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Mechanical Properties and Failure of Biopolymers: Atomistic Reactions to Macroscale Response

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Abstract

The behavior of chemical bonding under various mechanical loadings is an intriguing mechanochemical property of biological materials, and the property plays a critical role in determining their deformation and failure mechanisms. Because of their astonishing mechanical properties and roles in constituting the basis of a variety of physiologically relevant materials, biological protein materials have been intensively studied. Understanding the relation between chemical bond networks (structures) and their mechanical properties offers great possibilities to enable new materials design in nanotechnology and new medical treatments for human diseases. Here we focus on how the chemical bonds in biological systems affect mechanical properties and how they change during mechanical deformation and failure. Three representative cases of biomaterials related to the human diseases are discussed in case studies, including: amyloids, intermediate filaments, and collagen, each describing mechanochemical features and how they relate to the pathological conditions at multiple scales.

Keywords

Amyloidosis; Biological materials; Bone; Lamin

1 Introduction

Biological materials are intriguing because of their mechanical properties and diverse physiological roles. Most biomaterials have hierarchical structures and they derive their mechanical properties from multiple mechanisms at different scales, ranging from nano- to macro-levels. Typically, each level of structure features its own failure mechanism and, thus, failure of biomaterial is typically complicated and dissipates large amounts of energy at different scales. Previous studies have attempted to reveal various toughening mechanisms of biological materials, and much progress has been made over the past decade or so. A prominent mechanism is when a collection of weak sacrificial bonds dissipates a large amount of energy in silk, which leads to a very high fracture toughness [1], and concentrated stress at cracks or flaws is redistributed because of the inelasticity of materials in nacre [2]. Many biological materials exhibit a combination of these and other mechanisms that exist

concurrently, enabled by the existence of hierarchical structures. However, the fundamental deformation and fracture mechanisms of many biological protein materials remain unknown for several reasons. First, in many cases, the information about the structural assembly at each hierarchy is not well known. Second, the deformation behavior and the fracture properties at a lower hierarchy level affect the mechanical property of a higher hierarchy level nonlinearly and in complicated ways. Finally, the responses of all hierarchy levels to the macroscale mechanical loads can change under different loading conditions, and hence be altered during the use of a material or as an organism evolves [3]. Because materials failure basically arises from the chemical bond breaking, studying the behaviors of chemical bonds is critical to understanding their deformation and failure mechanisms. This connection between the chemical scale and meso- and macro-level properties remains a fertile area of materials science and engineering.

The robustness of biomaterials is important for performing their biological functions which work in various physiological conditions. Over 6,000 diseases are directly or indirectly caused by genetic mutations [4]. Because the components of biomaterials are complex and numerous, it is challenging to specify the roles of all components and to distinguish the important parts from others. Thus, it is useful to focus on their mutations and effects causing serious diseases to understand their properties. Even a single base in a genetic sequence changed by random mutation can cause serious disease in the human body because of the reordering of the protein's finely tuned chemical bond network, weakening the structural stability and altering the mechanical properties of the associated biopolymer.

Moreover, some diseases are linked to reactions with foreign peptide deposits in tissues (such as Alzheimer's disease, Parkinson's disease, etc.) [5]. Other diseases, such as Hutchinson–Gilford progeria syndrome (HGPS, a genetically-caused rapid ageing disease), are triggered by a genetic defect occurring in the building block of the nuclear membrane structure of lamins [6–8]. Genetic disorders in collagenous tissues, such as *Osteogenesis imperfecta*, have been linked to the variation of material structure caused by mutations in genes that encode the tropocollagen molecules [9–11].

In a given system, the deformation and fracture processes where the thermodynamic state variables (e.g., the energy and stress) change because of mechanical loadings is a very intriguing topic in mechanochemistry [12]. In particular, failure mechanisms and their criteria have been examined extensively for engineering applications. Not only for engineering materials such as metal and silicon, but also for biomaterials, a detailed knowledge of their mechanical properties is crucial for understanding their functions and malfunctions in the human body. For instance, the mechanical robustness of amyloid fibrils makes it difficult to decompose them in the human body [13]. Hutchison–Gilford progeria changes the deformation and failure mechanisms of cells from allowing large deformation to localizing stress [14, 15].

The deformation and fracture accompanying the remodeling of chemical bonds in biological systems, the mechanochemical properties, provide a set of interesting research questions with significant practical impacts. However, studying mechano-chemical processes from a purely experimental point of view can be quite difficult because of the limitation of

experiments caused by associated time- and length-scales [12]. To be specific, because the newly created surfaces during fracture can feature a high reactivity because of unsatisfied valences on atoms or missing counterparts, structural relaxation occurs on relatively short time scales via rearranging of chemical bonds. The brevity of these time scales makes the phenomena elusive to experimental approaches. However, advances in molecular dynamics simulations, including powerful techniques such as steered molecular dynamics (SMD), metadynamics, and replica exchange molecular dynamics (REMD), make it possible to analyze the stable structures of biomaterials and the behavior of chemical bonds under extreme loading on an extremely short time scale (i.e., femtoseconds to nanoseconds).

Several earlier computational and experimental studies have revealed how chemical bonds behave in biological systems during deformation and failure. Although their structures and mechanical properties are significantly different, they share some common important features in terms of their mechanochemical behavior. In biological materials, three different types of interaction are crucial for their mechanical properties and structures: covalent bonds, ionic bonds, and hydrogen bonds. These bonds behave differently under external loads, affecting the stability and mechanical properties of biological systems. Because of their complex chemical bond networks, the different types of interactions interplay to maximize the resistance to failure of materials or weaken mechanical properties in the cases related to diseases. The key mechanochemical features of three representative materials, amyloids, intermediate filaments, and collagen molecules, which closely relate to human diseases and the different types of bonds related to their features are summarized in Fig. 1.

In the following we focus on describing the behavior of these chemical bonds of amyloids, intermediate filaments, and collagen under mechanical loads on the atomic length scale. Understanding the complicated behavior and interplay of these chemical bonds in biological materials can help us to analyze and predict the mechanochemical processes in other bio/nano-materials, leading to the development of new medical treatments, detecting tools for diseases, and new strategies to design biomedical materials with novel functions [16–19].

2 Mechanochemical Mechanisms of Amyloidosis

2.1 Introduction

In general, the failure of proteins to remain in their native functional conformational state leads to various human diseases. These misfolded proteins can no longer play their ordinary roles in organisms [20]. Amyloidosis is a disease caused by the deposition of misfolded proteins called amyloids. These misfolded proteins are polypeptide molecules with highly ordered and stable β -sheets.

Alzheimer's disease is a representative amyloidosis, which is a neurodegenerative disorder where the deposition and self-assembly of amyloid may block cell-to-cell signaling at synapses. Once amyloid plaques are formed, they typically grow uncontrollably. This formation leads to neurotoxicity hindering the normal biological function of native tissues. Based on the knowledge of mechanical stabilities and rigidities of amyloid plaques, new treatments of amyloidosis, such as weakening the mechanical strength of the amyloid

deposits, could be developed. This exemplifies a materials-driven approach to develop a treatment that invokes controlled failure as a driving mechanism.

Most protein polypeptides possibly form amyloids through a structural transition from their native soluble conformation into insoluble fibrillar depositions [18], but their formation, including their forming rate and their structural stability, mainly depends on their sequence [21]. In addition to the effect of sequence, there are several factors affecting the formation of amyloids. Because the aggregation of amyloid occurs in aqueous solution, the hydrophobicity of side chains determines the aggregation propensity of a sequence; a series of hydrophobic residues facilitates the aggregation. Previous studies have shown that replacement of hydrophobic residues of peptides (e.g., 17–19 residues of A β (1-43)) by hydrophilic residues can reduce aggregation and enhance solubility [22–24]. Another critical factor of aggregation is a net charge of the polypeptide. Because a high net charge can make the protein unstable in a compact folded state because of the intramolecular repulsions, decreasing the positive net charge of the protein by mutations leads to an accelerated formation of β -sheet containing aggregates [25, 26].

Although amyloids are associated with the neurodegenerative disorders in organs, because of their exceptional sturdiness, strength, and stability under diverse conditions, their highly ordered hierarchical structures can be utilized as a powerful tool for creating future devices or interesting nanostructured molecular materials. For example, several studies have shown the possibility of using amyloid fibrils as a template for fabrication of conductive nanowires [16–19]. These great potentials for technological applications increase the interest in amyloid protein material from various fields of science.

2.2 Formation and Structure

Many mechanisms of aggregation of amyloids have been studied and provide us with the insight into ways to control amyloid formation. Among the variety of protein amyloids, β -amyloids (i.e., A β (1-40), A β (1-42), A β (1-43)) have been considered critical to understanding Alzheimer's disease [27]. The plaques in brain tissue with Alzheimer's disease are predominantly comprised of two A β amyloids, A β (1-40) and A β (1-42).

The structural information about the amyloid fibrils can be obtained by imaging technique such as TEM (Transmission Electron Microscopy), AFM (Atomic Force Microscopy), and X-ray diffraction [28, 29]. The analysis of amyloid structure is challenging because of their extremely large size and the difficulty of forming crystallized structures for NMR (Nuclear Magnetic Resonance) spectroscopy.

However, the progress in methods to grow microcrystals of small peptide fragments and in preparation of amyloid fibrils for ssNMR (Solid State Nuclear Magnetic Resonance) spectroscopy has contributed to identifying the structure of amyloid fibrils [30]. Human A β (1-40) is composed of 40 amino acids: Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val (DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV), and A β (1-40) peptide can form fibrils from the peptide solutions purified with 1.0 mM at pH 7.4 by incubation for 5–24 days [31]. In the models obtained from the

ssNMR data and by the energy minimization of the molecular structure with CHARMM force field, residues 1–8 are fully disordered, and the peptide conformation is composed of two β -strands, separated by a 180° turned bend formed by residues 25–29. The cross- β unit is a double-layered structure, with parallel β -sheets formed by residues 12–24 and 30–40. The assembly of these double-layered β -sheets using hydrogen bonds constitutes a long fibril with an axis coinciding with the direction of the intermolecular backbone (Fig. 2a) [30].

The self-assembled amyloid fibrils exhibit distinct morphologies, often characterized as twisted or parallel assemblies of finer filaments in TEM images (Fig. 2c). The morphology of A β (1–40) depends subtly on synthetic conditions, showing significantly different toxicities in neuronal cell cultures [32]. This shows that the structures of amyloid fibrils are not determined solely by the amino acid sequence. However, the sequence of the peptides is important as it controls the formation of the basic structural motifs. Because A β (1–42) has a different sequence, the structure shows different features from A β (1–40). A β (1–42) also can be synthesized and form fibrils in vitro with slightly different conditions from that of A β (1–40) and the structural information can be analyzed by the ssNMR as well. Unlike A β (1–40), it has been revealed that residues 1–17 are disordered and residues 18–26 and 31–42 form two intermolecular β -sheets in A β (1–42) [33].

2.3 Mechanochemical Properties

The molecular mechanism determining the chemical response induced by mechanical loading is crucial to identifying the mechanochemical properties of materials. Although it is challenging to address directly the behavior of the chemical bonds experimentally, the behavior of chemical bonds is reflected in mechanical properties in deformation. Thus, it is crucial to make an effort to measure the mechanical properties of materials and compare them with the computational results. From the computational methods, we could find reliable mechanisms that explain the behavior of chemical bonds on the atomic scale.

The AFM indentation method has been used to measure the elastic properties of molecular materials with high spatial resolution [34, 35]. The reported Young's modulus of amyloid fibrils by AFM indentation ranges from a few tens of MPa [36, 37] to a few tens of GPa [38, 39] depending on the sample size of fibrils, experimental approach, and loading conditions.

Because the mechanical properties of amyloid fibrils are derived from AFM nanoindentation measurements on the basis of assumptions about the geometry of the fibrils, they cannot provide highly accurate information about deformation. Because of the vague information on structures, to determine how different loading conditions affect the amyloid fibrils' structures and mechanical properties, we must resort to simulation. Thanks to the development of the molecular dynamics approach, one can obtain the Young's modulus of a biological system computationally and literally see how the system deforms and fails under various loading conditions.

Molecular dynamics simulations have been used to study the structure and mechanical properties of the amyloid fibrils, revealing the deformation mechanism of the compressive and tensile loading of amyloid fibrils [19, 40]. The results have shown good agreement with

experimental findings, showing that the computational method is reliable for studying amyloid fibrils under various loading conditions such as compression and tension.

Under both compression and tension, the Young's modulus of amyloid fibrils increases as the compressive or tensile strain is increased. Because the major chemical bonds supporting the structural stability of amyloid fibrils are the hydrogen bonds in the β -sheets, the mechanical response to the external load comes from the adjustment of the hydrogen bond network. The crucial deformation mechanism is associated with the torsional twisting of amyloid fibrils along the fibril axis which occurs with change in the density of the hydrogen bonds between β -sheets.

Under compressive loading, the number of hydrogen bonds increases because of the contraction of the distance among the β -strands, resulting in self-strengthening. This outstanding self-strengthening mechanical response to the compression could explain why the amyloid is so stable and can withstand high stresses without failure. On the other hand, as the amyloid fibril stretches under the tensile loading, the average distance between interlayers increases. The increased distance leads to a continuous decrease of hydrogen bond density until the fibril fails. However, the energy of amyloid fibrils still increases (Fig. 2b) in spite of the continuous decrease in the number of hydrogen bonds during the deformation. The increase in energy is coupled with a torsional motion of the amyloid fibrils, showing a higher Young's modulus than under compression. Although the fibrils have little chance to increase the number of hydrogen bonds under the tension, they can enhance their stiffness produced by the behavior of the covalent bonds rather than hydrogen bonds. Both compression and tension are associated with a torsional twisting deformation, in which a variation of the interlayer twist angle is proportional to both the applied stress and the measured strain. As described above, the different chemical and structural rearrangements of the amyloid fibrils play a primary role in strengthening their stiffness in responding to both compressive and tensile loading.

In addition to the Young's modulus, measured by atomic force microscopy and spectroscopy, the strength of amyloid fibrils is found to range from a few hundred megapascals (MPa) to one gigapascal (GPa) [38]. Recent molecular dynamics simulations studied the failure mechanism of amyloid fibrils under tensile stress [41], demonstrating a length dependency of the failure strain and stress (Fig. 2b). As described above, the number of hydrogen bonds continuously decreases as the tensile strain increases. Intriguingly, during the failure, the density of hydrogen bonds increases because of rearrangements and stabilization of the structures of broken parts of fibrils, making it difficult to break the whole system. As the amyloid fibril breaks into two parts, it is required to break a number of hydrogen bonds between the β -sheets. However, only one set of hydrogen bonds breaks and others recover from damage after the amyloid fibril fails. This partial self-healing mechanism prevents the whole system from breaking into several parts.

Longer amyloid fibrils are more likely to be brittle and weak than shorter ones (Fig. 2b). Because the hydrogen bond interaction is a relatively short-range force (~ 4 Å), the critical strain and critical stress of the shorter amyloid fibrils can have higher values than the longer amyloids based on the fibrils' elastic response under tensile loading. Previous studies have

shown that the number of hydrogen bonds that break simultaneously can be determined theoretically, providing a critical strand length of hydrogen bond clusters [42]. Beyond the critical length, the deformation of the system is more likely to be localized and the failure force of the system decreases to a certain value. On the other hand, under the critical length, the failure of the system is governed by the displacement rather than the critical strength. The reported theoretical critical length and reference failure strength of amyloid fibrils are about 1,000 nm and 175 pN, respectively [42]. These critical length and strength predictions mainly originate from the behavior of hydrogen bond clusters at the nanoscale, and are critical to understanding their ability to develop a significant mechanical strength in human brain tissue.

2.4 Mutation Effects

The stability and mechanical properties of amyloid fibrils result from the highly regular stacking of β -sheets and the dense network of hydrogen bonds. Because the sequence of the peptide plays a key role in aggregation, it is worth studying the effects of mutations to clarify the role of chemical bonds in structural stability and formation by comparing the properties and structures between normal and abnormal amyloid fibrils. The salt bridge between aspartate (D) 23 and lysine (K) 28 (Fig. 2a) is the key to understanding geometric properties of amyloid fibrils, as disrupting these salt bridges by replacing the amino acid with glycine (G) affects the stability and mechanical properties of amyloid fibrils [43].

The mutations damaging the salt bridge by replacing one of the residues (D23 or K28) or both (D23K28) by G can change three important features of amyloid fibrils: the length periodicity (Fig. 2d), failure strain, and Young's modulus. The periodicity is defined as the length of the amyloid fibrils needed to cover a complete twist turn. The D23 increases the twisting angle between β -sheets from 1.32° to 3.11° , decreasing the periodicity length to less than half. Removing one of the salt bridges is the primary reason for the change in the twisting angle, because the twisting angle of D23K28 remains less changed at 1.72° .

The modification of chemical bond networks is crucial to determining the pathways of the mechanochemical behavior. This changes the critical strain and the Young's modulus of the amyloid fibril. Interestingly, the mutations weaken the ability of fibrils to rearrange their internal structure through the twisting movement described in Sect. 2.3. For example, the angle change at 0.4% strain decreases from 23% to 7% in K28 and from 23% to 2% in D23, the D23K28 unwinding under the compression. The Young's modulus of the mutated fibrils shows significant variation from about 4 GPa (D23 and K28) to 19 GPa (D23K28). It is evident that because of changes in their structure and chemical characteristics, the mutated components of the amyloid fibrils can have significant influence on the aggregation and mechanical properties of the amyloid fibrils.

3 Intermediate Filaments: Structure, Mechanics, and Disease

3.1 Introduction

The intermediate filament protein (IF protein) is a major component of animal cells along with microfilaments and microtubules. Compared to microtubules and micro-filaments, a single intermediate filament tends to be rather flexible, extensible, and tough [44].

IF proteins assemble into a filament with a diameter of 8–12 nm which is the intermediate size between those of microtubules (25 nm) and microfilaments (7–9 nm). Whereas microtubules and microfilaments are more likely to be related to many basic cellular functions [44, 45], intermediate filaments play a key role in mechanical properties of tissues and cells, such as the stiffness and maximum strain, and the mechanical integrity of various tissues such as muscle, liver, and skin [44, 46].

More than 65 functional genes are related to the intermediate filaments and at least 30 diseases are related to the mutations in these genes [47]. The intermediate filaments gene family can be classified into five distinct types (I–V) based on their primary structure, gene, and their regulated tissue expression pattern [48, 49].

Intermediate filament protein types I and II are acidic keratin and basic keratin, found in epithelial cells. Vimentin, desmin, glial fibrillary acidic protein, synemin, and peripherin comprise the type III intermediate filaments. The type IV intermediate filaments include nestin and syncoilin, which are located in nervous systems and mostly found in neurons. The nuclear intermediate filaments, lamin A, its splice variant lamin C, and lamin B1 and B2 (type V), form a filamentous support inside the inner nuclear membrane [50].

Each intermediate filament type is closely related to a genetic disease associated with certain mutations. For example, HGPS is a rare genetic disease caused by a structural defect in the lamina nuclear membrane changing the mechanical properties at the cellular level [6]. This genetic disease causes the segmental premature aging in children, who suffer from failing to reach full stature and experience hair loss, thin wrinkled skin, and joint stiffness. Patients usually die of cardiovascular disease or stroke in their early teens [51]. Various skin diseases have also been associated with keratin mutations, which cause cytoplasmic aggregates and weaken the mechanical properties of epithelial cells [52, 53].

As with other biological materials, the change of nanostructure and the mechanical properties of the intermediate filaments are crucial to understanding diseases related to the intermediate filament mutations. Because the mechanical properties reflect the chemical bond network at the crack surfaces or in the entire system, the modification of structures by mutations severely affects how the chemical bonds at the crack surface interact and respond to mechanical loads [15, 49, 54]. Understanding how the different types of bonds in intermediate filaments react together under various mechanical loadings can give us opportunities to control and utilize the properties for medical applications.

3.2 Formation and Structure

Although various primary proteins form a variety of intermediate filaments, all intermediate filaments have a similar structural design. The basic building block, a monomer of IF protein, is a long central α -helix, called 'rod' domain, interrupted by 'linkers' and flanked by an N-terminal 'head' and a C-terminal 'tail' domain as shown in Fig. 3a [49, 55, 56]. Two monomers twist around each other to form a coiled-coil dimer, which is stabilized by a hydrophobic left-handed stripe that winds around the axis of each α -helix [57]. This rod-like domain controls the mechanical property of the single intermediate filament. Despite these similarities, there are distinct differences at the tail domain between nuclear IF proteins (e.g., lamin) and cytoplasmic IF proteins (e.g., vimentin) [58].

Although physical features of intermediate filaments look similar, different types of intermediates form different types of crosslinks affecting the elastic properties of the filament networks. The strong covalent bonds among the individual filaments are formed in keratin [59, 60], or divalent cations such as Ca^{2+} and Mg^{2+} glue vimentin intermediate filaments through coulombic interactions [61].

Vimentin filaments are the most widely distributed type among all IFs and are the focal point in this section. Their basic unit is a long monomer made up of 466 amino acid residues consisting of four main segments: 1A, 1B, 2A, and 2B divided by three linkers: L1, L12, and L2 as shown in Fig. 3a. Segment 2A (residues 264–282) is the shortest of all the segments. Segment 2B with 115 amino acids (residues 291–405) features an α -helical coiled-coil geometry for the major segment. One of the discontinuities found in heptad repeats of an α -helical coiled-coil protein is the 'stutter' region as pointed out in Fig. 3a [62, 63]. It can be seen that the stutter is located at the end of the eighth heptad, in the vicinity of residue 351 [64]. An analysis of the amino acid sequence revealed that the vicinity of the stutter region is hydrophilic, showing a parallel arrangement of two coils in the α -helix rather than a coiled-coil configuration. Although parts of the vimentin dimer structure have been revealed separately in independent experiments, a complete atomistic-level model of the basic constituents of this kind of protein material remains elusive.

The known structures largely comprise the rod-like domain of the vimentin dimer, as illustrated in Fig. 3a. However, some segments have unknown structures. Experimental work using diffraction techniques has identified certain structural characteristics, and work based on Electron Paramagnetic Resonance measurements have revealed that the local stiffness of the L1 and L2 domains (both helical structures) is close to that of the rod-like domain [65]. There appear to be persistent experimental challenges in identifying the remaining parts of vimentin IF structures. However, the intrinsic limitations in experimental methods call for the development of complementary computational methods that can provide a more controlled condition to assess the relation between the nanostructure and the nanomechanics of this class of materials.

3.3 Mechanochemical Properties

To understand the mechanical properties of intermediate filament networks, knowledge of the mechanical behavior of single intermediate filaments is required. The mechanical

properties of coiled-coil α -helix dimers, basic building block of vimentin intermediate filaments, under tensile loading were studied in molecular dynamics simulations [66, 67]. The stretching process can be divided into three regimes (Fig. 3b). Because the coiled-coil structure has multiple hydrogen bonds, the number of hydrogen bonds could be a critical measure to distinguish different regimes of the deformation. In the first regime at small strains (<50%), most hydrogen bonds are stretched, and their mechanical response shows elastic behavior as described in Fig. 3b. Unfolding of the α -helices leads to the second plateau regime (around up to 90%). The third strain-hardening regime (leading up to 180%) is associated with the stretching covalent bonds once all hidden length in the protein is exhausted. This highly nonlinear mechanical feature of coiled-coil α -helices results from the structural transitions of the chemical bond network which is changing because of the mechanical loading.

The different stages of mechanical response of the intermediate filaments show another important feature of the biological materials. In the second regime, the hydrogen bonds rupture and the α -helices unfold; the unfolded structure is stretched in the third regime. The transition between these two regimes and the stress-strain behavior depends on the loading rate. In other words, the mechanochemical properties of intermediate filaments show rate dependency (Fig. 3b). At low loading speed, the α -helices unfold smoothly by rupturing hydrogen bonds without covalent bond stretching. At high loading speed, the covalent bonds extend in their folded structure because there is less rupturing of hydrogen bonds. Thus, different levels of pulling speeds control the ratio between hydrogen bond rupturing and covalent bond stretching.

The ratio in which two different types of bonds respond to the mechanical loading is governed by the molecules' intermediate structures. It is a notable observation that mechanochemical properties can be controlled through the modification of structures, and the structures can, in turn, also be controlled by loading speeds. The intermediate filament protects its structure from rapid and small deformation (such as an impact) by utilizing the strong covalent bonds. The intermediate filament can also maintain its structure in spite of large and smooth deformation by recovering broken hydrogen bonds and folding upon release of the mechanical load.

In addition to the behavior of the hydrogen bonds, an α - β structural transition is observed in both experiment and computational simulation in the final regime (Fig. 3c) [67–69]. Because of rearrangement of the β -sheet structure during deformation, the stress-strain behavior shows a plateau region (Fig. 3c IV) before rupture, corresponding to the sticky sliding of β -strands with respect to each other. In the plateau region, a large amount of energy is dissipated by forming and breaking multiple hydrogen bonds as the protein fiber fails.

Different types of crosslinks play a key role in the higher structure of intermediate filament networks. As shown in previous studies, the intermediate filament network is involved not only in the mechanical role but also in the cellular role. It is intriguing that the intermediate filament network can withstand extreme deformation >100% without rupture, even with the existence of defects [14, 70]. This extreme capacity for deformation mainly results from the highly nonlinear properties of the coiled-coil structures by sacrificing individual protein

filaments [65]. Also, the flaw-tolerant characteristic results from the stiffening behavior during the secondary structural transitions for a single filament. In addition to the properties of a single filament, the crosslinks between intermediate filaments are also critical to the mechanical behavior of the intermediate filament network [42].

3.4 Mutation Effects

The important role of lamins in genetic diseases has been identified by recent studies [49, 71, 72]. For example, HGPS is a rare genetic disease causing premature aging early in life and leading to cardiovascular disease and death before age 16 years. The A- and B-type lamins comprise the intermediate filament that is the main component of the nucleoskeleton at the inner face of the nuclear membrane of the cell [73]. The *LMNA* gene encodes several A-type lamin proteins, mainly Lamin A and C. The *LMNB1* and *LMNB2* gene encode B-type lamins (lamins B1 and B2). Among the various lamin proteins, lamin A has received significant attention for its mechanical role in biology. Because the loss of lamin A affects nuclear mechanics more than other lamins in HGPS, a single point mutation in the *LMNA* gene activates a cryptic splice site, causing 50 amino acids encoded by exon 11 to be deleted, and the resulting mutant protein is called D50 lamin A (D50 LA) [7].

The properties of the hierarchical structure where individual intermediate filaments strongly form crosslinks with each other and flexibility of lamin filaments lead to the extremely large capacity of the larger-scale protein network to deform without failure. Replica exchange MD simulations and experiments reveals that D50 LA tail domain is more compact and displays less heterogeneity than the mature lamin A tail domain [74]. The compact and stable lamin may lead to more stiffness and reduced toughness. The increased stiffness could reduce the viability of cells by impairing their ability to recover from damage [75].

4 Collagenous Diseases

4.1 Introduction

Collagenous protein is the most abundant biological material in the human body. The three main types (I, II, and III) of collagen form 80–90% of the collagen in people. Type I collagen is a crucial structural protein material which maintains the integrity of many tissues such as bones, teeth, tendons, and ligaments. Many tissue disorders are associated with structural alterations of tropocollagen molecules that can be caused by over 300 mutations.

One of the most intriguing tissues composed of collagen protein is bone. Bone is extremely tough yet light, and can adapt to mechanical stimulus by changing its structure and repairing itself. Bone is, similar to other biomaterials, a hierarchical composite material composed of the soft tropocollagen and strong hydroxyapatite minerals (HAP). The hierarchical structures allow bone to have extreme toughness, dissipating high deformation energy without crack propagation. Distinct toughening mechanisms govern the mechanical properties at each level of hierarchy.

Osteogenesis imperfecta (OI) is a genetic disease characterized by high bone fragility, also referred to as “brittle bone syndrome.” Patients with OI exhibit associated symptoms such as short stature and loose joints. The origin of the disease is the changes of the structure of

tropocollagen molecules caused by the substitution of a single glycine amino acid. The OI mutations lead to serious reductions in the ultimate strength and the critical strain of OIM (Osteogenesis imperfecta murine) collagen [76].

A basic building block of the bone is the mineralized collagen fibril. With various percentages of mineralization, different types of bones have specialized properties for their own purposes. As with other biomaterials, the atomistic mechanical properties of its basic constitutive units are strongly correlated with their chemical bond network induced by their structural stability, and their reactivity during deformation and fracture.

4.2 Formation and Structure

Several hierarchical structure levels play a role in the superior mechanical properties of bone. The most fundamental building block is a polypeptide composed of a series of amino acids. Three strands of polypeptide assemble into the triple helical tropocollagen molecules. The molecular structure of mineralized-collagen fibrils is shown in Fig. 4a. The molecular segments repeat within a single unit cell called the 'D period' (length $D = 67$ nm). The D period consists of an overlap region ($0.46D = 30.8$ nm) and a gap region ($0.54D = 36.2$ nm) [77]. Because of their staggered geometry, which creates the gap regions, the tropocollagen molecules form collagen fibrils mineralized by the hydroxyapatite crystals mainly in those gaps [77].

The mechanism of nucleation of the minerals in bone has been intensively studied. Although it is not clearly understood, previous studies show that amorphous calcium phosphate (ACP) is a precursor phase in bone formation. After ACP is deposited as a first-formed mineral phase, it transforms into matured mineral phase, HAP [5, 78].

Recent studies revealed the process of mineralization of type I collagen by using horse tendon in solutions contacting CaCl_2 , K_2HPO_4 , and polyaspartic acid (pAsp). The time-resolved study shows that mineralized calcium phosphate particles are found close to the gap regions after 24 h. After ACP diffuses into fibrils, it develops to the oriented apatite (plate-shaped crystals with 2–5 nm thick, 15–55 nm long and 5–25 nm wide) (Fig. 4b) [79].

The structure of HAP ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is shown in Fig. 4a. The basic unit comprises pairs of positively charged calcium ions (C-sites) and clusters of six negatively charged phosphates (P-sites). Because the collagen protein is not stiff, the distribution of HAP in mineralized-collagen fibril plays a key role in the stiffness of bone. On the other hand, the mineral is less tough than the collagen molecule. Bone is naturally designed to utilize the unique properties of both minerals and proteins.

4.3 Mechanochemical Properties

Because tropocollagen is one of the basic components of bone tissue, it is critical to understand the mechanical properties of a single tropocollagen molecule. The Young's modulus of the tropocollagen has been estimated both in experiments and molecular dynamics simulations. The Young's modulus of tropocollagen has a wide range of values [80] and this wide variation comes from the tropocollagen nanostructure. A full atomistic

simulation performed by Gautieri et al. reveals that tropocollagen has three different deformation stages.

Initially, the tropocollagen molecules unwind with rotation in the first stage and then the hydrogen bonds break, and release energy in the second stage. The behavior of tropocollagen molecules in the second stage, where multiple hydrogen bonds break, is the major mechanism mediating the deformation of collagen fibrils. This process may allow the bone to dissipate large amounts of energy through large deformation. In the final stage, the backbones of molecules are stretched and the mechanical response is stronger than in previous stages because the deformation is mainly related to the covalent bonds. It is also revealing that the Young's modulus depends on the loading rate (Fig. 5a), as with other biological materials described in previous chapters, and the dependency results from the not fully unfolded structure of tropocollagen molecules. Figure 5a shows how the structure changes with different loading speeds. Although the tropocollagen molecules can fully unwind under low-speed loading, partially unfolded structures are observed at high-speed loading. The effects of differently deformed structures at different loading speeds demonstrate the nonlinear elasticity or hyperelasticity of the collagen materials.

Because the mineral has higher modulus than the collagen molecules, it is commonly believed that the presence of HAP is important to the stiffness of bone [81, 82]. The mechanical and failure properties of bone depend on the relative amount of the mineral deposited in the collagen fibrils [83, 84]. From tests on various bone samples of mammals, it is known that the Young's modulus of the mineralized-collagen fibrils increases linearly with the mineral contents [81].

A full-atomistic mineralized-collagen fibril atomic model for MD simulations was recently developed by Nair et al. (Fig. 4a) [85]. They performed tensile tests with different volume fractions of mineralization. Their study reveals that mineral content increases the stiffness of the mineralized-collagen fibrils (Fig. 4c). These simulations show that the mineral crystals can withstand four times more stress than the collagen, and the collagen can deform more than the mineral. Because the HAP is largely deposited in the gap regions, as the HAP percentage increases, the gap regions become stiffer because of the strong ionic forces in the HAP. On the other hand, the collagen can deform more than the mineral because of its softness under the external loadings. It is evident that the volume fraction of HAP is critical for the stiffness of mineralized-collagen fibrils. In addition, the different stiffness between the gap region and the overlap region can lead to the different structural deformation states, affecting the kind of unfolding that accompanies the hydrogen bond breaking and strong electrostatic force between the HAP and proteins. The variation may allow the mineralized-collagen fibrils to have a wide range of stiffness and critical strain points. Similar to the intermediate filaments, this variation can play a critical role in toughening bone at the macroscale.

4.4 Mutation Effects

Because the structure of the tropocollagen depends on its amino acid components and sequence, the mutations replacing an amino acid affect the tropocollagen structures. This structural difference leads to changes in the intermolecular interactions in the system,

affecting the collagen fibril packing and the space of the gap regions, thus changing the mechanical properties of bone.

Molecular dynamics studies of the effects of OI at the molecular level have shown a reduction in the Young's modulus of up to 15% [86] (Fig. 5b). The structural changes caused by OI mutations induce localized stress concentration at the collagen fibril level, leading to lower stiffness and more brittleness than observed in normal collagen fibrils. It was suggested that the decrease of the Young's modulus is strongly correlated with the density of the hydrogen bonds. Figure 5c shows the effects of the mutated acids on the hydrogen bond density of stabilized structures. As the density of hydrogen bonds decreases, the collagen molecules become softer, and this effect is more pronounced as the severity of mutation increases (Fig. 5b). Also, as a result of elongated equilibrium distance between the collagen molecules by the mutation, the adhesion energy between the molecules decreases. This weakened intermolecular interaction results in the reduction of the intermolecular crosslinks in the collagen fibrils. Molecular level studies clearly show that even a single substitution induced by mutations has a significant effect on the higher level of structures, critical for understanding the mechanical properties and the catastrophic failure mechanism of bone.

Because of the different structures of the collagen proteins, the deposition of the mineral in the gap regions can change. The X-ray diffraction data show that the mineral plates in OI mineralized-collagen fibrils are more structurally disordered and thinner than those of normal fibrils. The qualities of mineral crystals, such as shape, thickness, and alignment, are related to a drastic change in the ability of tropocollagen to bind to the minerals [87]. However, how these kinds of qualities affect the mechanical properties of bone is still little understood. Modeling the different mineral qualities in the mineralized-collagen fibrils remains challenging because the mechanism of crystallization from the amorphous structures is not clear yet. It would be interesting for future studies to clarify the crystallization process and how the mechanical properties change.

Although the main mechanism of crystallization is not yet known, we can see how the quality of mineral is important in the fibrils. In a previous study, how the interaction between minerals and collagens changes as a function of HAP thickness is reported [88]. Although the interaction between collagens and minerals is crucial for understanding the mechanical properties of mineralized-collagen fibrils, the strength and stiffness of each component should be considered individually to obtain an accurate description of mechanical behavior. However, the changes in those mechanical properties produced by the mineral qualities have not yet been studied in atomic simulations. Here, we aim to shed light on the importance of the qualities of minerals to the study of mineralized-collagen fibrils of OI bone by performing simple tensile tests with different thicknesses of HAP minerals. Based on experiments on mice and rats, the earliest measurable thickness of HAP is under 2 nm and it increases to more than 3 nm in 3 months [89]. Because the average thickness of mineral crystals of OI mice is around 1.7 ~ 1.8 nm [87], we performed tensile tests of HAP with different thicknesses ($T = 1.7, 3.4, 5.1$ nm).

Tensile tests are performed under different environments, in a vacuum and in water. Although our model is extremely simplified, the results show meaningful effects of mineral

thickness usually ignored up to now. We observed that the mechanical properties such as Young's modulus, strength, and critical strain are sensitive to thicknesses less than 5 nm. As the thickness increases, the sensitivity decreases and the mechanical properties become closer to those of bulk materials. In the vacuum, the critical strength of 1.7 nm HAP does not show a peak point (~2.0 GPa under 8% tensile strain) because the structure is not so well ordered as with the bulk crystals because of the lack of other layers in the thickness direction. Because the ionic interaction based on Coulombic interaction reaches a longer distance than other interactions, it is important to have enough layers to be stabilized in a specific structure. However, because of the thinness of the minerals, the interactions with environments such as proteins and water can disturb the structure, making the minerals lose their bulk properties.

On the other hand, in the water, we observe a clearer critical strength point of 1.7 nm HAP than that of 1.7 nm HAP in the vacuum. This means the mechanical properties are affected by the interactions between HAP and the environments. Thin HAP (under 2 nm) shows more ductility and fluidity because of the relatively disordered structure. Although the structure is more amorphous and the structural deformation occurs across the entire system during the tensile deformation with the thin HAP, the deformation and failure of HAP become more localized as the thickness of HAP increases. Because of the thinness of the minerals, the interactions with environments such as proteins and water can disturb or stabilize the HAP structure, making the minerals lose or gain their bulk properties.

We note that the drastic difference in the strength of 3.4 and 1.7 nm samples of HAP is observed in both vacuum and water (50% and 30% difference in vacuum and water, respectively). When the forces HAP can withstand in the fibrils with the real size are considered, the effect of thickness is more significant. In the case of 15 nm width, the forces drop from 200 to 50 nN in vacuum, and from 250 to 80 nN in water. Because the mutation of collagen molecules changes the structure of collagen and, thus, the thickness of HAP, the brittleness of OI bone may derive from the failure of HAP crystal. Although we do not consider the changes of shapes, grains, or alignments of HAP produced by the collagen proteins, mineral crystal qualities and their roles are intriguing subjects for future study.

The single point mutant of collagen and volume of mineral phase have a significant effect on the mechanical properties and toughness of bone. Although previous experiments suggest mutations change the mineral quality, the role of mineral quality is still unclear. We performed tensile tests of HAP with different thicknesses to address the importance of HAP quality in mineralized-collagen fibrils. Even though the simulation is limited to only the thickness effect of HAP, it has been shown that not only volumetric factor but also the quality of the minerals has effects on the strength, stiffness, critical strain, and failure mechanism of mineralized-collagen fibrils. To determine in more detail the roles of the quality of mineral, understanding the formation mechanism of HAP in vivo and more precise information about HAP are essential for future work.

4.5 Simulation Details

In our simulations we used the empirical force fields for the interaction among ions of HAP reported earlier [90, 91]. Two-body ionic short-range interaction potential of the

Buckingham type and Coulombic interaction term were adopted. A Morse potential was adopted for both O-H and P-O bond interactions. The harmonic three-body potential was used to describe the tetrahedral configuration of oxygen ions around phosphorus. The force fields describe the structures and the mechanical properties of HAP reasonably well, and show excellent agreement with the experimental data. For the interactions between water molecules and ions of HAP, we adopted the same approach as in the previous study [92].

We generated the HAP crystal structures using Visual Molecular Dynamics (VMD) [93]. Because we aim to test the effects of thickness (in the y direction), we build a slab system by inserting a vacuum layer (4 nm, Fig. 6a) and a water layer (4 nm, Fig. 6b) around the HAP slab. After energy minimization, the temperature of the system is initially set to 5 K with a Berendsen thermostat for 10,000 steps. Then we apply a Berendsen barostat to relax the simulation box for 30,000 steps. Then we gradually increase the temperature to room temperature (300 K). We relaxed the system with the additional 70,000 steps. After relaxation, we fixed the edge part of the longest side (the z direction) and deformed the system in the z direction as quickly as 1.0 m/s. We performed the same tensile test for the bulk material with the same geometry of 1.7 nm to show that the weakening strength mainly comes from the finite thickness. All relaxations and running simulations were performed with a 0.5 fs time step. Our MD simulations were performed using LAMMPS [94] and VMD was used for visualization.

5 Remarks

Biological materials are important not only because of their physiological roles but also because they hold significant potential for future applications in various areas of technology, such as nanodevices. In this review, we have shown that the mechanochemical properties of biomaterials are closely related to their structure and loading conditions. Because of the mechanical stimuli, the behavior of different types of chemical bonds can either enhance or weaken the mechanical properties of biopolymers by affecting the failure and deformation mechanism at each deformation stage. The breaking and reforming of hydrogen bonds play the most crucial role in defining the stability and capacity of protein materials to withstand extreme mechanical loadings. First, the hydrogen bonds allow the system to have large deformation by continuously changing the structures under the slow loading (e.g., unwinding the collagen and the coiled-coil alpha helix in the intermediate filaments). Second, the weak hydrogen bonds can be relatively more easily recovered than most engineering materials after the loading is removed, enabling the system to recover from the damage (e.g., failure of amyloid fibrils under the tensile loading). Finally, the hydrogen bond network controls the point where the covalent bonds in the system start to withstand the external loadings (such as rate dependency of the collagen fibrils and intermediate filaments).

We also discussed the mutation effects on mechanical properties of the materials. Basically, the mutation can change the stabilized structure of biomaterials, affecting the chemical bond networks. The altered structures can change the mechanochemical properties of materials under the various external loadings, weakening or enhancing the strength and toughness of materials. In addition, mutations can change the density of crosslinks between the basic

units, critical to determining the properties at a higher hierarchical level of the biological systems.

Because the rupture and fracture occur over an extremely short time span, computational methods such as molecular dynamics and newly developed MD techniques become essential tools to analyze the structure of materials and the relations between mechanochemical processes and loading conditions. Through the simple MD simulations of HAP crystals, we have shown that the thickness of HAP can affect the behavior of ionic bonds under the tensile loadings, showing the importance of the chemical environments of HAP crystals and the crystal quality. The details of the effects of the HAP crystal quality and the nucleation mechanism would make an interesting follow-up study in the future.

The hierarchical structures of biological materials are critical to understanding the mechanical properties in the different level of structures. As discussed above, the single mutation of the most fundamental building block of biomaterials affects the chemical bond network, density, and structure. The fact that different types of bonds respond differently to mechanical loadings and interact with each other makes the biomaterials extremely intriguing. The knowledge of mechanochemical properties and processes under various loading conditions provide valuable opportunities and insight for new materials design and applications in the future.

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Biomaterials	Mechanochemical Features	Related chemical bonds			
		covalent	ionic	hydrogen	salt-bridge
Amyloid	self-strengthening			✓	
	length dependency			✓	
	periodicity			✓	✓
Intermediate Filaments (IF)	alpha-beta transition			✓	
	crosslink (IF network)	✓	✓		✓
Mineralized Collagen (MC)	mineral stiffening	✓	✓	✓	
	thickness dependency		✓		
IF & MC	rate dependency	✓		✓	

Fig. 1.

Summary of major chemical bonds and mechanochemical features in biological materials discussed in this review. The mechanical properties result from interplay between chemical bonds and deformation of biomaterials. The effect of each chemical bond becomes distinct or obscure because of the states of deformation and loading

Fig. 2.

Structures of normal and mutated amyloid fibrils and mechanical responses under tensile loadings. **(a)** Visualization of molecular structure of twofold symmetric amyloid A β (1-40) fibril showing the characteristic twisting structure and one layers of amyloid fibril with highlighted salt bridge (charged side chains) site at residue D23 (aspartate) and K28 (lysine). **(b)** Size dependency of critical strain under tensile loading: the energy density as function of the applied strain with different lengths, $L_{20} = 91.87$, $L_{30} = 144.33$ and $L_{40} = 189.75\text{\AA}$. (Adapted with permission from [95]. Copyright (2010), with permission from Elsevier). **(c)** TEM images of amyloid A β (1-40) fibril with two different morphologies: twisted and parallel after synthesized (*left*), TEM images of sonicated amyloid fibrils fragments (*right*) [32]. **(d)** Periodicity of mutated and normal A β (1-40) fibrils (figures in **a** and **d**). (Reprinted with permission from [43]. Copyright (2010) American Chemical Society)

Fig. 3. Schematic view of the human vimentin protein and force-strain curves of coiled-coil intermediate filament under tensile loadings. **(a)** Schematic representation of vimentin structure. **(b)** Force-strain behaviors of a coiled-coil α -helical structures revealing the loading rate dependency of the molecular-level stiffness under tensile loading. (Reprinted from [66], with kind permission from Springer Science and Business Media). **(c)** α - β secondary structural transition of coiled-coil α -helix under tensile loading. (Reprinted from [67])

Fig. 4.

(a) Collagen fibril model with 0%, 20%, and 40% mineralization. (b) Cryogenic TEM image of different stages of mineralization in vitro of type I collagen of horse tendon in the presence of $10 \mu\text{g ml}^{-1}$ polyaspartic acid (pAsp) which is an inhibitor of hydroxyapatite crystallization [96]; mineralization calcium phosphate particles were found outside the fibrils after 24 h (*white arrows*), plate-shape crystal (2–5 nm thick, 15–55 nm long and 5–25 nm wide) after 72 h. (Reprinted by permission from Macmillan Publishers Ltd: [79], Copyright (2010)). (c) Modulus-strain curves showing HAP volume dependency in simulation. (a and c are reprinted from [43])

Fig. 5.

(a) Force-strain curves of tropocollagen under tensile loading showing rate dependency. (Reprinted from [80], Copyright (2009), with permission from Elsevier). (b) Young's modulus of a single tropocollagen molecule, as a function of the amino acid replacing and as a function of OI severity. (Reprinted from [86], Copyright (2009), with permission from Elsevier). (c) Effects of mutation types on interchain hydrogen bonds. (Reproduced from [97], with permission of The Royal Society of Chemistry)

Fig. 6. The relaxed structure of HAP mineral crystals: **(a)** in a vacuum; **(b)** in water. **(c, d)** Stress–strain curves of HAP as a function of different thicknesses