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Research paper

Deformation rate controls elasticity and unfolding pathway of single tropocollagen molecules

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ABSTRACT

Collagen is an important structural protein in vertebrates and is responsible for the integrity of many tissues like bone, teeth, cartilage and tendon. The mechanical properties of these tissues are primarily determined by their hierarchical arrangement and the role of the collagen matrix in their structures. Here we report a series of Steered Molecular Dynamics (SMD) simulations in explicit solvent, used to elucidate the influence of the pulling rate on the Young's modulus of individual tropocollagen molecules. We stretch a collagen peptide model sequence $[(\text{Gly-Pro-Hyp})_{10}]_3$ with pulling rates ranging from 0.01 to 100 m/s, reaching much smaller deformation rates than reported in earlier SMD studies. Our results clearly demonstrate a strong influence of the loading velocity on the observed mechanical properties. Most notably, we find that Young's modulus converges to a constant value of approximately 4 GPa tangent modulus at 8% tensile strain when the initially crimped molecule is straightened out, for pulling rates below 0.5 m/s. This enables us for the first time to predict the elastic properties of a single tropocollagen molecule at physiologically and experimentally relevant pulling rates, directly from atomistic-level calculations. At deformation rates larger than 0.5 m/s, Young's modulus increases continuously and approaches values in excess of 15 GPa for deformation rates larger than 100 m/s. The analyses of the molecular deformation mechanisms show that the tropocollagen molecule unfolds in distinctly different ways, depending on the loading rate, which explains the observation of different values of Young's modulus at different loading rates. For low pulling rates, the triple helix first uncoils completely at 10%–20% strain, then undergoes some recoiling in the opposite direction, and finally straightens for strains larger than 30%. At intermediate rates, the molecule uncoils linearly with increasing strain up to 35% strain. Finally, at higher velocities the triple helix does not uncoil during stretching.

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1. Introduction

Collagen is the most abundant structural protein component and is responsible for the structural integrity of tissues like bone, tendon, ligament, cartilage, teeth, skin, cornea and blood vessels. In many of these tissues, collagen is organized in complex hierarchical structures, resulting in the specific organization and mechanical properties of the aforementioned tissues. Nonetheless, the basic building block and the lowest hierarchical level of all of these tissues is the triple helical tropocollagen molecule (Alberts et al., 2002).

More than 20 types of tropocollagen molecules have been reported, but the most common one in the human body is the type I collagen molecule, which is found mainly in bone and tendons. Tropocollagen is a rod-like fibrous protein about 300 nm long and 1.5 nm in diameter, made up of three polypeptide strands, each of which is a left-handed chain, not to be confused with the commonly occurring alpha helix, which is right-handed. These three left-handed helices are twisted together into a right-handed coiled coil, a triple helix or "super helix", a cooperative quaternary structure stabilized by numerous H-bonds.

A unique feature of tropocollagen is the regular organization of amino acids in each of the three chains: the sequence is based on the strictly repeating triplets of the general form (Glycine-X-Y). This kind of regular repetition and high Glycine (Gly, G) content is never found in globular proteins, while it can be recognized in a few other fibrous proteins, such as silk fibroin, in which the sequence is $(\text{Glycine-Alanine})_n$. Because glycine is the smallest amino acid, it plays a unique role in fibrous structural proteins: in collagen, Gly is required at every third position because the assembly of the triple helix puts this residue at the interior (axis) of the helix, where there is no space for a larger side group than glycine's single hydrogen atom. In fact, the replacement of one Gly residue by another residue anywhere along the chain results in pathological conditions known as *Osteogenesis Imperfecta* and the *Ehlers-Danlos Syndrome*, which are characterized by fragile bones, weak tendons, and thin skin (Byers, 2000, 2001; Mooney and Klein, 2002). In spite of the compulsory requirement of having Gly in every third position, the X and Y positions can accommodate any amino acid residue. These residues have a structural role in determining triple-helix stability and a functional role in specifying intermolecular interactions. Proline (Pro, P) and Hydroxyproline (Hyp, O) are the most common residues in the X and Y positions respectively, as well as the most stabilizing: Pro and Hyp rings, with their geometrically constrained carboxyl and secondary amino groups, they account for the tendency of the individual polypeptide strands to form left-handed helices spontaneously, without any intra-chain hydrogen bonding (Jenkins and Raines, 2002).

The mechanical properties of tissues like bone and tendons are primarily determined by their hierarchical structures and the role of collagen in these structures. Most research in this field was focused on the macroscopic overall mechanical properties of collagen fibers tissues, without explicitly considering the molecular nanoscale structure. On the other hand, few studies have been reported which analyze the mechanical properties and the deformation mechanisms of tropocollagen under mechanical load. Harley et al. (1977) used the Brillouin scattering technique, estimating the Young's modulus of collagen to be 9.0 GPa, whereas Cusack and Miller (1979) obtained 5.1 GPa. By X-ray diffraction, Sasaki and Odajima (1996) measured a value of 2.9 GPa. In recent years, optical trapping methods have also been used, reporting elastic moduli between 0.35 and 12.2 GPa, depending on the adapted triple-helical radius (Sun et al., 2002). The results obtained with experimental techniques depend on the biological sample, but the main limitation is the lack of details about nanoscale phenomena. To overcome these limitations single molecules have also been tested via molecular simulations, using collagen-like models obtained by x-ray crystallography (Buehler, 2006a,b, 2008). Using molecular mechanics an elastic modulus of 2.4 GPa was reported (Vesentini et al., 2005), and similar results where published by another group (Lorenzo and Caffarena, 2005) who found a value of 4.8 GPa obtained by performing steered molecular dynamics (SMD) simulations. With a similar technique, one of the authors of this paper assessed the Young's modulus of single tropocollagen molecules ranging from 6.99 to 18.82 GPa for varying deformation rates (Buehler, 2006a,b; Buehler and Wong, 2007).

The range of mechanical properties reported for tropocollagen molecules (0.35–18 GPa) is rather broad (see Table 1). Furthermore, there is a large gap between deformation rates used in SMD simulations (on the order of nm/ps), and those used in AFM and optical trapping experiments, which are of the order of μm/s (Bozec and Horton, 2005) (these deformation rates are more likely to be close to the biologically relevant deformation rates).

This review suggests that there is a critical need to establish a firm understanding of the reason for this broad range of values. Furthermore, as of today, there is no clear understanding and no analysis that illustrates the precise atomistic mechanisms during mechanically stimulated unfolding of collagen molecules. The aim of the study reported here is thus to establish a clear understanding of the influence of the pulling rate on the Young's modulus Y of individual tropocollagen molecules, as well as to perform a detailed analysis of the unfolding pathway under mechanical load.

2. Methods

The mechanical properties of tropocollagen molecules are investigated using SMD simulations, submitting the tropocollagen molecule to traction along its principal axis. Using the software THeBuScr (Triple Helical Collagen Building Script) (Rainey and Goh, 2004a,b), we built a model of the tropocollagen molecule. We choose the simplest model of collagen, with only Gly-Pro-Hyp triplets on each of the three chains. The collagen model we use, $[(\text{Gly-Pro-Hyp})_{10}]_3$, is truncated to 30 amino acids per chain in order to reduce computational costs and for comparison reasons, since peptides of comparable length were used both in computational and experimental studies (Bella et al., 1994; Persikov et al., 2000; Lorenzo and Caffarena, 2005; Vesentini et al., 2005; Buehler, 2006a,b). This leads to short length tropocollagen segments with a length of approximately 8 nm.

Due to its short length (shorter than its persistence length of approximately 10–20 nm (Sun et al., 2004; Buehler and Wong, 2007)) entropic elasticity effects are not considered here. This is consistent with the experimental data obtained for uncrimped, straight molecules.

Molecular dynamics simulations are performed using the GROMACS code (Berendsen et al., 1995; Vandrunen et al., 1995) and the GROMOS96 43a1 force field. This force field has been successfully used in earlier studies to simulate tropocollagen molecules (Grigera, 2002; Lorenzo and Caffarena, 2005).

The proteins are solvated in a 15 nm × 3 nm × 3 nm water box using single point charge (SPC) water molecules for solvent. SETTLE (for water) and LINCS algorithms are used to constrain covalent bond lengths involving hydrogen atoms, thus allowing a time step of 2 fs. Non-bond interactions are computed using a cutoff for neighbour lists at 10 Å, with a switching function between 0.8 and 0.9 nm for Van der Waals interactions. The Particle-Mesh Ewald sums (PME) method is applied to describe electrostatic interactions. The preliminary system energy minimization is performed by using a steepest descent algorithm. The system is equilibrated at a temperature of 310 K (37 °C) for 1,200 ps of molecular dynamics. The whole protein is held fixed for the first 200 ps by restraining the atomic positions, and thereafter only the first and the last C_α-atom of each chain are restrained for the following 1000 ps.

To perform the SMD simulations, the centre of mass of the three N-terminal C_α-atoms is kept fixed by means of a strong harmonic restraint with a spring constant of 3×10^5 kJ mol^{-1} nm^{-2}, while the centre of mass of the three C-terminal C_α (the pulled group of atoms) is linked to a spring with an elastic constant $k_{spring} = 4,000$ kJ mol^{-1} nm^{-2}, which is moved along the molecular axis with velocities from 0.01 to 100 m/s.

All molecular dynamics simulations are carried out in an *NPT* ensemble, with the systems coupled to a heat bath at 310 K (coupling constant of 0.1 ps and Berendsen thermostat) and to an hydrostatic bath at 1 atm (coupling constant of 0.5 ps and Berendsen barostat).

The force applied to the tropocollagen molecule by the virtual spring is:

$$F(t) = k_{spring}(x_{spring}(t) - x_{pull}(t)), \tag{1}$$

where x_{spring} and x_{pull} represent spring and pulled group positions, respectively. The elastic constant of tropocollagen, k_{TC}, is calculated by fitting the force F versus ΔL relationship (the parameter L is the tropocollagen molecular length) and by considering the value of its derivative at 8% strain (for applied strains smaller than 8%, the tropocollagen molecule is crimped so it is not under tension). The Young's modulus Y is calculated from:

$$Y = \frac{\sigma}{\varepsilon} = \frac{F/A}{\Delta L/L_0} = \frac{F}{\Delta L} \times \frac{L_0}{A} = k_{TC} \times \frac{L_0}{A}. \tag{2}$$

The strain is given by

$$\varepsilon = \frac{L - L_0}{L_0}, \tag{3}$$

where ε is the tensile strain in the measured direction. In Eqs. (2) and (3), L and L_0 are the current and the initial tropocollagen length, respectively, and A is the cross-sectional area of the tropocollagen molecule, obtained from the ratio between the molecular volume and L_0. In order to estimate the statistical accuracy of the determined elastic moduli, we perform five simulations with different initial configurations for each case studied and determine the averaged value.

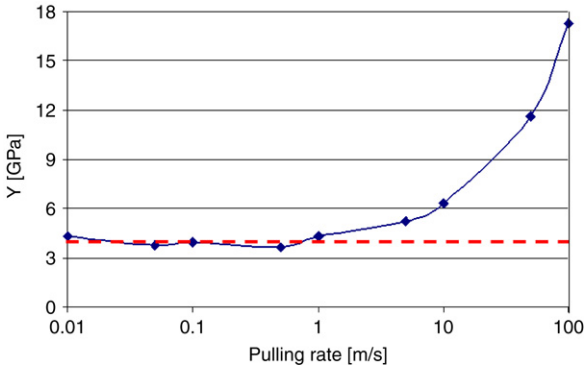

Fig. 1 – Young's modulus of a single tropocollagen molecule, fully solvated, as a function of pulling rate, evaluated at 8% tensile strain of the molecule when the initially crimped molecule has been straightened out. The trend shows that the elastic modulus of the tropocollagen molecule is rate dependent, but it converges to a value close to 4 GPa for slow deformation. Once the converged value is reached (indicated with the red, dashed line), the fluctuations are within the error bars of the measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Due to computational limitations, molecular dynamics simulations are restricted with respect to the time scales that can be reached, limiting overall time spans in such studies to tens of nanoseconds. Due to this limitation, many earlier molecular dynamics simulation results of dynamically stretching tropocollagen molecules have been carried out at large deformation rates, exceeding several m/s (see also discussion in the Introduction section).

3. Computational results

In this study, the mechanical behaviour of tropocollagen is evaluated in terms of its Young's modulus for different loading rates (Fig. 1). For pulling rates lower than 0.5 m/s there is a flat regime in which the mechanical properties of tropocollagen are independent of the loading rate. For intermediate velocities, between 0.5 and 10 m/s, a heel region can be recognized, where the mechanical properties start to increase with the pulling velocity, while at higher rates a steep increase of Y is observed. We obtain a standard deviation of ±0.2 GPa from the five simulations studied at each pulling velocity, for pulling velocities below 1 m/s.

The simulation trajectories suggest that tropocollagen molecules unfold in different pathways, depending on the deformation rate. In particular, when pulled at low rates, the

Fig. 2 – Tropocollagen molecules at large deformation, comparing the effect of slow deformation (0.1 m/s, upper part) with rapid deformation (100 m/s, lower part). If deformed slowly, the protein uncoils so that when the backbone begins to be stretched the three chains are arranged in parallel since they have lost their triple helical structure. If the loading is fast, the collagen peptide remains in a coiled coil configuration when the protein backbone is stretched. This leads to higher mechanical forces.

protein unfolds before the chains' backbones are eventually stretched, whereas for higher velocities it fails to unfold, keeping the coiled coil configuration even at high strain (see Fig. 2).

In Fig. 3 we depict the molecular rotation, calculated considering the relative rotation of the molecular ends as a function of the applied strain. The tropocollagen molecule begins in a coiled coil configuration, and then unfolds as the molecule is stretched. Notably, three different unfolding pathways can be recognized: for low pulling rates, the triple helix uncoils completely at 10%–20% strain, then undergoes some recoiling in the opposite direction, and finally straightens for strains larger than 30%. At intermediate rates, the molecule unfolds linearly with strain up to 35% strain. On the other hand, at higher velocities the triple helix does not uncoil even at large strain (Fig. 2). These three different unfolding pathways are very well matched with the rate-dependence of mechanical properties (Fig. 1), suggesting that the higher Y found for pulling rates ≥50 m/s are due to the kept coiled coil configuration, which otherwise is lost during the deformation at slower loadings, in particular lower than 1 m/s. The recoiling in the opposite direction is an interesting phenomenon that, to the best of our knowledge, is described here for the first time. However, the underlying molecular mechanism for this phenomenon is not completely clear. A possible explanation could be the moment of inertia that forces the molecule to continue its rotation before being straightened by the external force.

Since the results concerning the mechanical properties converge for pulling rates slower than 0.5 m/s, we investigate additional details of molecular unfolding using the case of 0.1 m/s. For this case, when the tropocollagen molecule is loaded, the force remains negligible until ≈10% of strain. For larger strains, it increases progressively up to 20 nN at 40% strain (see Fig. 4). This behaviour can be explained by monitoring the simulation trajectory, which shows that the molecule is slightly bent in the initial configuration (see Fig. 5). Thus during the initial stage (up to 8% strain) the molecule is straightened and only after this point does it begin to be stretched, leading to an increased force (this observation is the basis for the analysis of Young's modulus at 8% strain, as discussed above).

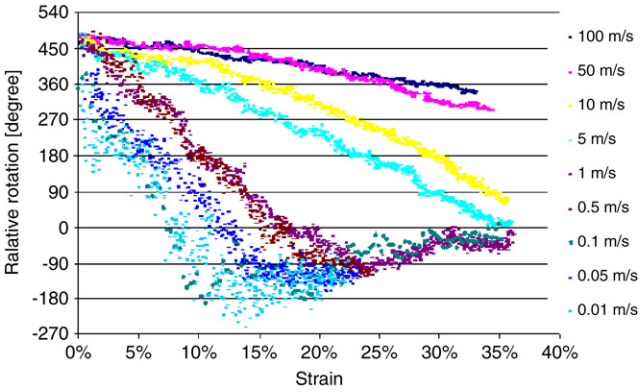

Fig. 3 – End-to-end rotation during loading. Three different types of behaviour depending on loading rate can be recognized, matching the trend of Young's modulus. If tropocollagen is pulled at high velocities (approximately 50 and 100 m/s) the molecule maintains its triple helical configuration even at large strains. At intermediate rates (approximately 5 and 10 m/s), the molecule uncoils with deformation, while reaching a straightened configuration only at large strain. At pulling rates below 1 m/s, the molecule completely uncoils at 10–20% strain, then rotates again in the opposite direction and then straightens at higher strain. In particular, for pulling rates lower than 0.1 m/s the rotation dynamics is independent on loading rate.

Fig. 4 – Force vs. strain for tropocollagen pulled at different velocities. At low deformation rates, when the elastic properties have converged to a finite value (as seen in Fig. 1) the force is negligible until approximately 8%–10% tensile strain is reached, then it increases gradually and, for strains larger than 35%, the force increases linearly with strain. This illustrates that the tensile behavior of single tropocollagen molecules is highly nonlinear. The continuous change of the modulus from small values at small strains to larger values (and thus higher stiffness) at larger strain may explain the range of values reported in the literature.

The investigation of the H-bond history leads to similar results. As shown in Fig. 6, in the undeformed situation the number of H-bonds is close to 30. This can be explained