Journal of Biomechanics xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Effect on the mechanical properties of type I collagen of intra-molecular lysine-arginine derived advanced glycation end-product cross-linking

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ARTICLE INFO

Article history:
Accepted 22 November 2017
Available online xxxx

Keywords:
Collagen
Molecular dynamics
Ageing
Glycation
Protein cross-linking
Molecular biomechanics

ABSTRACT

Non-enzymatic advanced glycation end product (AGE) cross-linking of collagen molecules has been hypothesised to result in significant changes to the mechanical properties of the connective tissues within the body, potentially resulting in a number of age related diseases. We have investigated the effect of two of these cross-links, glucosepane and DOGDIC, on the tensile and lateral moduli of the collagen molecule through the use of a steered molecular dynamics approach, using previously identified preferential formation sites for intra-molecular cross-links. Our results show that the presence of intra-molecular AGE cross-links increases the tensile and lateral Young's moduli in the low strain domain by between 3.0–8.5% and 2.9–60.3% respectively, with little effect exhibited at higher strains.

1. Introduction

Collagen is one of the major contributors to the mechanical properties of mammalian tissues. There are currently 28 different human collagen types (Kadler et al., 2007), with the fibril-forming type I collagen being the predominant form. Type I collagen is typically found in connective tissues such as tendon, ligament, bone, skin and the cornea of the eyes of vertebrates (Bhattacharjee and Bansal, 2005). Type I collagen provides not only the tensile strength to these connective tissues, but it also serves as a structural framework for the attachment of cell and other extracellular matrix (ECM) biomolecules (Sweeney et al., 2008). The functional integrity of collagen within the collagenous tissues is vital for the normal functioning of the body.

With collagen making up a significant proportion of connective tissues, approximately 90% in some cases (Kannus, 2000), its biomechanical and energy storage properties are of utmost importance (Franchi et al., 2007). The mechanical functions of the supramolecular structure in collagenous tissues are optimised for the direction and magnitude of load. Tendons have uni-directional tensile strength, a consequence of fibre alignment in thick bundles parallel to the long axis of the tendon (Silver et al., 2003). In skin, the fibres form an isotropic network capable of managing multi-directional forces (Ottani et al., 2001). The forces experienced by the collagenous tissues vary greatly in magnitude and direction. Applied forces can be sporadic, sustained or repetitive. For example, a runner's Achilles tendon can experience peak forces of 11.4 times their body weight (Dixon and Kerwin, 2002), bearing over 2000 cyclic loading events during a 5 km run (Lichtwark et al., 2013).

The hierarchical structure of the collagen supra-molecular assembly results in several mechanical properties over different scales: the molecular scale, i.e. the response of the collagen molecule to strain; the fibrillar scale, with the response of fibrils to an applied load; the microscale, which incorporates the response of a collagen fibre; and finally the macroscale, where the mechanics of the whole collagenous tissue are considered. At the molecular scale, a number of atomistic and coarse-grained molecular dynamics simulations have been conducted to probe the response of the molecule to an applied load (Bhowmik et al., 2007; Buehler, 2006; Gautieri et al., 2009), with a small number of experimental studies probing single molecule responses to a load (Bozec and Horton, 2005; Sun et al., 2002). At the microfibril and fibril level the amount of experimentally determined data increases, with experiments using a wide variety of techniques such as a micro-electromechanical systems device (Eppell et al., 2006), X-ray diffraction (Sasaki and Odajima, 1996a) and AFM (Van Der Rijt et al., 2006; Wenger et al., 2007). The results of these studies have

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https://doi.org/10.1016/j.jbiomech.2017.11.021
0021-9290/

shown that as the hierarchical scale increases, Young's modulus (YM) decreases significantly, with the molecular level ranging from 2 to 9 GPa, whereas on the tissue scale, the modulus varies between 0.001 and 1 GPa, depending on the tissue type (Sherman et al., 2015). The most likely reason for this variation is the inter-fibrillar sliding and the straightening and reorientation of the fibrils/fibres (Parry, 1988).

Due to the size and complexity of collagen molecules, to date, very few computational investigations have been conducted. Most investigations have utilised collagen-like peptides (CLPs), which are triple helical peptides of typically 30 residues in length. CLPs have similar properties to collagen and due to their reduced size are more amenable to study. The first computational study to probe the mechanical properties of the collagen molecule was conducted by Lorenzo and Caffarena (2005), when they conducted a steered molecular dynamics approach, testing the molecular response of short 29–30 amino acid collagen-like peptides, based on a model with two springs in series (Lorenzo and Caffarena, 2005). However, this study used a polarisable continuum model, which was shown to be insufficient in a later study on the participation of structural water in carrying load (Zhang et al., 2007), thereby altering the mechanical properties. Two other studies have been conducted since, investigating several elements, e.g. how helical hierarchy controls collagen deformation (Pradhan et al., 2012) and the role of the mature enzymatic cross-links (Kwansa et al., 2014). Yet to date, no such study has been conducted to investigate the effect that age-related non-enzymatic cross-links have on the mechanical properties of collagen.

Enzymatic cross-links are initiated by the enzyme lysyl oxidase and form inter-molecularly between the collagen molecules at defined locations, to provide functional stability to the collagen fibril (Reiser et al., 1992). Unlike enzymatic cross-links, non-enzymatic cross-links are considered pathological, disrupting normal biological function and altering the mechanical properties of the tissue (Reddy, 2004; Schalkwijk et al., 2004). Non-enzymatic cross-links, most commonly advanced glycation end products (AGEs), are formed through a series of successive chemical reactions between a reducing sugar, such as glucose (an aldose) or fructose (a ketose), and a protein or lipid (Biemel et al., 2001; Vistoli et al., 2013). To date only a few physiologically relevant AGEs have been characterised from tissues ex vivo, most notably lysine-lysine and lysine-arginine cross-link forming AGEs (Biemel et al., 2002). The concentration of AGEs have been shown to steadily increase with normal ageing (Simm et al., 2007; Thorpe et al., 2010), particularly in tissues with low turnover rates, such as type I collagen whose half-life can be up to 200 years in tendon (Heinemeier et al., 2013). Studies have shown that the mechanical and biological functions of collagen are disrupted or altered upon formation of AGE cross-links, which may account for some of the age-related mechanical decline of collagenous tissues (Monnier et al., 2005). Reddy et al. found that *in vitro* incubation of rabbit Achilles tendon in ribose increased levels of the AGE pentosidine, as well as increasing the YM by 159%, from 24.89 ± 1.52 MPa to 6 5.087 ± 14.41 MPa, suggesting that the presence of AGE cross-links increases the stiffness of soft tissue (Reddy, 2004).

In previous work, we have identified the preferential intra-molecular DOGDIC and glucosepane AGE formation sites within the collagen molecule, and discussed their potential impact on the biological function of collagenous tissues (Collier et al., 2016, 2015). Glucosepane is the most abundant lysine-arginine derived cross-linking AGE, forming a 7 membered ring containing two hydroxyl groups (Fig. 1A). DOGDIC is a hexose-derived lysine-arginine cross-linking AGE, which forms a 5-membered ring at the guanidine functional group of arginine, with an aliphatic carbon chain extending out which contains three hydroxyl groups, as seen in Fig. 1B. In this work we aim to expand upon those findings by probing, through the use of steered molecular dynamics simulations, the effect of the presence of these cross-links at the identified sites on the mechanical properties of the collagen molecule. We expect that the presence of an AGE cross-link within the collagen molecule will result in an increase in stiffness, due to interference with the normal extension mechanism.

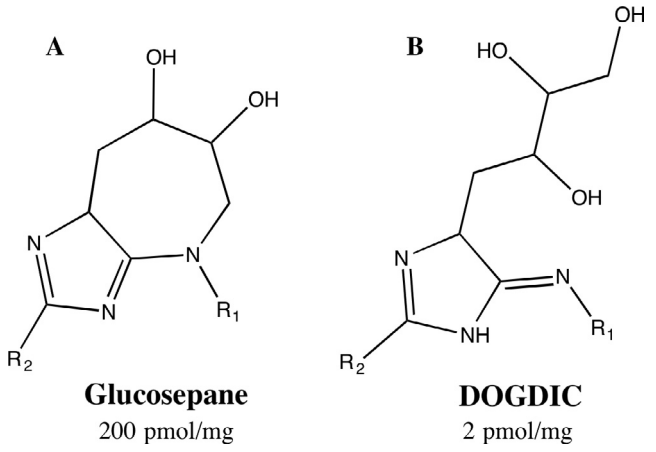

Fig. 1. Schematic image of Lysine (R_1) and Arginine (R_2) Cross-linking AGEs, (A) Glucosepane, (B) DOGDIC, with their concentration in human albumin serum as determined by Biemel et al., reported below each structure (Biemel et al., 2002).

2. Results and discussion

Using a steered molecular dynamics (MD) approach we have probed the effect of AGE cross-linking on the tensile and lateral mechanical properties of the collagen molecules. Sets of wild type (native) and cross-linked collagen-like peptides (CLPs) were created by taking short collagen sections from our previous simulations, 7 residues either side of the eleven identified preferential intra-molecular cross-linking locations (Collier et al., 2016, 2015). Six constant velocity steered MD simulations (n = 6) were conducted to probe the mechanical properties along the principal axis of the collagen molecule and transverse to the principal axis.

The YM for the models were then calculated in two strain regions, low strain (0–15%) and intermediate strains (20–40%). Values are reported for the eleven different CLPs as relative differences between the wild type and cross-linked models. Relative values will remove the effect of variation in the primary sequence on the reported mechanical properties (Uzel and Buehler, 2009). The absolute values for the tensile YM of the wild-type collagen varied between 3 and 4.5 GPa, which is in good agreement with previously reported values (Sherman et al., 2015).

In the low strain domain an increase in the calculated value for the tensile YM was obtained for both AGEs, DOGDIC and glucosepane, with values increasing by between 3.0 and 8.5% (Fig. 2A and B). Upon cross-linking, all of the potential sites exhibit an increase in the YM which is independent of the AGE type, although the magnitude of the effect is very dependent on the local environment. The major contributor to the increase in YM is the increase in number of hydrogen-bonds present, typically three or more, between the AGE and the collagen peptide, which would otherwise be absent in the wild type collagen.

At intermediate strains (20–40% strain), no statistically significant increase is observed in the tensile YM on introduction of an AGE between polypeptide chains (Fig. 2C and D). A decrease in YM, as seen for G4, G6, D1, D2 and D4, is likely the result of inaccuracies in the cross-sectional area of the peptide. In the intermediate strain domain, the hydrogen-bonds are no longer present due

Please cite this article in press as: Collier, T.A., et al. Effect on the mechanical properties of type I collagen of intra-molecular lysine-arginine derived advanced glycation end-product cross-linking. J. Biomech. (2017), https://doi.org/10.1016/j.jbiomech.2017.11.021