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Research paper

Molecular structure, mechanical behavior and failure mechanism of the C-terminal cross-link domain in type I collagen

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ABSTRACT

Collagen is a key constituent in structural materials found in biology, including bone, tendon, skin and blood vessels. Here we report a first molecular level model of an entire overlap region of a C-terminal cross-linked type I collagen assembly and carry out a nanomechanical characterization based on large-scale molecular dynamics simulation in explicit water solvent. Our results show that the deformation mechanism and strength of the structure are greatly affected by the presence of the cross-link, and by the specific loading condition of how the stretching is applied. We find that the presence of a cross-link results in greater strength during deformation as complete intermolecular slip is prevented, and thereby particularly affects larger deformation levels. Conversely, the lack of a cross-link results in the onset of intermolecular sliding during deformation and as a result an overall weaker structure is obtained. Through a detailed analysis of the distribution of deformation by calculating the molecular strain we show that the location of largest strains does not occur around the covalent bonding region, but is found in regions further away from this location. The insight developed from understanding collagenous materials from a fundamental molecular level upwards could play a role in advancing our understanding of physiological and disease states of connective tissues, and also enable the development of new scaffolding material for applications in regenerative medicine and biologically inspired materials.

1. Introduction

Cross-linking of molecules, fibrils and fibers is a critical feature in numerous connective tissues such as bone, tendon, cartilage or blood vessels (Gelse et al., 2003; Fratzl and Weinkamer, 2007; Gupta et al., 2010). In these tissues, the mechanical strength is primarily provided by strong and elastic molecules such as collagen and elastin (Alberts et al.,

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2002; Buehler and Yung, 2009), which arrange into highly organized hierarchical structures (Fratzl and Weinkamer, 2007; Gelse et al., 2003; Gupta et al., 2010; Ortiz and Boyce, 2008) where the formation of intermolecular covalent cross-links is important in enhancing the mechanical stability of the resulting tissues. However, when the cross-link density increases, as found in elderly individuals, effects such as skin wrinkling, cartilage impairment or bone embrittlement as in osteoporosis and diabetes can occur (Bailey, 2001; Saito and Marumo, 2009). This illustrates the role of cross-links in the alteration of material properties in changes of physiological states and disease states, which can eventually lead to a breakdown of key material components of biological systems (Buehler and Yung, 2009). The effects of increased cross-link density on increasing the level of brittleness of collagenous tissues was confirmed by earlier coarse-grained computational simulations (Buehler, 2008), which investigated the influence of the cross-link density on the strength and the failure mechanism of a two-dimensional bead–spring representation of a collagen microfibril (Buehler, 2006). This study (Buehler, 2008) showed that the increase in cross-link density results in a severely altered mechanical behavior, leading to stronger but more brittle collagen fibrils. This altered mechanical response could have important effects on tissue viability. However, a molecular level understanding of the nanomechanical behavior of cross-linking in collagen fibrils has remained elusive, partly due to the lack of appropriate molecular models with atomistic resolution that would have enabled a systematic analysis. Here we report a study that overcomes this limitation, by developing first a molecular model of a cross-link in a model fibril of type I collagen, and then by applying the model to probe the deformation mechanisms under mechanical loading.

Our study focuses on type I collagen, a fibrous type of collagen and the most abundant of all collagen types in our body. Collagen is defined as a coiled-coil trimer molecule, each of which is composed of the repeated sequence of amino acids Gly–X–Y, where X and Y are commonly found to be proline and hydroxyproline amino acids, respectively. In type I collagen, these tropocollagen molecules self-assemble into pentameric right-handed twisted microfibrils (Orgel et al., 2006) in a staggered fashion, with an axial periodicity of approximately 67 nm (Piez and Trus, 1981). These microfibrils further arrange into fibers that can reach a diameter of several hundreds of nanometers. The structures are stabilized through intermolecular cross-links, formed between telopeptides and adjacent triple helical chains through lysine–lysine covalent bonding (Knott and Bailey, 1998; Light and Bailey, 1980; Reiser et al., 1992). Telopeptides, present at both N- and C-termini, are non-helical domains that remain after the cleavage of propeptides (Prockop and Kivirikko, 1995). The formation of these cross-links is mediated by the enzyme lysine oxidase (Eyre et al., 2008). Using X-ray diffraction on rat-tail tendon collagen fibrils, it was recently shown that the C-terminal cross-link occur between lysine 17 and lysine 87 in adjacent molecules, and more importantly, it was suggested that the telopeptide takes a folded configuration as shown in the schematic in Fig. 1(a) (Orgel et al., 2001). By utilizing these experimental advances as a guide, we build a full-atomistic model of the entire overlap region of two collagen molecules, incorporating a covalent cross-link between the above mentioned lysine residues. Our objective is to generate a simple model system that provides generic insight into the deformation mechanisms and associated mechanical behavior of cross-linked collagen fibrils, for which a high level of control about the underlying genetic sequence and structural arrangement is possible in our *in silico* approach.

2. Materials and methods

2.1. Molecular structure

To enable a high level of control over the genetic sequence, the triple helical collagen domains are created using the software THeBuScr (Rainey and Goh, 2004) by inputting the following sequences (these sequences are part of the complete sequence of type I collagen from GenBank RefSeq NM_000088.3 and NM_000089.3 that can be found in www.le.ac.uk/ge/collagen/COL1A1_numbering.pdf and www.le.ac.uk/ge/collagen/COL1A2_numbering.pdf). For the α_1 chains in the C-terminus region:

GARGPAGPQGPRGDKGETGEQGDRGIKGHRGFSGLQGPPGPPGSP
GEQGPSGASGPAGPRGPPGSAGAPGKDGLNGLPGPIGPPGPRGRTG
DAGPVGPPGPPGPPGPPGPP.

For the α_2 chain in the C-terminus region:

GPSGPQGIRGDKGEPGEKGPRGLPGLKGHNGLQGLPGIAGHHGDQ
GAPGSVGPAGPRGPAGPSGPAGKDGRTGHPGTVGPAGIRGPQGHQ
GPAGPPGPPGPPGPPGVSGGG.

For the α_1 chains in the N-terminus region:

GPMGPSGPRGLPGPPGAPGPQGFQGPPGEPGEPGASGPMGPRGPP
GPPGKNGDDGEAGKPGRPGERGPPGPQGARGLPGTAGLPGM**K**GH
RGFSGLDGAKGDAGPAGPKGEPGSPGENGAPGQMGPR.

For the α_2 chain in the N-terminus region:

GPMGLMGPRGPPGAAGAPGPQGFQGPAGEPGEPGQTGPAGARGP
AGPPGKAGEDGHPGKPGRPGERGVVGPQGARGFPGTPGLPGFKGI
RGHNGLDGLKGQPGAPGVKGEPGAPGENGTPGQTGAR.

In the collagen sequence pattern G–X–Y, each proline in the Y position is substituted by a hydroxyproline. Because the force field used here does not account for hydroxylysine, no post-translation modification was considered for the lysines. The C-telopeptides are generated with the Nanoengineer program (http://nanoengineer-1.com). The following sequences are used: SAGFDFSFLPQPPQE**K**AHDGGRYYRA for the α_1 chains, YDFGYDGDFYRA for the α_2 chain.

The three telopeptide segments are attached to the C-terminus region by concatenating both structure files (Protein Data Base [PDB] format). Then, they are positioned in space by manual manipulation using Visual Molecular Dynamics (VMD) (Humphrey et al., 1996) in order to reach an approximate alignment with the C-terminus region. In the same fashion, the N-terminus region is juxtaposed to the C-terminus region in order to achieve an overlap of 30 nm, according to experimental data (Orgel et al., 2001). Consequently, the telopeptides are shaped so as to reach the particular pattern suggested in Orgel et al. (2001) as displayed in Fig. 1(a). This process involves successive displacements of