Matrix Biology 34 (2014) 161–169

Contents lists available at ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

Mechanical recruitment of N- and C-crosslinks in collagen type I

Albert L. Kwansa [a,b], Raffaella De Vita [b], Joseph W. Freeman [a,c,*]

[a] Virginia Polytechnic Institute and State University, Virginia Tech-Wake Forest School of Biomedical Engineering and Sciences, Musculoskeletal Tissue Regeneration Laboratory, 330 Kelly Hall, 325 Stanger St., Blacksburg, VA 24061, USA
[b] Virginia Polytechnic Institute and State University, Department of Engineering Science and Mechanics, Mechanics of Soft Biological Systems Laboratory, 202 Norris Hall, 495 Old Turner St., Blacksburg, VA 24061, USA
[c] Rutgers University, Department of Biomedical Engineering, Musculoskeletal Tissue Regeneration Laboratory, 317 BME Building, 599 Taylor Rd., Piscataway, NJ 08854, USA

ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form 25 October 2013
Accepted 25 October 2013
Available online 21 November 2013

Keywords:
Collagen type I
Crosslink
Molecular dynamics
Molecular model
Telopeptide

ABSTRACT

Collagen type I is an extracellular matrix protein found in connective tissues such as tendon, ligament, bone, skin, and the cornea of the eyes, where it functions to provide tensile strength; it also serves as a scaffold for cells and other extracellular matrix components. A single collagen type I molecule is composed of three amino acid chains that form a triple helix for most of the molecule's length; non-triple-helical extensions called N- and C-telopeptides are located at the amino/N-terminal and carboxy/C-terminal ends of the molecule, respectively. In two of the three chains, the C-telopeptide has been reported to possess a hair-pin/hook conformation, while the three N-telopeptides display a more extended structure. These telopeptides are crucial for the formation of enzymatic covalent crosslinks that form in collagens near their N- and C-ends. Such crosslinks provide structural integrity, strength, and stiffness to collagenous tissues. However, deformation mechanisms of N- and C-crosslinks and functional roles for the N- and C-telopeptide conformations are not yet well known. By performing molecular dynamics simulations, we demonstrated that two dehydro-hydroxylysino-norleucine crosslinks, positioned at the N- and C-crosslinking sites, exhibited a two-stage response to the mechanical deformation of their parent molecules. We observed that the N-crosslink served as the first responder to mechanical deformation, followed by the C-crosslink. The results of our simulations suggest a mechanical recruitment mechanism for N- and C-crosslinks. Understanding this mechanism will be crucial for the development of larger-scale predictive models of the mechanical behavior of native collagenous tissues, engineered tissues, and collagen-based materials.

1. Introduction

Collagens are extracellular matrix (ECM) proteins that are found in nearly all eukaryotic organisms except for plants and protozoa (Urich, 1994). In mammals, collagens are especially abundant and can comprise up to one-third of all protein in the body by weight (Williams, 1978). There are approximately 27 different types of collagens that have been identified (von der Mark, 2006); type I collagen is the most prevalent and is found in vertebrate connective tissues such as tendon, ligament, bone, skin, and the cornea of the eyes (von der Mark, 2006). Type I collagen functions to provide tensile strength to these connective tissues, and it serves as a structural framework for cells and other ECM components such as fibronectin, proteoglycans, and bone mineral (Sweeney et al., 2008).

Type I collagen is a rod-shaped molecule with a length of ~300 nm and a diameter of ~1.5 nm (Birk and Bruckner, 2005). It is composed of three amino acid chains (often called α-chains), each containing just over 1000 amino acids; there are two α1 chains and one α2 chain. The entire molecule is composed of an N-telopeptide domain at the beginning, a triple-helical domain, and a C-telopeptide domain at the end (The UniProt Consortium, 2012). The N-telopeptides have been reported to display an extended coil structure; and the longer α1 C-telopeptides a hair-pin/hook structure (Orgel et al., 2006).

Fibril-forming collagens (e.g., types I, II, III, V, and XI) are able to assemble into structures called fibrils, which are stabilized by covalent bonds called crosslinks. There are enzymatic crosslinks and non-enzymatic crosslinks. Enzymatic crosslink formation is regulated by lysyl hydroxylase and lysyl oxidase enzymes. Lysyl hydroxylases are intracellular enzymes that convert specific lysines to hydroxylysines, and lysyl oxidases are extracellular enzymes that convert the side chain ε-amino group of telopeptide lysines and hydroxylysines into an aldehyde group (Avery and Bailey, 2008; Kagan and Ryvkin, 2011). These aldehyde groups can react readily with other ε-amino groups to form these crosslinks (Knott and Bailey, 1998). There are several types

Abbreviations: α, alpha value; ANOVA, analysis of variance; Cα, alpha carbon; CHARMM/CHARMm, Chemistry at HARvard Molecular Mechanics; deH-HLNL, dehydro-hydroxylysino-norleucine; deH-LNL, dehydro-lysino-norleucine; ECM, extracellular matrix; GBIS, generalized Born implicit solvent; NAMD, NAnoscale Molecular Dynamics; *P*, p-value; RMS, root-mean-square; SMD, steered molecular dynamics; TCL, tool command language; VMD, Visual Molecular Dynamics.

* Corresponding author at: Rutgers University, Department of Biomedical Engineering, Musculoskeletal Tissue Regeneration Laboratory, 317 BME Building, 599 Taylor Rd., Piscataway, NJ 08854, USA. Tel.: +1 848 445 6595.
E-mail address: joseph.freeman@rutgers.edu (J.W. Freeman).

0945-053X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.matbio.2013.10.012

of enzymatic crosslinks that can form (e.g., immature, divalent crosslinks and mature, trivalent crosslinks), depending upon factors such as tissue type, age, and health (Eyre et al., 1984a, 1984b; Fujii et al., 1994; Sims and Bailey, 1992). Non-enzymatic crosslinks tend to form in old age or in certain disease states (e.g., diabetes) outside of the regulation of enzymes (Avery and Bailey, 2008). The focus of this paper is on one of the enzymatic crosslinks called dehydro-hydroxylysino-norleucine (deH-HLNL). deH-HLNL involves the reaction of a lysine aldehyde (from an N- or C-telopeptide domain) to a hydroxylysine (from the triple-helical domain) (see Fig. 1 for the chemical structure of deH-HLNL and its precursor amino acids).

Steered molecular dynamics (SMD) and moving constraints are two extensions of molecular dynamics that allow for constant force pulling (e.g., constant force SMD) or constant velocity pulling (e.g., constant velocity SMD and moving constraints). In moving constraints and constant velocity SMD, each pulled atom or the center of mass of a group of pulled atoms, respectively, is connected through a virtual spring (k = stiffness) to a virtual atom that moves at a constant velocity (v = velocity) (Bhandarkar et al., 2011). The use of virtual springs and virtual atoms is meant to provide an analog to position-controlled atomic force microscopy (AFM) (Isralewitz et al., 2001a). Constant velocity SMD and its related techniques have been used to simulate the mechanical functions of proteins and protein unfolding pathways (Isralewitz et al., 2001b), and to predict the Young's modulus of collagen-like molecules (Gautieri et al., 2009; Lorenzo and Caffarena, 2005).

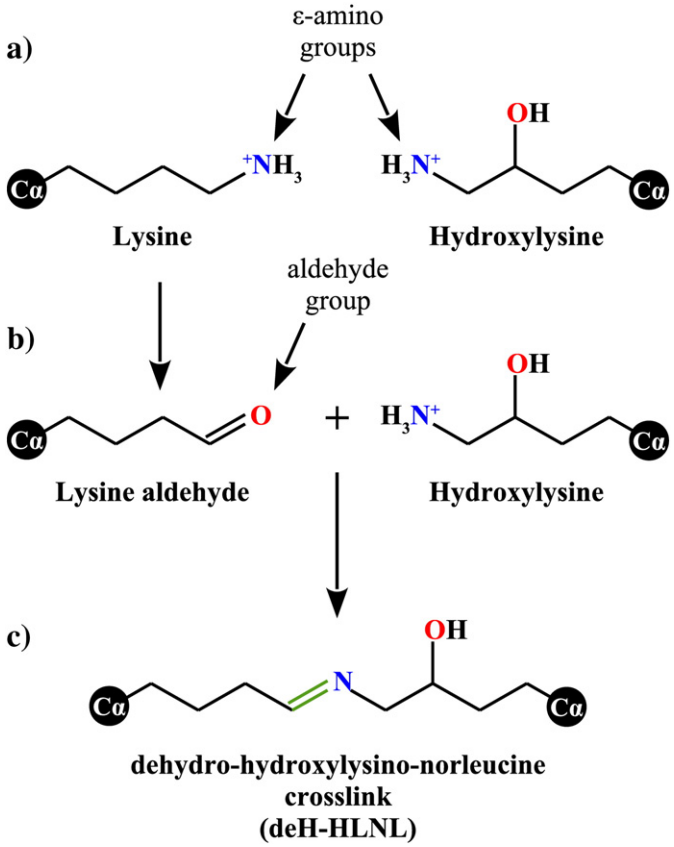

Fig. 1. A schematic of the formation of the enzymatic crosslink dehydro-hydroxylysino-norleucine (deH-HLNL) from the amino acids lysine and hydroxylysine (a modification of lysine). (a) shows the precursor amino acids lysine and hydroxylysine. (b) shows the product of the conversion of lysine to a lysine aldehyde. (c) shows the product of the reaction between this lysine aldehyde and hydroxylysine. In the case of deH-HLNL, the lysine is derived from one of the N- or C-telopeptide domains, while the hydroxylysine is derived from the main triple-helical domain. In the online color version of this figure, colors are used to further distinguish oxygen atoms (red), nitrogen atoms (blue), and the newly formed covalent bond within the deH-HLNL crosslink (green) (i.e., a carbon-nitrogen double bond).

The pulling velocity and the stiffness of the virtual springs have been shown to influence constant velocity pulling simulations. For instance, faster pulling rates can lead to over-estimated mechanical properties (Gautieri et al., 2009). Furthermore, overly compliant virtual springs can lead to under-estimated mechanical properties, and overly stiff virtual springs can result in increased numerical noise (Lorenzo and Caffarena, 2005). One, therefore, typically seeks a sufficiently reduced pulling velocity (i.e., closer to what might be employed experimentally or experienced physiologically) and sufficiently stiff virtual springs. There is a balance, however, that must be sought when choosing a pulling velocity, since a reduced pulling velocity necessitates a greater number of simulation timesteps to reach the same level of deformation, which increases the computational demands of one's simulation (e.g., time and/or computing resources).

Collagen and collagen-like molecules have been investigated through experiment and simulation; however, only a few investigations have been carried out on the mechanism through which collagen crosslinks respond to deformation. Uzel and Buehler conducted constant velocity SMD simulations (k = 4000 kJ/mol/nm^2 = ~9.56 kcal/mol/Å^2 (Gautieri et al., 2009); v = 1 m/s) with a 30-nm-long collagen type I molecular model containing an enzymatic C-terminal lysine-lysine crosslink called dehydro-lysino-norleucine (deH-LNL). They reported that this C-crosslink exhibited an initial delayed response due to the unfolding and straightening of the C-telopeptide. It was also reported that the crosslink contributed more to load-bearing at higher levels of deformation (Uzel and Buehler, 2011). In another study, Bourne and Torzilli investigated how perpendicular forces applied to a crosslink affect the conformation of its parent collagen molecule. Constant velocity SMD (k = 1 kcal/mol/Å^2; v = not reported) was used to pull on a non-enzymatic crosslink precursor amino acid (arginine) in a direction perpendicular to the long axis of a collagen-like molecule. It was reported that the collagen-like molecule offered little resistance to the perpendicular forces applied, which led to molecular bending and conformational disruption of the triple helix before covalent failure might be expected. It was thus suggested that these conformational disruptions, in response to crosslink loading, present an additional mechanism of damage within collagen proteins (Bourne and Torzilli, 2011). Interesting and important insights have been reported in these studies that have already been employed in the development of a rheological model of a crosslink's load-deformation behavior (Uzel and Buehler, 2011) and a finite-element model of unmineralized and mineralized fibrillar collagen (Hambli and Barkaoui, 2012).

In order to contribute to this area of research, we sought to investigate the influence of crosslink location (i.e., N vs. C-crosslinking site) using deH-HLNL as a model crosslink. deH-HLNL is an enzymatic lysine-hydroxylysine crosslink that has been detected in connective tissues such as skin (Saito et al., 1997; Sims and Bailey, 1992), tendon, and ligament (Fujii et al., 1994). deH-HLNL is especially abundant in developing (i.e., immature) and healing tissues. We used molecular dynamics to model the deformation of a ~23-nm-long molecular model in representation of the ends of two crosslinked collagen type I molecules with two deH-HLNL crosslinks positioned at the N- and C-crosslinking sites; these simulations were designed to model modes of molecular deformation that have been proposed through the X-ray diffraction of bovine Achilles tendons (Sasaki and Odajima, 1996).

2. Results and discussion

2.1. Higher spring constants improve the accuracy of the observed pulling velocity

In order to select an appropriate spring constant for the virtual springs, a spring constant study was conducted (k = 0.01, 0.1, 1, 10, 100, 1000, 9999 kcal/mol/Å^2 with v = 100 m/s). We found that stiffer