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Mechanical Deformation Mechanisms and Properties of Amyloid Fibrils

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Abstract

Amyloid fibrils have recently received attention due to their remarkable mechanical properties that are highly correlated with the biological functions of amyloid fibrils. We have studied the mechanical deformation mechanisms and properties of amyloid fibrils as a function of their length scales by using atomistic simulations. It is shown that the length of amyloid fibril plays a role on its deformation and fracture mechanisms in such a way that the competition between shear and bending deformations is highly dependent on the fibril length, and that as the fibril length increases, so does the bending strength of the fibril while its shear strength decreases. The dependence of rupture force for amyloid fibril on its length is elucidated using Bell model, which suggests that the rupture force of the fibril is determined from the hydrogen bond rupture mechanism that critically depends on the fibril length. We have measured the toughness of amyloid fibrils, which is shown to depend on the fibril length. In particular, the toughness of the fibril with its length of ~3 nm is estimated as ~30 kcal/mol·nm^3, comparable to that of a spider silk crystal with its length of ~2 nm. Moreover, we have shown the important role of pulling rate on the mechanical deformation mechanisms and properties of amyloid fibrils. It is found that as the pulling rate increases, so does the contribution of shear effect to the elastic deformation of amyloid fibril with its length of <10 nm. However, we found that the deformation mechanism of amyloid fibril with its length of >15 nm is almost independent of pulling rate. Our study sheds light on the role of the length scale of amyloid fibrils and pulling rate in their mechanical behaviors and properties, which may provide insights into how the excellent mechanical properties of protein fibrils can be determined.

Introduction

Amyloid fibrils that are formed as a highly ordered β sheet-rich one-dimensional nanoscale structure via protein aggregation,[1-3] have been reported to play a pivotal role in the expression of not only neurodegenerative diseases such as Alzheimer's and Parkinson's diseases,[4] but also non-neurodegenerative diseases such as type II diabetes.[5,6] Amyloid-induced disease, for instance type II diabetes, stems from the deposition of toxic protein

aggregates, that is, amyloids with their various length scales ranging from oligomer to fibril, onto a specific organ such as pancreas.[5,6] Specifically, the deposition of islet amyloids results in the apoptosis of β-cells, which are responsible for insulin secretion, and consequently leads to the type II diabetes. The formation of islet amyloid fibril is due to the aggregation of human islet amyloid polypeptide (hIAPP) consisting of 37 amino acids.[5] When hIAPP chains are aggregated to form the amyloid oligomers and fibrils, 10 amino acids (i.e. 20^{th} to 29^{th} residues with the sequence of "SNNFGAILSS") in hIAPP play a crucial role on protein aggregation mechanism such as hydrogen bonding between hIAPP chains necessary for the aggregation.[7,8] This sequence "SNNFGAILSS" abbreviated as $hIAPP_{20-29}$ is referred to as amyloidogenic core. Moreover, it has been reported that the peptide chains consisting only of this amyloidogenic core, i.e. $hIAPP_{20-29}$, can be assembled so as to form the amyloid fibril.[7-9]

In recent years, the amyloid fibrils have been reported to exhibit the remarkable mechanical properties even though the fibrils are formed by the aggregation of mechanically weak protein chains or peptide chains.[10] This remarkable mechanical property is due to the fact that the amyloid fibril is constructed via hydrogen bonding between peptide chains. The hydrogen bonds between peptide chains in the formation of the fibril serve as a chemical glue that particularly allows an amyloid fibril to resist a mechanical force.[11] In general, the mechanical properties of protein materials are ascribed to the ability of hydrogen bonds and their network to sustain a protein structure even under a force.[12-14] A recent study by Buehler and coworkers[15] has shown that the excellent mechanical properties of β sheet crystal, which constitutes the key element of a spider silk, originate from the geometric confinement of hydrogen bonds, which allows the crystal to effectively resist to a force. This may imply that the geometry of hydrogen bonds sustaining the structure of β sheet-rich fibrils such as amyloid fibrils gives rise to their remarkable mechanical properties as reported in recent studies by Solar and Buehler.[16,17]

It should be noted that these remarkable mechanical properties of amyloid fibrils are highly correlated with their biological functions.[10] For instance, the amyloid-driven pathology such as type II diabetes is ascribed to the mechanical properties of amyloid fibril.[18] In particular, the ability of amyloid fibril to disrupt a cell membrane leading to cellular apoptosis[19] is attributed to the elastic modulus of the amyloid fibril measured in the order of 10 GPa being much higher by about three orders of magnitude than that of a cell membrane,[20] which results in the amyloid fibril-driven mechanical disruption of the cell membrane. Moreover, prion infectivity has recently been found to be closely related to the fracture toughness of prion fibril.[21,22] In addition, as described in our previous work,[23] the size-dependent elastic properties of prion amyloids provide an insight into the critical size of prion amyloid that plays a role in prion infectivity. Furthermore, the excellent mechanical properties of amyloid fibrils have led researchers to develop biomimetic materials whose mechanical properties can be tuned.[24] For instance, researchers at Cambridge have developed a nanostructured film made of amyloid fibrils and reported the remarkable elastic modulus of such a film being close to that of the most rigid proteinaceous materials such as keratin and collagen.[25] A recent study by Mezzenga and coworkers[26] has reported the development of nanoscale composite materials by coupling graphene sheet and amyloid fibril, where amyloid fibril serves as a linker between graphene sheets. In their work,[26] the mechanical properties of such composite materials can be tuned by the enzyme-driven degradation of amyloid fibrils. These previous studies[24-26] suggest the necessity to investigate the mechanical

properties of amyloid fibrils for not only understanding the origin of the biological functions of amyloid fibrils but also developing a novel biomimetic or composite materials whose material properties can be controlled.

Even though single-molecule experiments such as atomic force microscope (AFM) experiment,[27,28] optical tweezer-based manipulation,[29] and fluorescence imaging[30] have allowed the mechanical characterization of protein materials such as protein fibrils,[31-33] these single-molecule experiments are unable to provide the detailed insight into the structural deformation of a protein molecule at atomistic scale in response to a force. Such a detailed insight into the mechanical deformation and dynamics of a protein structure can be gained by computational simulations such as molecular dynamics (MD) simulations. For instance, MD simulations have recently been utilized to study the mechanical properties of protein materials with their various length scales ranging from a single protein molecule to self-assembled protein fibrils.[34] We have previously characterized elastic moduli of amyloid fibrils by measuring their thermal fluctuation behaviors using MD simulations.[18] Among MD simulations, steered molecular dynamics (SMD) simulation has recently been considered due to its ability to characterize the mechanical deformation mechanisms of various protein materials in response to a force.[12] For last two decades, SMD simulations have been widely used for investigating the unfolding mechanism of a single protein molecule in response to a force applied to the termini of the protein molecule.[14,35-38] In recent years, SMD simulations have been also taken into account to understand the mechanical behavior of self-assembled protein materials such as protein fibrils.[34] For example, Buehler and coworkers[15] have utilized SMD simulations to study the mechanical deformation mechanisms and properties of a β sheet crystal that is a key ingredient of spider silk. They have also characterized the mechanical properties of amyloid fibrils by using SMD simulations and found that these properties are comparable to those of mechanically strong protein materials.[17] In addition, they have reported that the mechanical properties of amyloid fibrils are highly correlated with collective rupture behavior of hydrogen bonds that sustain the cross-β structure of amyloid fibril.[16] Recent studies by Harris, et al.[39,40] have provided the mechanical deformation behavior of amyloid fibril by using SMD simulation, and shown the role of amino acid sequence on the mechanical deformation of amyloid fibril. Moreover, a previous study by Lee, et al.[41] has suggested the structural deformation of amyloid fibrils in response to a force applied to the fibril, and found the role of structural polymorphism (i.e. steric zipper pattern) on the fracture behavior of amyloid fibrils.

Despite aforementioned recent studies reporting the mechanical behavior of amyloid fibrils by using SMD simulations, it has not been fully understood yet how the length scale of amyloid fibril affects its mechanical deformation behavior. Recent studies[23,42,43] have reported that, by using coarse-grained model such as elastic network model (ENM), the bending rigidity of amyloid fibril is critically dependent on its length. Specifically, this length-dependent bending elasticity of amyloid fibril is attributed to the length-dependent deformation modes, that is, the competition between shear and bending deformations. Despite ENM reporting the length-dependent elastic properties of amyloid fibrils, ENM cannot provide the *in situ* mechanical deformation behavior of amyloid fibril such as its time-dependent deformation mechanism in response to a force applied to the fibril. Moreover, as previous studies[33,44-46] report that the mechanical strength (e.g. unfolding force) of a protein molecule is significantly dependent on the deformation rate at which the protein molecule is pulled, the mechanical deformation behavior of amyloid fibril may be governed by the pulling rate. Furthermore, recent reports[47,48] suggest that the elastic property

(e.g. Young's modulus) of a protein molecule depends on the deformation rate. These studies[33,44-48] imply that the loading rate may affect the mechanical deformation mechanisms and properties of amyloid fibrils.

In this work, we study the mechanical deformation behaviors and properties of amyloid fibrils by using all-atom explicit water SMD simulations. Our work is aimed towards unveiling how the mechanical deformation behaviors and properties of amyloid fibrils are incorporated into their length scales as well as the deformation rate. It is shown that the mechanical properties of the fibril (e.g. stiffness and toughness) are critically dependent on its length in such a way that as the length of the fibril decreases, such mechanical properties are critically enhanced. We theoretically elucidate the role of the length scale of amyloid fibrils on their mechanical properties (i.e. elastic modulus and rupture force) using analytical models such as Timoshenko beam model (for analysis of elastic modulus) and Bell model (to analyze the rupture force), and compare these theoretically predicted mechanical properties with those obtained from SMD simulations. Moreover, it is found that the deformation rate makes a critical impact on not only the mechanical properties of amyloid fibrils but also their deformation mechanisms. Our work may provide the design principles of amyloid fibrils, which highlight the importance of the length scale of amyloid fibril and deformation rate in understanding the mechanical deformation of amyloid fibril and its mechanical properties.

Results and Discussion

In order to understand the mechanical deformation mechanisms of amyloid fibrils, we have utilized steered molecular dynamics (SMD) simulations that allow us to mechanically deform the fibril based on a mechanical pulling of the fibril. As described in Methods and Material, both ends of the fibril are fixed whereas a β strand that locates in the middle layer of the fibril is extended with a pulling speed (rate) of v = 0.005 Å/ps (Fig. 1a), otherwise specified. Here, we pull a single β strand in the middle layer of the fibril rather than all β strands in the middle layer in order to mimic AFM-based pulling experiment of amyloid fibril.[49] Specifically, in AFM pulling experiment,[49] a chemically modified AFM tip is likely to pull a single β strand in the layer of amyloid fibril. It is shown that for an amyloid fibril with its length (L) of 3.41 nm, when a β strand in the middle layer is extended, this β strand becomes to be pulled out from the fibril (Fig. 1b). On the other hand, the extension of a β strand in the middle layer of a long fibril (with its length of 8.28 nm) results in the bending deformation of the fibril rather than just pull-out of that β strand (Fig. 1c). This suggests that the length scale of amyloid fibril plays a critical role in the mechanical deformation mechanism of the fibril in such a way that the mechanical deformation of a short fibril is dominated by shear deformation, whereas the bending deformation mostly governs the mechanical behavior of a long fibril. This is consistent with previous findings[15,23,42,50] reporting that the competition between shear and bending deformations is a key deformation mechanism in a protein fibril (or fiber).

Based on SMD trajectory of deformed amyloid fibrils, we have obtained the force-displacement relationships of the fibrils (Fig. 2), which is useful in analyzing the mechanical properties of the fibrils. As shown in Fig. 2, we observe the significant force peaks in the force-displacement curve for a short amyloid fibril, while the magnitude of these force peaks is critically decreasing as the fibril length increases. This suggests that the length scale of amyloid fibril governs not only the deformation mechanism of the fibril but also the mechanical response

(property) of the fibril. The discernible force peak for a short fibril (e.g. L = 3.41 nm) is attributed to its deformation mechanism such that shear deformation is a major source of the mechanical deformation of the fibril. As already described in Fig. 1, the mechanical deformation of a long fibril is mostly attributed to the bending deformation rather than the shear deformation. In addition, a large force peak found for a short fibril may be due to the cooperative rupture of hydrogen bonds, but this rupture mechanism cannot be applicable for a long fibril. In particular, when a long fibril is mechanically bent, the hydrogen bonds are likely to be ruptured one by one, which may lead to the different force curve of the long fibril from that of a short fibril (for details see below).

In order to determine the elastic properties such as bending rigidity, we have considered the linear region of a force-displacement curve just before the fracture of amyloid fibril is initiated. The stiffness of amyloid fibril is defined as the slope of the force-displacement curve, i.e. $k = dF/du$, where F and u represent the force and displacement, respectively. Based on the force-displacement curves of amyloid fibrils with their various length scales, we found the correlation between the length of the fibril and its stiffness (Fig. 3a). In particular, as the length of a fibril increases, the stiffness becomes to decrease. This length-dependent behavior of the stiffness has been also reported in the case of spider silk crystal[15]. The stiffness of amyloid fibrils found in this work is comparable to that of protein fibrils (e.g. spider silk crystal) reported in the literature[15,17,51] (Table 1 and 2). This length-dependent behavior of the stiffness of the fibril can be elucidated from elasticity theory, particularly Timoshenko beam model. Specifically, as reported in the literature[15,23,42,50], the relationship between the stiffness and length of the fibril originates from the competitive deformation mechanisms such as shear versus bending deformation modes. This relationship is represented in the following equation:

$$k = \frac{D}{L^3}\left(1 + \frac{a}{b}\frac{cD}{G_S A L^2}\right)^{-1} \quad (1)$$

where k, D, G_S, L, and A represent the stiffness, bending rigidity, shear modulus, length, and cross-sectional area of an amyloid fibril, respectively, while a and b are boundary condition-dependent constants, and c is a constant that depends on the geometry of amyloid fibril. From Timoshenko beam model, i.e. Eq. (1), the bending rigidity and shear modulus of hIAPP amyloid fibrils are measured as $D = 7.73 \times 10^{-26}$ N·m^2 and G_S = 5.97 GPa, respectively. These values of bending rigidity and shear modulus are comparable to those of spider silk crystal and other amyloid fibrils found in the literature[15,17,42,43,51,52] (Table 1 and 2). For instance, the bending rigidity and shear modulus of spider silk crystal are estimated as $D = 2.8 \times 10^{-26}$ N·m^2 and G_S = 4.6 GPa, respectively.[15] This clearly shows that the elastic properties of hIAPP amyloid fibril is comparable to those of spider silk crystal (Table 1). This may be attributed to the fact that both protein fibrils (i.e. spider silk crystal and amyloid fibril) exhibit the structural feature in that both fibrils are formed by protein aggregation based on parallel stacking of β strands, even though the amino acid sequence of the β strand for the amyloid fibril is different from that for spider silk crystal. This implies a design principle that the mechanical properties of β sheet-rich protein materials are encoded in their hydrogen bond networks. In order to gain insight into the role of shear effect on the mechanical (elastic) deformation of amyloid fibrils with respect to their length, we introduce the dimensionless parameter, which provides how shear effect contributes to the deformation of the fibril, defined as[42]

$$\psi = \frac{a}{b}\frac{cEI}{G_S A L^2} \quad (2)$$

Fig. 3b depicts the dimensionless parameter ψ as a function of the fibril length. For an amyloid fibril with its length of 3.41 nm, ψ is almost close to 1, which suggests that shear effect is responsible for the mechanical deformation of the fibril. When the length of the fibril is 5.36 nm, ψ is less than 1, indicating that shear effect becomes to play a minor role on the mechanical deformation of the fibril. As shown in Fig. 3b, as the fibril length increases, the parameter ψ becomes smaller, which implies that shear effect does play a vital role on the deformation of only a short amyloid fibril.

Now, we proceed to study the mechanical strength of amyloid fibrils by using SMD simulations. In particular, the mechanical strength of the fibril can be defined by measuring the maximum force at which the fracture of amyloid fibril begins. This maximum force is defined as the first force peak (referred to as "rupture force"), at which significant hydrogen bonds sustaining the fibril structure start to be ruptured, in the force-displacement curve. Fig. 4a shows the rupture force of amyloid fibril as a function of its length (L). The rupture force at L = 3.41 nm is measured as ~1.7 nN, whereas the rupture force at L = 17.05 nm is estimated as ~0.6 nN. This indicates that the fracture force of amyloid fibril is determined from its length in such a way that as the fibril length decreases, the rupture force of the fibril increases. This may be ascribed to the deformation mechanisms of amyloid fibril such that the mechanical deformation of a long fibril is governed by bending deformation while shear effect only dominates the deformation of a short fibril. That is, the higher rupture force of a shorter fibril is due to the negligible role of bending mode on the mechanical deformation of such a fibril. Here, we note that this length-dependent behavior of the rupture force is also found for the spider silk crystal (Fig. 4a). For gaining more detailed insight into the role of the fibril length on the rupture force, we measure the number of fractured hydrogen bonds during the deformation of amyloid fibril as a function of its length. As shown in Fig. 4b, it is interestingly found that the shear deformation of a short amyloid fibril (e.g. L = 3.41 nm) leads to the simultaneous rupture of several hydrogen bonds, whereas the bending deformation of a long fibril (e.g. L = 17.05 nm) results in the rupture of a single hydrogen bond. This indicates that the number of fractured hydrogen bonds, which determines the mechanical strength of protein material, is significantly dependent on the fibril length. For quantitative analysis, we consider the Bell model with assuming that the deformation of amyloid fibril follows the quasi-static process (that neglects the effect of pulling speed). Bell model[53] with ignoring the effect of pulling speed provides the rupture force of amyloid fibril in the form of[54]

$$F = \frac{1}{\Delta x_b}\left[k_B T \ln\left(\frac{1}{\omega\tau}\right) + N_{HB}\Delta E_{HB}^0 \right] \quad (3)$$

where F is the rupture force, Δx_b is the pulling distance at the moment of rupture ($\Delta x_b \approx 4$ Å), k_B and T are the Boltzmann constant and absolute temperature, respectively, τ is the characteristic timescale of hydrogen bond rupture ($\tau \approx 20$ ps), ω is the natural frequency of a hydrogen bond ($\omega = 10^{13}$ s^{-1}), N_{HB} is the number of fractured hydrogen bonds, and ΔE_{HB}^0 is the energy required to break a single hydrogen bond ($\Delta E_{HB}^0 = 2.83$ kcal/mol).[54] From Bell model without effect of pulling speed as depicted in Eq. (3), the rupture force of the fibril with its length of 3.41

nm is predicted as F = ~0.6 nN, which is comparable to that (i.e. F = 0.65 nN) obtained from SMD simulation (Fig. 4a). For a long fibril with its length of 17.05 nm, the Bell model without effect of pulling speed predicts the rupture force of F = ~0.9 nN, while SMD simulation provides the rupture force of F = 1.75 nN. The discrepancy between rupture forces measured by a theoretical model given by Eq. (3) and SMD simulation for a long fibril is attributed to the fact that the model depicted in Eq. (3) neglects the effect of pulling speed that critically affects the measured rupture force (for detail, see below). This clearly demonstrates that, as shown in Fig. 4, the length scale of amyloid fibril is a key factor in determining the force-driven bond rupture mechanisms, and consequently, the mechanical strength (e.g. rupture force) of the fibril.

In order to further understand the role of deformation mode on the mechanical strength of amyloid fibrils, we have taken into account the maximum bending stress ($\sigma_b^{\max}$) and shear stress ($\tau_{\max}$), which the fibril can exert, as follows.[55]

$$\sigma_b^{\max} = \frac{3F_0(L)\cdot L}{bh^2} \tag{4.a}$$

$$\tau_{\max} = \frac{F_0(L)}{bh} \tag{4.b}$$

where $F_0(L)$ is a length-dependent rupture force, at which amyloid fibril starts to be fractured, while b and h indicate the width and thickness of the fibril, respectively. The maximum bending stress (referred to as "bending strength") is evaluated as $\sigma_b^{\max}$ = 4 GPa at L = 3.41 nm, whereas $\sigma_b^{\max}$ = ~8 GPa at L = 17.05 nm (Fig 5a). The maximum shear stress (or referred to as "shear strength") is measured as $\tau_{\max}$ = 0.8 GPa at L = 3.41 nm, whereas it is estimated as $\tau_{\max}$ = 0.3 GPa at L = 17.05 nm (Fig 5b). This obviously shows that the mechanical strength of an amyloid fibril is highly dependent on its length in such a way that as the fibril length increases, so does the bending strength of the fibril whereas the shear strength of the fibril decreases. This is consistent with the length-dependent deformation mechanism that the fibril length determines the competitive deformation mechanisms such as shear versus bending deformations. Here, it should be noted that the bending strength of hIAPP fibril estimated by our atomistic simulations is larger by about one order of magnitude than that of insulin fibril measured by AFM bending experiment[51] (Table 2). This may be due to the discrepancy between experimental and simulation conditions such that atomistic simulations typically utilize the pulling rate being larger by a few orders of magnitude than that used in AFM experiments. The role of deformation (i.e. pulling) rate on the mechanical properties of amyloid fibrils is demonstrated later.

Moreover, we measure the toughness and resilience of the fibril, since the protein fibril has recently been reported to exhibit remarkable toughness which is much larger than that of any other man-made materials such as composite material (e.g. Kevlar).[15,56-58] Here, the toughness (W_T) of amyloid fibril is defined as

$$W_T = V^{-1}\int_0^{u_{\max}} F(u)\,du \tag{5.a}$$

where V is the volume of an amyloid fibril, $F(u)$ is the displacement-dependent force, and $u_{\max}$ is the maximum displacement at which the fibril is completely broken. Here, it should be noted that the toughness of a fibril, defined

by Eq. (5a), means the dissipated energy during the mechanical deformation of the fibril. The resilience (W_R) of the fibril is denoted as

$$W_R = V^{-1}\int_0^{u^*} F(u)du \qquad (5.b)$$

Here, u^* is the displacement at which the rupture of hydrogen bonds sustaining the fibril structure is initiated. It is shown in Fig. 6 that both the toughness and resilience of amyloid fibril is decreasing with increasing its length. As shown in Table 1, the toughness and resilience of the fibril with its length of 3.41 nm are comparable to those of a spider silk β sheet crystal with its length of ~2 nm (ref.[15]). Specifically, the toughness and resilience of hIAPP amyloid fibril with its length of 3.41 to 17.05 nm are measured as W_T = 3.9 to 26.2 kcal·mol^{-1}·nm^{-3} and W_R = 2.23 to 8.23 kcal·mol^{-1}·nm^{-3}, respectively, while the toughness and resilience of spider silk crystal with its length of 1.87 to 6.56 nm are estimated as W_T = 9 to 29 kcal·mol^{-1}·nm^{-3} and W_R = 4.5 to 9 kcal·mol^{-1}·nm^{-3} (ref[15]), respectively. For a given length, the toughness and resilience of amyloid fibril are comparable to those of spider silk crystal. This suggests that once protein (or peptide) chains are aggregated to form a β sheet-rich crystal through parallel stacking of β strands, such a crystal exhibits the excellent toughness even up to W_T = ~30 kcal/mol·nm^3 regardless of the amino acid sequence of the crystal. This highlights a design principle that the mechanical properties of β sheet-rich protein materials are encoded in the hydrogen bond networks (between β strands), which allows for the formation of cross-β structure for the β sheet-rich crystal (fibril). Moreover, the toughness of amyloid fibrils is almost independent of a pulling rate (not shown). This suggests that the toughness of a β sheet-rich protein material is determined only from its length scale, which provides the size of a protein material as a key design parameter in determining the mechanical toughness of the protein material.

We note that the pulling rate (i.e. rate of bending displacement) considered in this work is in a range of 5 × 10^{-3} to 5 × 10^{-2} Å/ps, which is larger by about four orders of magnitude than the pulling speed typically used in a single-molecule experiment.[32] As reported by many researchers[31,38,44,45,59-61] after the pioneering work of Bell,[53] the force associated with the rupture of chemical bonds such as hydrogen bonds is critically dependent on the pulling speed. In particular, the process of bond rupture is described by the crossing of energy barrier from bonded state to ruptured state. From Bell's theory, the mean rupture force involving in the bond rupture is represented in the form of $\langle F_u \rangle = (k_BT/\Delta x_b)\ln(Kv\Delta x_b k_0^{-1}e^{-\gamma})$,[60,61] where k_B, T, γ, K, v, Δx_b, and k_0 indicate the Boltzmann's constant, absolute temperature, Euler-Mascheroni constant ($\gamma \approx 0.5772$), stiffness of a protein molecule, pulling rate, energy barrier width, and kinetic rate at zero force, respectively. Fig. 7a shows the force (i.e. "rupture force") at which an amyloid fibril is fractured as a function of pulling speed. The rupture force of an amyloid fibril is linearly proportional to the logarithm of displacement (i.e. pulling) rate, which is consistent with Bell's theory. Moreover, since the mechanical strengths such as bending strength and shear strength is proportional to the rupture force (Eq. 3), these strengths are also linearly proportional to the logarithm of displacement rate (Fig. 7b and c). This suggests that the mechanical strength of amyloid fibril may depend on the experimental (simulation) condition such as the rate at which the fibril is deformed.

Moreover, we have also studied the effect of pulling rate on the elastic properties of amyloid fibrils. As described above, when a hIAPP amyloid fibril is bent with the rate of 5×10^{-3} Å/ps, the bending rigidity of the fibril is estimated as $D = 0.77 \times 10^{-25}$ Nm^2, which is comparable to the bending rigidity of an insulin fibril measured by AFM bending experiment.[51] We also note that the bending rigidity of hIAPP fibril measured at pulling speed of 5×10^{-3} Å/ps is comparable to that estimated by elastic network model as reported in our previous work.[42] Table 2 summarizes the mechanical properties of amyloid and protein fibrils. However, the bending rigidity of hIAPP fibril evaluated at pulling speed of 5×10^{-2} Å/ps is obtained as $D = 3.77 \times 10^{-25}$ Nm^2, which is larger by about one order of magnitude than the bending rigidities of not only insulin fibril measured by AFM experiment but also hIAPP fibril evaluated by a our SMD simulation using the pulling rate of 5×10^{-3} Å/ps. This clearly elucidates the significant role of the pulling rate on the mechanical properties of amyloid fibrils such as their bending rigidity, as shown in Fig. 8a. The logarithmic dependence of bending rigidity on the displacement rate is consistent with a recent finding by Grubmuller and coworkers[47] showing that the elastic property of a protein assembly (e.g. viral capsid) is dependent on the logarithm of pulling speed. In addition, we have also shown the dimensionless parameter ψ, which quantitatively describes the contribution of shear effect to the elastic deformation of amyloid fibril, as a function of pulling rate (Fig. 8b). For a short fibril (i.e. with its length of ~3 nm), as the pulling speed increases, so does the contribution of shear effect to the elastic deformation of amyloid fibril. Even for an amyloid fibril with its length of <10 nm, the contribution of shear effect is increasing as the pulling rate increases. This suggests that as the pulling speed increases, the fracture of amyloid fibril is likely to begin via an avalanche-like ruptures of hydrogen bonds (i.e. all-or-none fashion of bond ruptures) due to the shear deformation. On the other hand, for an amyloid fibril with its length of >15 nm, the role of shear effect in the elastic deformation of the amyloid fibril is almost independent of pulling speed. This indicates that deformation mechanism (i.e. shear versus bending deformations) of amyloid fibril is determined by both the fibril length and pulling speed. Our work suggests that mechanical deformation mechanisms and properties of protein fibrils are critically dependent on not only the length scale of amyloid fibril but also the experimental (or simulation) conditions at which the fibril is deformed.

Conclusion

In this work, we have studied the mechanical deformation mechanisms and properties of amyloid fibrils by using atomistic simulations. We have shown that the length scale of amyloid fibrils plays a critical role on their mechanical behaviors and properties in such a way that the competition between shear and bending deformations is highly dependent on the length of amyloid fibrils. In particular, the shear deformation is a key deformation mechanism for a short amyloid fibril (e.g. with its length of ~3 nm), while the bending deformation becomes more dominant as the fibril length increases. Moreover, it is found that the mechanical strengths of amyloid fibrils are also dependent on their length scales such that the shear strength of the fibril is maximized at the length of ~3 nm, while the bending strength increases as the fibril length increases. Our study shows that the length-dependent mechanical strength such as rupture force of amyloid fibril can be theoretically analyzed using Bell model with assuming the quasi-static deformation of the fibril. It is observed that the length-dependent rupture force is due to the fact that the rupture mechanisms of hydrogen bonds for a cross-β structure in the fibril critically depend on the fibril length.

Furthermore, we have measured the mechanical toughness (i.e. energy dissipated during the deformation) of amyloid fibrils, which is shown to depend on the fibril length. The maximum toughness is estimated as ~30 kcal/mol·nm^3 at the fibril length of ~3 nm, which is comparable to the toughness of spider silk β sheet crystal with its length of ~2 nm though the amino acid sequence of amyloid fibril is different from that of spider silk β sheet crystal. This indicates that the toughness of β sheet-rich materials such as amyloid fibril and spider silk β sheet crystal is governed by their length scales but is independent on their amino acid sequence. It may be attributed to the fact that the fracture mechanism of a β sheet-rich fibril is determined from the cooperative rupture mechanism of hydrogen bonds which sustain the fibril structure. In other words, the toughness of amyloid fibril comparable to that of spider silk crystal is attributed to the structural feature of both amyloid fibril and spider silk crystal in that they are formed by protein aggregation through parallel stacking of β strands. In addition, we have shown that the mechanical properties such as bending rigidity and strengths of amyloid fibrils are critically dependent on the pulling rate (i.e. the rate of deformation), while the toughness of amyloid fibril is almost independent on the deformation rate. In addition, we have found that the pulling rate governs the mechanical deformation mechanisms and properties of amyloid fibrils. The effect of pulling rate on the mechanical deformation mechanism is evident for a short amyloid fibril with its length of <10 nm, whereas such pulling rate effect on the deformation mechanism is not significant for a long fibril with its length of >15 nm. This implies that the mechanical deformation mechanism of amyloid fibril is determined from both the length scale of amyloid fibril and the deformation rate. Our study highlights on the important role of the length scale of amyloid fibrils on their mechanical deformation mechanisms and properties, which provides an insight into the design rule showing how the remarkable mechanical properties of protein materials can be acquired from their structural feature such as the length scales. Our work can be further extended for not only studying the structure-property-function relationship of amyloid fibrils but also developing the design principles for biomimetic materials whose mechanical properties can be tuned based on the structural features of biomimetic materials such as their length scales.

Methods and Material

We constructed the molecular structure of hIAPP amyloid fibril with its length in a range between ~3 nm and ~17 nm based on the experimentally observed structure of $hIAPP_{20-29}$ that is provided in protein data bank (pdb) with pdb code of 2KIB (ref.[9]). Before we conduct the *in silico* mechanical test of hIAPP amyloid fibrils, we carried out equilibrium dynamics simulation in order to obtain the stable structure of hIAPP amyloid fibril by using NAMD package[62] with CHARMM27 force field.[63] Here, the equilibrium dynamics simulation was implemented under explicit solvent condition using TIP3P water box. The solvent (water) box was built large enough to solvate an amyloid fibril in such a way that the distance between water atoms on the surface of a solvent box and the outer atom of amyloid fibril is set to be 40 Å. Before conducting the equilibrium dynamics simulation, we carried out energy minimization process using conjugate gradient method with iteration step of 10^4. Subsequently, the equilibrium dynamic simulation was performed based on NPT ensemble at temperature of 310 K and pressure of 1 atm with time step of 2 fs based on SHAKE algorithm. The stability of the structure of hIAPP amyloid fibril was confirmed by measuring the root-mean-square distance.

For *in silico* mechanical test of an amyloid fibril, we consider the deformation of amyloid fibril such that its two ends are fixed while one β strand in the middle layer of amyloid fibril is pulled out (Fig. 1a). Here, an *in silico* mechanical test was performed with using SMD simulation (based on NVT ensemble) implemented in NAMD package. In order to deform the amyloid fibril by pulling out such a β strand, we connect a harmonic spring (with its force constant of 10 kcal/mol·Å^2) to the Cα atom of outer residue for the β strand in the middle layer of the fibril. Then, the harmonic spring is gradually extended with a pulling speed in a range of 0.005 Å/ps to 0.05 Å/ps. The force is measured from Hooke's law such as $F(t) = K_S(vt - X)$, where K_S is the force constant of a harmonic spring, v is the pulling speed, t is the time, and X is the distance that a β strand, which is pulled out, travels. Based on such a measured force, the relationship between force and displacement was obtained, which allows for extraction of the stiffness of an amyloid fibril.

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Tables

Table 1. Comparison between mechanical properties of hIAPP amyloid fibril and spider silk crystal based on SMD simulations. Here, SMD simulation results on spider silk crystal were adopted from ref. 15.

	hIAPP amyloid fibril	spider silk crystal
Sequence	SNNFGAILS	$(Gly\text{-}Ala)_3$
Number of β-strands per a layer perpendicular to the fibril axis	2	3
Length [nm]	3.41 ~ 17.05	1.87 ~ 6.56
Rupture force [nN]	0.65 ~ 1.75	1.5 ~ 2.23
Stiffness [N/m]	0.44 ~ 3.83	0.15 ~ 4.8
Toughness [$kcal \cdot mol^{-1} \cdot nm^{-3}$]	3.9 ~ 26.2	8 ~ 29
Resilience [$kcal \cdot mol^{-1} \cdot nm^{-3}$]	2.23 ~ 8.23	4.5 ~ 9
Bending rigidity [$\times 10^{-26}$ $N \cdot m^2$]	7.73	2.8
Shear modulus [GPa]	5.97	4.6
Pulling velocity [Å/ps]	0.005	0.0005

Table 2. Mechanical properties of protein fibrils

Material	Measurement Method	Length (nm)	Stiffness (N/m)	Bending Rigidity ($\times 10^{-26}$ N·m^2)	Shear Modulus (GPa)	Bending Strength (GPa)	Source
hIAPP$_{20\text{-}29}$ fibril	Steered Molecular Dynamics (Simulation)	3.41 ~ 17.05	0.44 ~ 4.2	7.73 ~ 37.7	5.97 ~ 6.71	4 ~ 8	this work
hIAPP$_{20\text{-}29}$ fibril	Elastic Network Model (Simulation)	10 ~ 300		~8	1.1		Ref. [42]
Aβ$_{1\text{-}40}$ fibril	Steered Molecular Dynamics (Simulation)	1.92 ~ 9.58	1 ~ 40	24.1	10.2		Ref. [17]
β helix	Steered Molecular Dynamics (Simulation)	3.73 ~ 10.72	0.2 ~ 4	6.2			Ref. [17]
Aβ$_{1\text{-}40}$ fibril	Cryo-electron microscope (Experiment)	500 ~ 1000		~13	0.0127		Ref. [52]
Aβ$_{1\text{-}40}$ fibril	Elastic Network Model (Simulation)	~30		21 ~ 63	4.3 ~ 5.6		Ref. [43]
insulin fibril	AFM Bending Test (Experiment)	> ~1500	~0.25	~9.1	~0.28	0.6 ± 0.4	Ref. [51]
insulin fibril	AFM Imaging (Experiment)	> ~2000		~17	~0.13		Ref. [51]
Spider silk β sheet crystal	Steered Molecular Dynamics (Simulation)	2 ~ 7	0.2 ~ 4	~2.8	4.6		Ref. [15]

Figure Captions

Fig. 1. *In silico* mechanical test of amyloid fibrils and their deformation mechanisms: (a) Boundary condition of a $hIAPP_{20-29}$ amyloid fibril is shown. Two ends of the fibril are fixed, while the Cα atom of an outer residue for a β strand in the middle layer of the fibril is pulled with a rate of 0.005 Å/ps via a harmonic spring (with its force constant of 10 kcal/mol·Å^2). **(b)** Deformation mechanism of a short amyloid fibril (with its length of 3.41 nm). The pulling of a β strand in the middle layer of a fibril leads to the shear-like deformation of the fibril. **(c)** Deformation behavior of a long fibril (with its length of 8.28 nm). The pulling of the β strand of the middle layer results in the bending-like deformation of the fibril.

Fig. 2. Force-displacement curves of hIAPP amyloid fibrils with their various length scales. As the length of amyloid fibril increases, the magnitude of a force peak appeared on the force-displacement curve is decreasing. The enlarged figure shows the high-resolution force-displacement curve of amyloid fibril with its length of 17.05 nm. The red line in the enlarged figure shows the slope of force-displacement curve to determine the stiffness of the amyloid fibril with its length of 17.05 nm.

Fig. 3. Elastic properties of hIAPP amyloid fibrils: (a) Stiffness of amyloid fibril as a function of its length. In addition, the stiffness of spider silk crystal is also dependent on its length. Solid lines indicate the theoretical prediction from Timoshenko beam model.. **(b)** Dimensionless parameter (ψ), which quantify the contribution of shear effect to the elastic deformation of amyloid fibril, with respect to its length.

Fig. 4. Fracture properties of hIAPP amyloid fibrils: (a) Rupture force, at which the fracture of amyloid fibril begins, is shown as a function of the fibril length. The rupture force of spider silk with respect to its length is also provided. In addition, the length-dependent rupture forces of amyloid fibril predicted from the Bell model with neglecting the effect of pulling speed [i.e. Eq. (3)] are provided in comparison with those obtained from SMD simulations. **(b)** Number of fractured hydrogen bonds as a function of the fibril length. The mechanical deformation of a short fibril leads to the simultaneous rupture of several hydrogen bonds in parallel, while the deformation of a long fibril results in the rupture of a single hydrogen bond rather than several hydrogen bonds.

Fig. 5. Mechanical strength of hIAPP amyloid fibrils: (a) Maximum bending stress acting on the amyloid fibril as a function of its length. **(b)** Maximum shear stress in the amyloid fibril with respect to its length.

Fig. 6. Toughness and resilience of amyloid fibrils as a function of their length scales. The toughness and resilience of amyloid fibril were compared with those of spider silk crystal. The comparable properties (i.e. toughness and resilience) between amyloid fibril and spider silk crystal is attributed to their structural feature in that both amyloid fibril and spider silk crystal are formed by protein aggregation through parallel stacking of β strands.

Fig. 7. Effect of pulling speed on the fracture properties of amyloid fibrils: (a) rupture force, **(b)** maximum bending stress, and **(c)** maximum shear stress are shown as a function of pulling rate. As the pulling speed increases, so do the fracture properties (i.e. rupture force, and maximum stresses) of amyloid fibrils.

Fig. 8. Effect of pulling speed on the elastic properties of amyloid fibrils: (a) bending rigidity, and **(b)** dimensionless parameter (showing the contribution of shear effect) are provided as a function of pulling speed.