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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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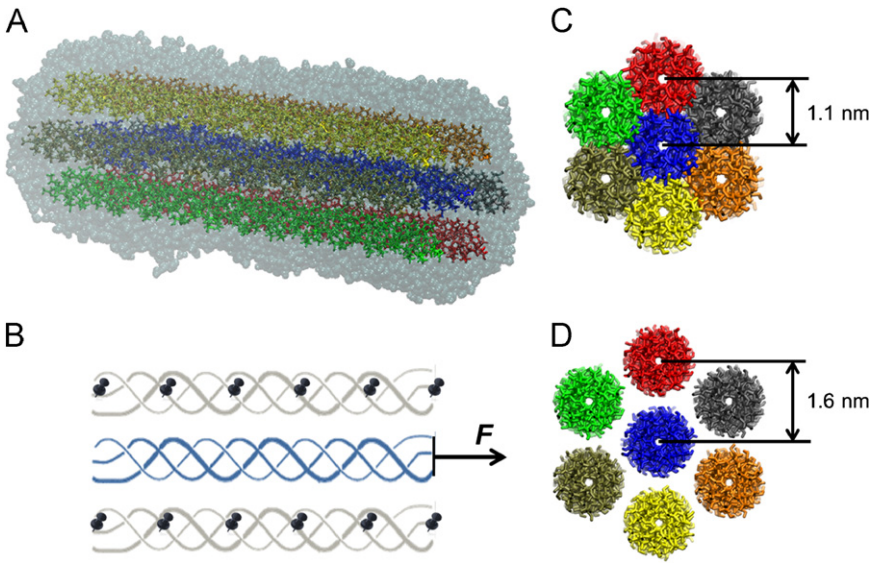

Fig. 1. Atomistic model of collagen microfibril. Molecular structure of the microfibril model used here, showing the seven collagen-like peptides solvated in a water layer. (A) Schematic of the loading conditions (B) the central molecule (blue) is pulled at one end by a virtual spring, while the surrounding 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