



Molecular biomechanics of collagen molecules

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Collagenous tissues, made of collagen molecules, such as tendon and bone, are intriguing materials that have the ability to respond to mechanical forces by altering their structures from the molecular level up, and convert them into biochemical signals that control many biological and pathological processes such as wound healing and tissue remodeling. It is clear that collagen synthesis and degradation are influenced by mechanical loading, and collagenous tissues have a remarkable built-in ability to alter the equilibrium between material formation and breakdown. However, how the mechanical force alters structures of collagen molecules and how the structural changes affect collagen degradation at molecular level is not well understood. The purpose of this article is to review the biomechanics of collagen, using a bottom-up approach that begins with the mechanics of collagen molecules. The current understanding of collagen degradation mechanisms is presented, followed by a discussion of recent studies on how mechanical force mediates collagen breakdown. Understanding the biomechanics of collagen molecules will provide the basis for understanding the mechanobiology of collagenous tissues. Addressing challenges in this field provides an opportunity for developing treatments, designing synthetic collagen materials for a variety of biomedical applications, and creating a new class of ‘smart’ structural materials that autonomously grow when needed, and break down when no longer required, with applications in nanotechnology, devices and civil engineering.

Introduction

Collagen molecules are the basic component of collagenous tissues, such as tendon and bone, which provide mechanical stability, elasticity and strength to organisms [1–7]. Unlike many engineering materials, collagen materials are ‘smart’ materials that have the ability to adapt their properties in response to mechanical forces through altering their structures from the molecular level up [8–10]. They are able to convert mechanical forces into biochemical signals that control many biological and pathological processes such as wound healing and tissue remodeling (Fig. 1). For example, appropriate physical training increases the cross-sectional area and the tensile strength of tendons [11–13], while inappropriate physical training can lead to tendon injuries

[14,15]. Due to the abilities of self-adapting and self-healing, how mechanical forces mediate the tissue remodeling and repairing process of collagen materials and understanding of their mechanotransduction mechanisms have recently attracted a lot of attention in the material research community.

Mechanical loading is important in collagenous tissue formation and remodeling [16]. It is understood that physical activity influences both collagen synthesis and degradation [9]. Fig. 2 shows an illustration of collagen synthesis and degradation after exercise. Both collagen formation and degradation increases initially after exercise in humans. A net collagen synthesis is found 36–72 h after exercise. These results show that collagen degradation is a fundamental event in connective tissue growth and remodeling [17]. At single molecule level, collagen molecules alter their structures in response to mechanical forces, providing signals to

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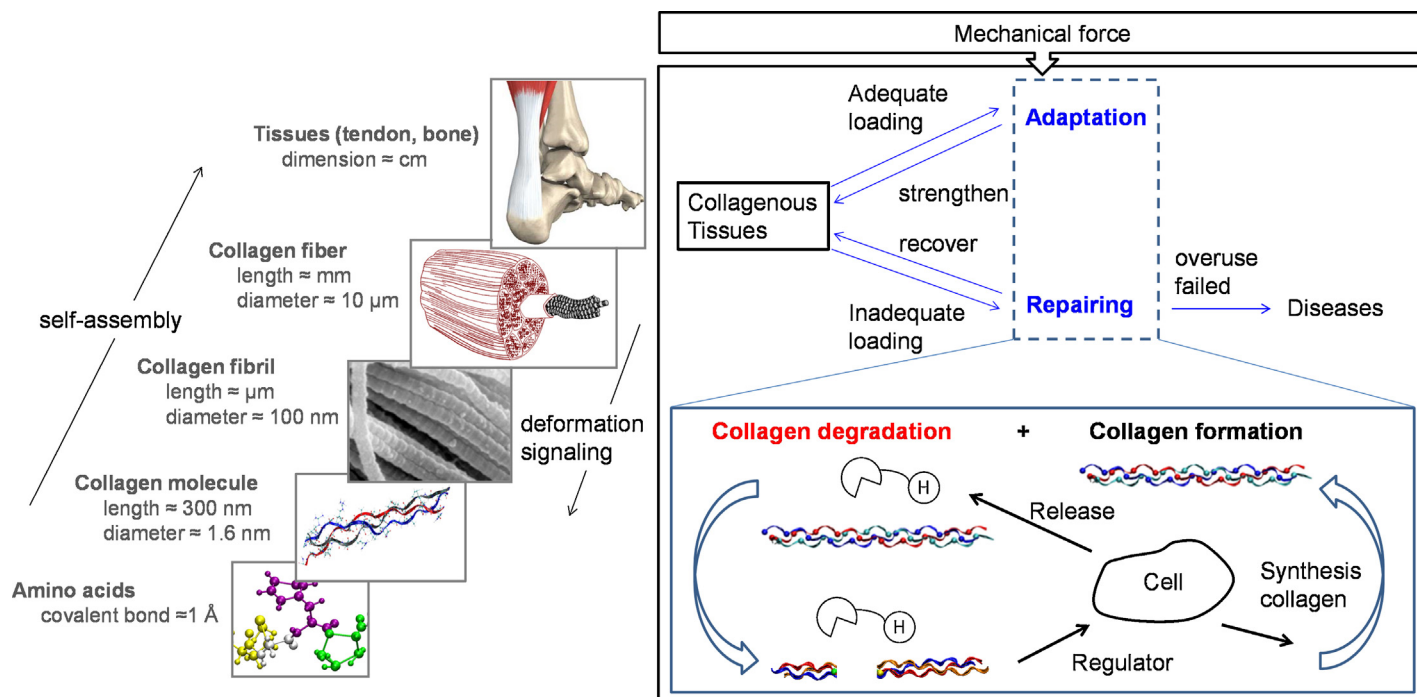


FIGURE 1

Structure of collagenous tissues and a schematic of biomechanics of collagen materials. Collagenous tissues are hierarchical structures composed of self-assembled collagen molecules. They are materials which can sense the mechanical forces, turning in to signals through deformation at molecule level, to enable tissue remodeling and repairing. Adequate loading enables the adaptation to strengthen the collagenous tissue while inadequate loading may lead to injuries. Hierarchical structure of connective tissues reprinted with permission from A. Gautieri, et al. *Nano Lett.* (2011). Copyright 2011 American Chemical Society.

mediate the collagen degradation rate. However, the biomechanics at collagen molecular level is still not well understood.

Recent technologies including experiments at single collagen molecule level and full atomistic simulations of collagen molecules have provided a way for us to reveal and understand the origin of pathological processes of collagenous tissues at the molecular level. Experiments have provided evidence that mechanical forces mediate the degradation rate of collagen molecules. In this paper, we review current understandings of the biomechanics of collagen molecules, which provide the basis of

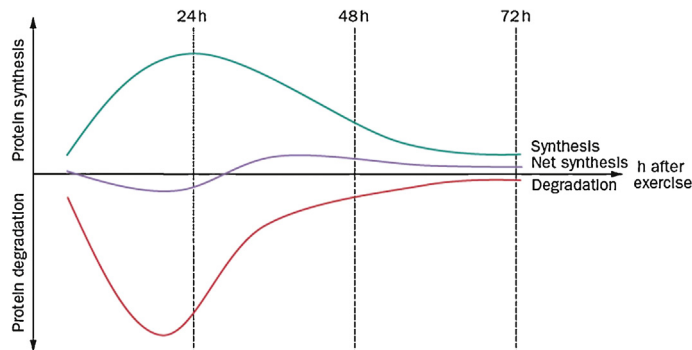


FIGURE 2

Illustration of collagen synthesis and degradation after exercise in humans, showing the direct coupling between synthesis and degradation to physical forces. Collagen formation and collagen degradation are two steps in the tissue remodeling and repairing processes. Net synthesis of collagen is found 36–72 h after exercise. Reprinted by permission from Macmillan Publishers Ltd.: *Nat. Rev. Rheumatol.* [9], copyright 2010.

understanding the mechanobiology of collagenous tissue. Structures and mechanics of collagen molecules will be reviewed first, followed by a review of collagen degradation mechanisms, and recent studies on how mechanical forces alter the structures of collagen molecules and thus mediate the degradation rate.

Structure and mechanical properties of collagen molecules

Collagen molecules are produced by cells and are self-assembled into hierarchical structures to form collagenous tissues [18–24]. The image on the left in Fig. 1 shows a schematic of the hierarchical structure of collagenous tissues [25]. The collagen molecule is a triple helical protein structure that consists of three chains with a characteristic repeating sequences $(\text{Gly-X-Y})_n$. A type I single collagen molecule has a diameter of about 1.6 nm with a length of about 300 nm. Collagen molecules form into collagen fibrils with a diameter of about 100 nm with a specific pattern known as D-period [22,25]. The collagen fibrils then form the fibers at the micron scale, which finally form the collagenous tissues. Collagen fibrils are the basic components of various collagenous tissues while the alignments of collagen fibrils and the components in a collagen fiber varies in different collagenous tissues to provide various mechanical and biological functions. For example, bone contains minerals to provide higher strength. In contrast, there is no mineral in tendon which can exhibit more strain in our daily activities. Collagen fibrils exhibit a parallel alignment in tendon and bone but align in different orientations in cornea [26] to support varied loading directions.

A statistical analysis of high-resolution X-ray crystal structures of triple-helical peptides has provided the molecular structure information of collagen molecules [27]. It has been revealed that the collagen molecule has a varied unit height of 0.853 nm for imino rich regions and 0.865 nm for amino rich regions and the inner radius is around 0.1 to 0.2 nm depending on the variation of the collagen sequences [27]. The collagen molecule is a heterogeneous structure along its twisting axis that the local conformation is controlled by the variation of sequences and each segment has varied mechanical and biological properties, and likely, biological functions.

A collagen molecule is flexible and has a worm-like chain behavior in response to mechanical forces below ~ 14 pN (Fig. 3) [28–30]. In this regime, the mechanics of single collagen molecule is controlled by entropic elasticity. The persistence length of collagen molecules are found to be in the range of 10–25 nm depending on the type of collagen. Experiments using optical tweezers to pull single collagen molecule show that the persistence length of type I collagen is 14.5 ± 0.73 nm [31] and the persistence length of type II collagen is 11.2 ± 8.4 nm [29]. Full atomistic simulations also reveal a similar range of the persistence lengths of collagen molecules

[30,32]. Although the persistence length is able to capture the overall force-displacement relation of a single collagen molecule (Fig. 3), the collagen molecule is known to be a heterogeneous material along its twisting axis due to the variation of sequences. The local conformations of the collagen molecule are found to vary and have various biological functions along the twisting axis [33].

Micro-unfolding regions have been identified in the collagen molecule [32,34,35], which are known to be important for biological functions such as collagen degradation. In the entropic elasticity regime of collagen molecules, the micro-unfolding regions are stretched firstly and exhibit larger deformations than the stable triple helix domains, leading to an inhomogeneous strain distribution [34] as shown in Fig. 3. This suggests that collagen molecules are able to respond to the mechanical forces by altering their structure with significant deformation at low mechanical force level, which is likely able to provide signals for altering its biological properties. For example, it has been shown that mechanical force is able to stabilize the structure of the cleavage site of collagen molecules and induces a molecular mechanism of force induced stabilization of collagen against enzymatic breakdown [34].

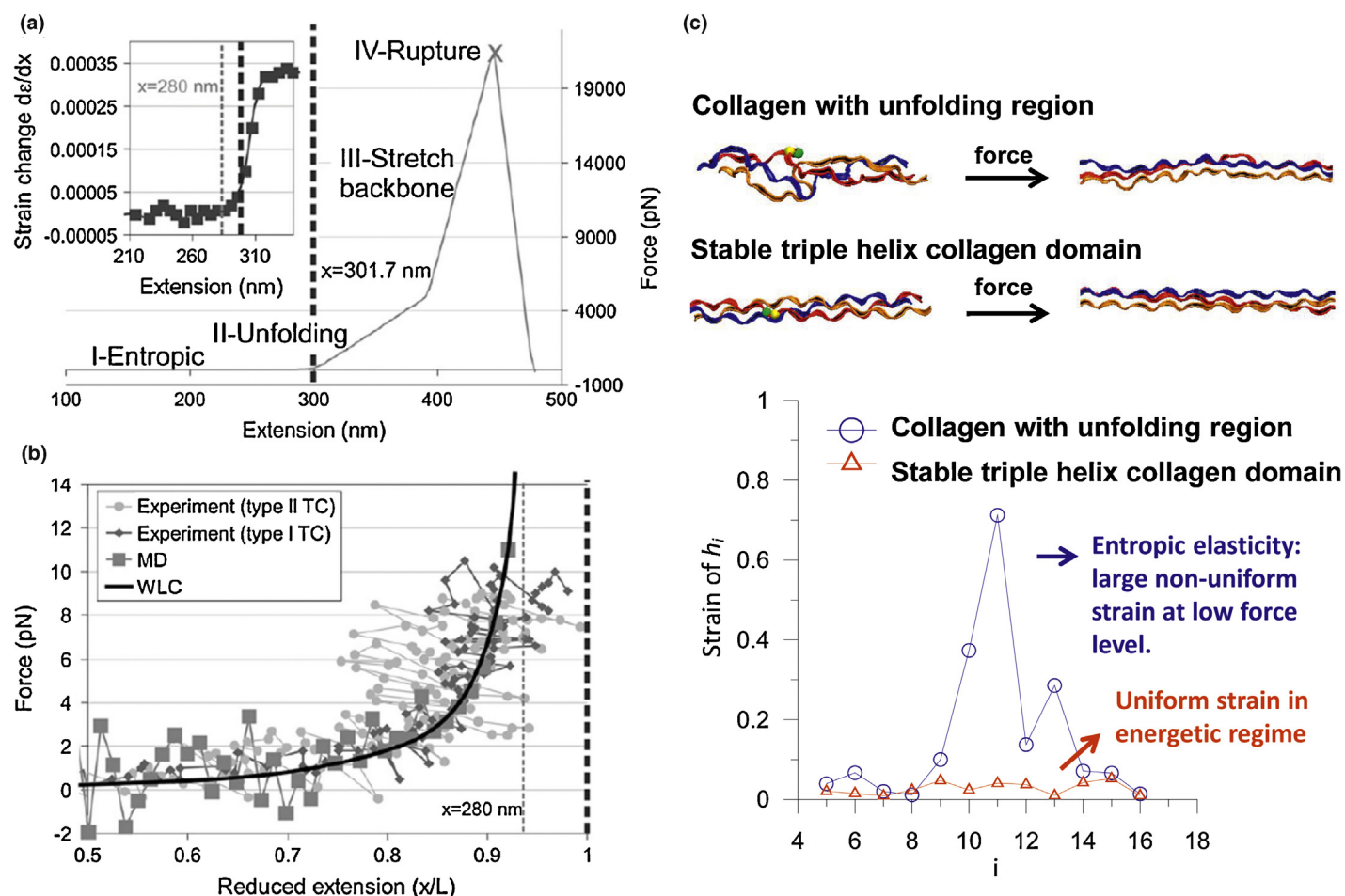


FIGURE 3

Mechanics of a single collagen molecule. (a) Typical force-displacement curve of a single collagen molecule. (b) The collagen molecule behaves like a flexible worm-like chain in response to a mechanical force below 14 pN. (c) A collagen molecule is an inhomogeneous material along its twisting axis. Each segment of a collagen molecule has specific mechanical and biological properties. In the entropic elasticity regime, unfolding regions of a collagen molecule exhibit large strain which provides biological signals in response of a low level force on the order of \sim pN. Once a collagen molecule is stretched beyond the entropic elasticity regime, it features a uniform strain distribution, as shown in the plot below. (a) and (b) reprinted from M.J. Buehler, S.Y. Wong, *Biophys. J.* 93 (2007) 37–43, copyright 2007, with permission from Elsevier.

The collagen molecule enters two linear regimes when it is stretched beyond the entropic elasticity regime [30] (Fig. 3). In the first linear regime, the collagen molecule exhibits uncurling of the triple helix over the entire length of collagen molecule. In this region, the collagen molecule has a uniform strain distribution along its entire domain once the micro-unfolding regions have been stretched out [34]. The Young's modulus of a collagen molecule is in the range of 3–7 GPa Young's established in earlier experimental and computational studies [34,36–40,25], which provides the mechanical strength of collagenous tissues for our daily activities. If a collagen molecule is further stretched, the collagen molecule becomes stiffer due to the stretching of backbone and eventually ruptures at higher mechanical loading which could lead to diseases.

Mechanisms of collagen degradation

Collagenases of the matrix metalloproteinase (MMP) family, including MMP-1, MMP-8, MMP-13 and membrane-bound MMP-14 [41], are major mammalian proteases involved in the physiological cleavage of collagen. MMPs consist of propeptide, catalytic and hemopexin domains as illustrated in Fig. 4. They play an important role in cleaving collagen into characteristic $3/4$ and $1/4$ fragments. For type I collagen, the specific cleavage site is after the 775th residue (Gly), in the sequences of G-1A for alpha-1 chain and G-1L for alpha-2 chain.

MMPs can only cleave a chain at the same time and the binding site of a stable triple helical structure is too narrow. Therefore it is widely accepted that MMPs are not able to cleave a stable triple helical structure. The cleavage site of collagen molecules must be in a vulnerable state to be cleaved. Two possible cleavage mechanisms have been proposed earlier. The first suggests that the collagen does not unwind by itself and MMPs unwind the collagen after binding [42]. In the second, a collagen molecule is believed to thermally unwind locally at the vicinity of the cleavage site before MMPs bind to it [43]. Without a *priori* assumption about the effect of MMPs on the local triple helix unwinding, these two models have been integrated into a more general mechanism previously as shown in Fig. 4 [44]. By setting $k_3 = k_{-3} = k_4 = k_{-4} = 0$, the scheme

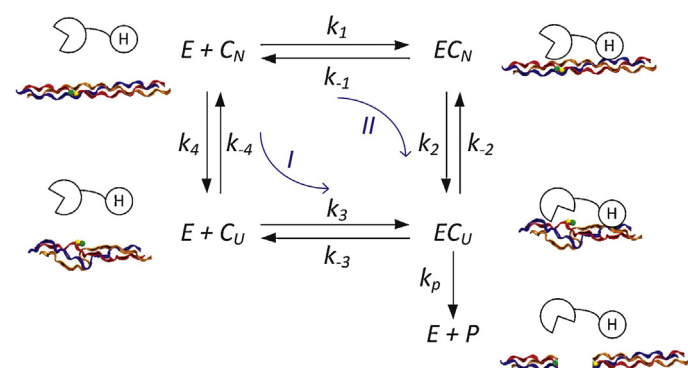


FIGURE 4

Molecular mechanisms of collagen degradation, including various pathways and possible mechanisms. The collagen molecule has to be in a vulnerable state to facilitate the degradation. Two mechanisms: (1) collagen molecule unfolds at the cleavage site before enzyme binding (path I); (2) enzyme unwinds collagen molecule after binding (path II), have been proposed to explain the degradation process. Reprinted from S.W. Chang, et al. Biophys. J. 33 (2012) 3852–3859, copyright 2012, with permission from Elsevier.

reduces to the first model, while with $k_2 = k_{-2} = 0$, the degradation scheme reduces to the second model.

There is evidence that both mechanisms exist and whether the cleavage site unwinds by itself depends on the thermal stability of the collagen molecule. Atomistic simulations, which serve as a tool that allows us to study the behavior at the vicinity of cleavage site with molecular details, have shown that there exists a vulnerable state of collagen at the cleavage site in the absence of MMPs [45–48], suggesting that the cleavage site could be thermally unfolded. On the other hand, recent experimental work has revealed that, for a stable triple helix, the hemopexin domain of MMP-1 binds to the cleavage site first, then a back-rotation of the catalytic domain leads to a “closed” conformation of MMP-1 and thus releases one chain out of the triple-helix, suggesting that the enzyme enables the unwinding of the cleavage site of the collagen molecules [49].

The MMPs are able to cleave the covalent bond between G-I/L while only one of the several other sites in the collagens that contain the same G-I/L bonds is hydrolyzed [50], suggesting that the local conformation at the vicinity of the cleavage site plays an important role in providing a recognition signal for MMPs since amino acid sequence alone is not sufficient for the high specificity of collagen recognition by MMPs [51,52]. Atomistic simulations of all G-I/L sites in type III collagen molecules have provided further evidences that local conformations of all sites have different vulnerability scores [47], indicating that the local conformation provides a recognition signal for enzymes.

The degradation varies in different types of collagen due to varied thermal stability and local conformation of the cleavage site. Experimental studies of human skin fibroblast collagenase have found large differences in the degradation rates, from 1.0 to 565 h^{-1} , for different types of collagen, including collagen type I, type II and type III [51]. Remarkably, the enzyme-substrate affinity is similar for all types of collagen. Han et al. also find similar enzyme-substrate affinity for type I heterotrimer and type I homotrimer which have very different degradation rates [44]. That is, the variations of the sequences of collagen molecules do not alter the binding affinity of enzyme but affect the proteolysis rate after enzyme binding.

Effects of mechanical force on collagen degradation

Degradation of collagen molecules is a crucial step for many biological and pathological processes such as wound healing, tissue remodeling, cancer invasion and organ morphogenesis [53–56]. Precisely regulated collagen degradation is required for normal physiological remodeling and repairing processes. Excessive or deficient degradations have been associated with many diseases. For example, accelerated breakdown of collagen may result in arthritis, atherosclerotic heart disease, tumor cell invasion, glomerulonephritis, and cell metastasis [57–64]. Deficient degradation of collagen has been shown to result increased trabecular bone in mice [65].

The chemical composition of a collagen molecule defines its material properties and how it alters its conformation in response to mechanical force. It is clear that mechanical force is able to alter the conformation of collagen molecule and thus mediates the collagen degradation rate (Fig. 5(a)). However, it remains a challenging question to understand whether mechanical force speeds

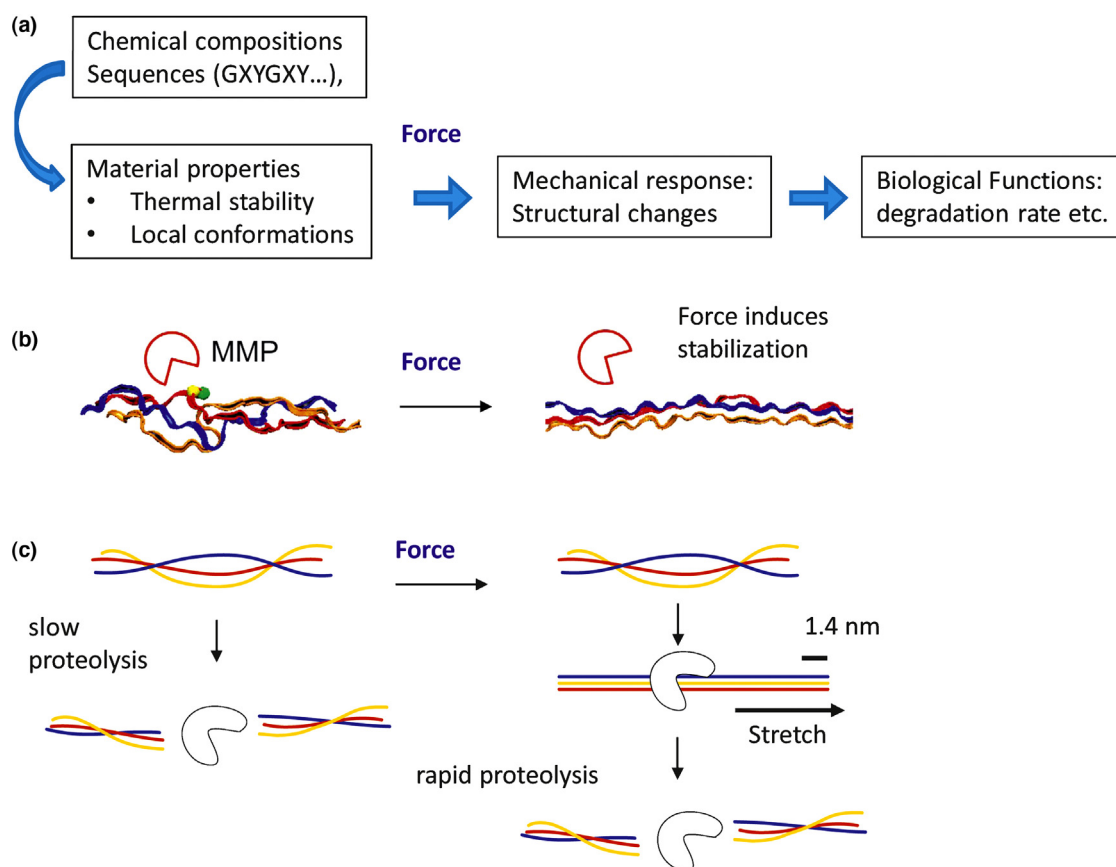


FIGURE 5

Potential mechanisms by which forces mediate the degradation rate. The chemical composition of a collagen molecule defines its mechanical properties. Mechanical force is able to alter the local conformation of a collagen molecule and thus mediates the collagen degradation rate. Two mechanisms have been proposed to explain how mechanical force slows down and speeds up the collagen molecule degradation. There exists two vulnerable states of the cleavage sites of collagen molecules, including unfolding (as shown in (b) before applying force) and unwinding of the cleavage site (as shown in (c) after applying force). (b) Mechanical force is able to slow down the degradation by enhancing the thermal stability of the collagen cleavage site if it is thermally unstable without applying force. (c) While on the other hand, mechanical force could enhance the degradation by unwinding the collagen cleavage site. Panel (c) adapted with permission from [73]. Copyright 2011 American Chemical Society.

up or slows down the degradation rate at different magnitudes of force and in different types of collagen molecules.

We summarize recent studies on mechanical effects on the collagen degradation rate in Table 1. Bhole et al. have shown that

mechanical strain enhances survivability of collagen micronetworks in the presence of collagenase [66]. The same mechanism has also been found for the reconstituted collagen fibrils in the presence of MMP-8 [67]. The fact that mechanical forces are able to

TABLE 1

Summary of recent experimental studies on mechanical force effects on collagen degradation rate.

Collagen type	Collagenase	Results	Effect on degradation rate (increase ↑ or decrease ↓)
Single collagen trimer peptide (GPQGIAGQGRGVVGL)	MMP-1	~10 pN induces a 100-fold increase in collagen degradation rate [73]	↑
Type I recombinant human collagen molecule	Bacterial collagenase	3–10 pN force slows enzymatic cleavage [71]	↓
Recombinant, post-translationally modified human collagen I	MMP-1	16 pN causes an 8-fold increase in collagen proteolysis rates [74]	↑
Recombinant, post-translationally modified human collagen I	Bacterial collagenase	16 pN force does not affect cleavage rates [74]	—
Reconstituted collagen fibrils	MMP-8	Mechanical strain stabilizes enzymatic degradation [67]	↓
Cornea and dissected from mature whole bovine eyes	Bacterial collagenase	Collagen degradation corresponds inversely to the tensile stress [69]	↓
Pepsin-extracted, bovine, type I, atelo-collagen monomers	Bacterial collagenase	Mechanical strain enhances survivability of collagen micronetworks [66]	↓

slow down the enzymatic cleavage has also been found in different conditions, including native tissue with dynamic loading, uniaxial tension on tendon *in vitro*, and at low force levels [68–71]. There is also evidence that mechanical force accelerates enzymatic degradation [72–74]. Experimental studies on single collagen molecule have shown that mechanical force is able to speed up the collagen degradation rate even with a low mechanical force level on the order of pN [73,74].

Two mechanisms have been proposed in the literature to explain how mechanical force speeds up and slows down the degradation rate. Mechanical forces can slow down the degradation rate by enhancing the thermal stability of the cleavage site of the collagen molecule [34,67,71] as illustrated in Fig. 5(b). On the other hand, Adhikari et al. have proposed a molecular mechanism that mechanical force pulls the collagen molecules to another vulnerable state which is more accessible to enzymatic breakdown as shown in Fig. 5(c) [73].

These two mechanisms suggest that there exists two vulnerable states of the cleavage sites of collagen molecules. One is the micro-unfolding conformation of the cleavage site, which has a lower thermal stability. The other is the unwinding conformation of the cleavage site of a collagen molecule. Mechanical force is able to stabilize a micro-unfolding conformation of the cleavage site [34] and therefore slow down the cleavage rate. On the other hand, the mechanical forces might unwind the conformation of the cleavage site and thus speed up the cleavage rate.

Summary

Collagen based materials such as connective tissues are fascinating ‘smart’ materials that can adapt their mechanical properties in response to mechanical loading. They achieve this, among other mechanisms, through their capacity to convert mechanical forces into biochemical signals that induce a host of downstream biological and pathological processes. In this article, we reviewed the biomechanics of collagen molecules including mechanical response of collagen molecules, degradation mechanisms and recent studies on how mechanical forces mediate the collagen degradation rate from a molecular mechanics point of view.

Collagen molecules have entropic and energetic elasticity behaviors. The entropic elasticity behavior of a collagen molecule allows mechanical forces in the physiological loading range to induce large deformation at specific segments with important biological functions while the energetic elasticity provides the strength of collagenous tissues for our daily activities. The local conformation of a collagen molecule and its deformation provides signals for the collagen degradation mechanism. Recent studies have revealed that mechanical force mediates the collagen degradation rate, which likely then initiates and alters the remodeling and repairing processes of collagen based materials. Altogether, the interplay of various mechanisms of elasticity (entropic versus energetic), local structural changes and instabilities, and the interaction with enzymes, poses a complex network of interactions that ultimately govern the mechanics of collagen.

More generally, the class of collagen based materials opens a great opportunity for a variety of biological, biomedical and pathological applications. Synthetic collagen based materials such as collagen scaffolds have already been shown to have many advantageous features for regenerative medicine. There is now

mounting evidence that the biomechanics of collagen molecules is the result of a coupled behavior of both its material and biological properties, but the precise mechanisms remain unclear. Future studies are required to carefully study how material properties of collagen molecules affect their biological functions. An interesting hypothesis could be developed based on the question on how the thermal stability of the collagen molecule affects the degradation rate. Understanding how mechanical forces alter the structure and degradation mechanism of a collagen molecule is required for designing and developing materials from the molecular scale upwards.

Future computational work could focus on a representation of the collagen-enzyme interaction, a direct simulation of the biochemical processes during the cutting of the triple helical structure, and the incorporation of larger-scale mesoscopic models to describe the evolution of gels under applied macroscopic stress. Translating some of the salient features of collagen – the capacity to autonomously form when needed (e.g. where mechanical forces grow), and break down when not (e.g. where mechanical forces have diminished) – could well serve as the basis of a new class of ‘smart’ structural materials that may be used in nanotechnology, microdevices and even as structural materials for infrastructure applications. Other interesting challenges include the question whether the described mechanisms in collagen can be combined with features of other protein materials, such as silk or elastin, for enhanced biological activity and other material properties. The use of bottom-up genetic engineering may provide a possible route to combine distinct domains from such sources into longer proteins.

Acknowledgements

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