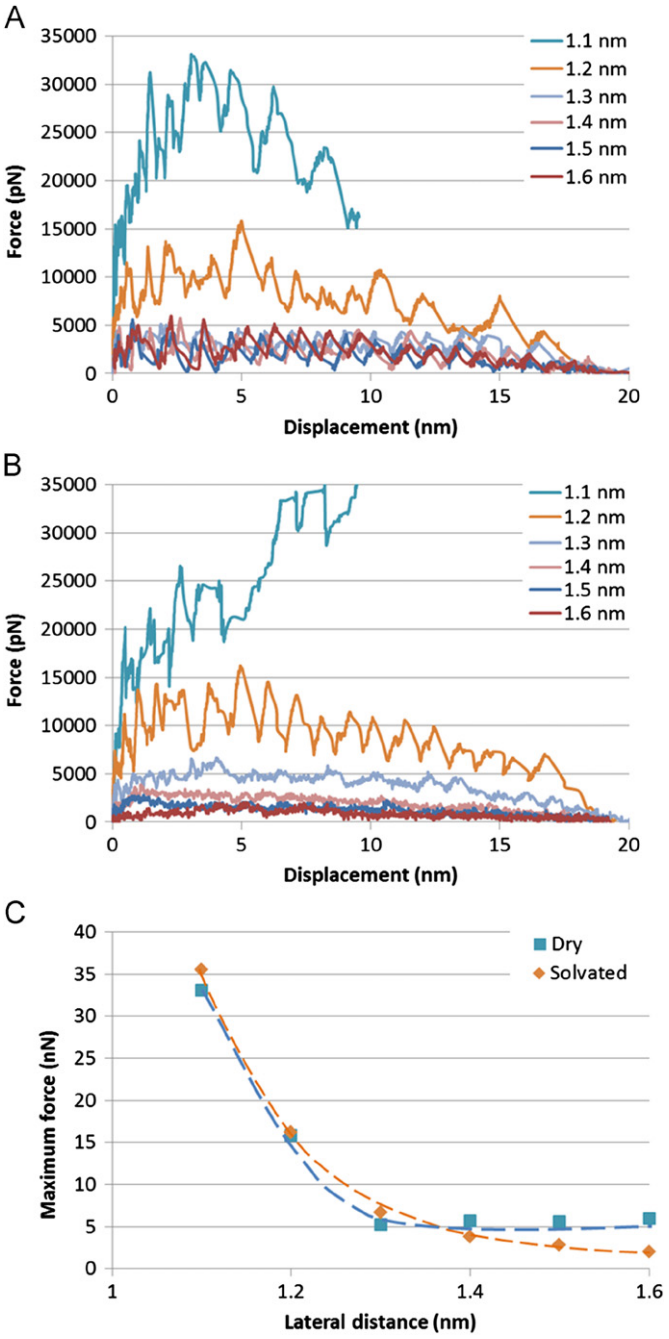

Fig. 2. Pulling test analysis. The force needed to pull the central molecule out of the microfibril is shown for the dry case, illustrating the characteristic sawtooth behavior. When the microfibril is hydrated (B) the pulling force shows a smoother trend, except for the case of the shortest intermolecular distance, for which water does not fit in the inner space of the microfibril. The maximum force (C) is shown to decrease with increasing lateral distance up to 1.3 nm, whereas further increase in the intermolecular distance has no (for the dry case) or minor effect on the force level.

of the lateral distance and decreases from ≈ 8 H-bonds to ≈ 0 H-bonds during the shearing simulation due to the decreasing overlap between the molecules (Fig. 3a).

The explicit water solvated microfibril shows a force trend similar to the dry case, with the average force decreasing with increasing intermolecular distance up to 1.3 nm, while for a lateral distance of 1.4 nm and above the force level remains almost constant at a value of 2.51 ± 0.76 nN (see Fig. 2b). However, in the presence of water, the sawtooth profile of the force observed in dry conditions is found only

for the two most densely packed microfibrils (lateral distance of 1.1 nm and 1.2 nm), whereas for larger intermolecular distances the force profile is smoother. The number of H-bonds formed between the central molecule and the surrounding ones is much lower (5.79 ± 1.85 H-bonds) compared to the dry case, except for the denser packing (1.1 nm and 1.2 nm), for which the number of H-bonds is comparable to the dry case (Fig. 3b).

4. Discussion

The characteristic sawtooth profile of the force observed in the sliding experiment of the dry microfibril can be attributed to the geometric structure of the surface of collagen molecules. Indeed, the collagen triple-helix shows a sawtooth-like profile, due to the alternation of bulky proline/hydroxyproline and small glycine residues. Thus, when the central collagen molecule slides within the microfibril, its protruding lateral chains interlock with the equivalent ones on the neighbor molecules (see Fig. 4). This hinders the sliding and causes the observed stick–slip movement. We note that this mechanism could be accentuated by the choice of Gly–Pro–Hyp triplets, and that type I collagen, which features a more varied sequence, may have a different profile and thus lead to a different force trend (future work may investigate this question). However, Pro and Hyp are very common amino acids in collagen and even other residues—which are larger than Gly—would provide a sawtooth like shape to collagen. The lateral interaction between collagen molecules is mediated by non-bonded interactions, in particular H-bonds. The number of H-bonds during the sliding shows a sawtooth trend as well, consistent with the stick–slip motion of the molecule (Fig. 3a). However, the number of the H-bonds does not show a significant variation with the intermolecular lateral distance. This is due to the fact that in the absence of water, and even at larger molecular distances, the central molecule bends due to the attraction of the neighbor molecules, thus forming approximately the same number of intermolecular H-bonds in all cases.

In the solvated microfibril, the sawtooth profile of the force is found only for the two most densely packed microfibrils (lateral distance of 1.1 nm and 1.2 nm). This effect can be attributed to the presence of water molecules that act as load mediators by forming water-bridges between the triple helices. Since the water molecules are very motile compared to collagen molecules they act as a lubricating medium, preventing the stick–slip motion observed in dry conditions. The change in mechanisms is also observed via monitoring of the number of direct H-bonds between the central molecule and the surrounding molecules (Fig. 3b), which show a significant decrease compared to the dry case, due to the fact that direct bonding between collagen molecules is partly substituted by water-mediated interactions. However, this phenomenon is not observed for the most densely packed microfibrils (lateral distance of 1.1 nm and 1.2 nm). In these cases, the packing of the molecules is too dense for water to fit in the intermolecular space and, as a consequence, the mechanical behavior shows the characteristics of the dry case, i.e. a sawtooth force profile and a higher number of direct H-bonds between collagen molecules.

In the case of the densest packing (intermolecular distance of 1.1 nm and 1.2 nm) the force needed to shear the central molecule is exceedingly high (in excess of 15 nN), suggesting that under these conditions intermolecular sliding is prevented. Experimental evidence suggests that deep dried samples of collagen tissue show an intermolecular distance of 1.1 nm (Fratzl et al., 1993). However, while it is known that molecular sliding occurs in hydrated samples, this may not be the case for dried samples. Indeed, wet and dry collagen fibrils show very different mechanical behavior (van der Rijt et al., 2006) and, although molecular sliding is one of the elongation mechanisms of collagen fibrils, the most important is the molecular stretching, as suggested in earlier experimental work (Sasaki and Odajima, 1996a). Thus, our results suggest that in dry samples (with

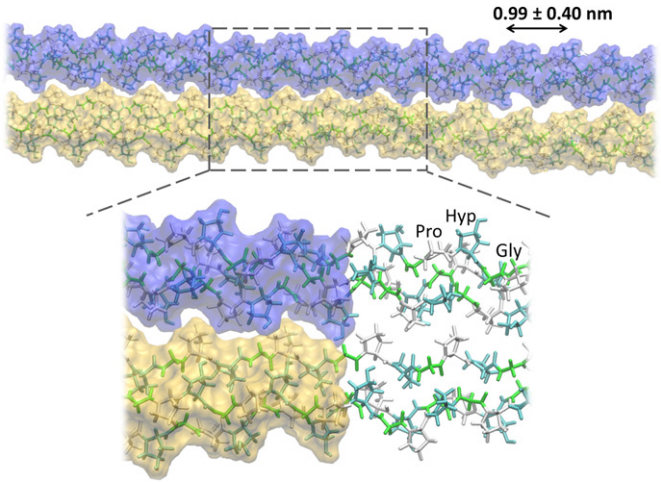

Fig. 4. Molecular mechanisms of deformation. The sawtooth behavior of the force, in particular for shorter intermolecular distances, can be attributed to the geometric features of the collagen molecule, which itself shows a sawtooth profile with a period of ≈ 1 nm (see upper panel). This distance agrees well with the period of the force peaks and leads to the observed stick–slip motion. The sawtooth profile is due to the alternation of bulky amino acids (Pro/Hyp) and smaller ones (Gly). The lower panel shows a close-up of the interface between two collagen molecules. The van der Waals surface of the two molecules is shown in blue and yellow, whereas amino acids are colored by residue type (Gly in green, Pro in white, Hyp in cyan color). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

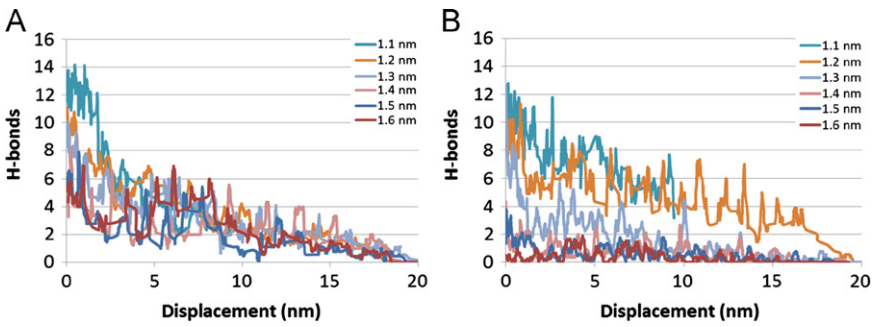

Fig. 3. H-bond analysis under applied displacement. Number of H-bonds between the central molecule and the surrounding ones, plotted as a function of molecule displacement during the shearing simulations in the dry (A) and hydrated case (B). The trend of intermolecular H-bonds shows features similar to the pulling force, with a decreasing value (due to decreasing overlap) and a series of peaks (due to the stick–slip motion). The number of H-bonds is not influenced by the intermolecular distance in the dry case, suggesting that the central molecule is always in contact with the surrounding molecules. On the other hand, in the presence of water (and for intermolecular distances higher than 1.2 nm) the number of H-bonds decreases, showing that at larger intermolecular distances water mediates the connection between the collagen molecules.

an experimentally observed intermolecular distance of 1.1 nm) the fibril elongation mechanism relies almost exclusively on molecular stretching, which could explain the higher mechanical properties found in dry fibrils. Furthermore, the observed trend for the shearing force as a function of the intermolecular distance agrees well with previous experimental observation (Leikin et al., 1997), which showed that the intermolecular forces between collagen molecules increase exponentially when the distance is below 1.4 nm and thus that shorter intermolecular distances are unprivileged.

The present work elucidates details of molecular interactions in collagenous fibrils when subjected to forces, and explains how the mechanical load is transferred between collagen molecules within fibrils, at atomistic level resolution and with full consideration of explicit solvent. Since fibrillar collagen is the major building block of many load-bearing vertebrates' tissues (such as bone, tendon, cartilage and skin), the findings of this research are significant to the advancement of our general understanding of the chemo-mechanical basis of load-bearing protein materials in biology. Our results show that the hydration state has a significant effect on fibril mechanics. Indeed, while the shearing force levels are similar in the dry and hydrated states, the presence of water reduces the stick–slip sliding, producing a smoother movement of the molecules. This means that water has a twofold role in collagen fibril mechanics, both as a load transfer medium and as a lubricant medium.

Conflict of interest statement

The authors declare no conflict of interest of any sort.

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