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DETERMINING THE MECHANICAL PROPERTIES OF AMYLOID-LIKE NANOSHEETS BY APPLYING PLATE THEORY ON ITS ELASTIC NETWORK MODEL

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Pathological prion proteins are amyloidogenic proteins that are known to disrupt the normal functioning cells inside the human cerebrum. Typically having a fibrillar shape, these proteins are known to infect other normally functioning prion proteins when it is fractured caused by external conditions. Moreover, prion proteins are known to change their conformation from fibrillar to nanosheet form according to the pH condition. As these proteins possess highly undegrading features and infectivity, studies about determining the pathological mechanism of prion proteins are carried out with effort. Even so, the information of amyloid-like HET-s prion protein, a fungal prion protein in *Podospora Anserina*, in its nanosheet form still remains elusive. In this study, we determined the mechanical and vibrational characteristics of HET-s nanosheet structure, which forms at pH ~4 and resembles a skewed plate, using in silico methods. Combining skewed plate theory with Rayleigh-Ritz approximation method into HET-s nanosheet structures at various length scale, we found that these nanosheets have mechanical properties comparable to those of previously reported biological 2-D nanomaterials. Our observation provides a detailed structural information on amyloid-like HET-s nanosheets, which may be related to its infectious characteristics.

Keywords: Elastic Network Model, Nanosheet, Plate Theory, Normal Mode Analysis

1. Introduction

Amyloids, an aggregation form of misfolded denatured β structure protein, is known to be involved in degenerative and neurodegenerative diseases such as Parkinson's syndrome, Creutzfeldt-Jakob disease, Alzheimer's disease and type II diabetes [1-3]. These accumulated amyloids originate from specific amyloid monomeric species under in vivo conditions initially and grow into various forms such as oligomers, fibrils and plaques [2, 4]. Of these amyloids, fibrils and plaque amyloid are known to have structural features with poor degradability under physiological conditions.

Studies on amyloid pathology suggest that prions, which are proteinaceous, toxic and infectious factors under the amyloid subclass, also affect cell function by converting normally functioning proteins to amyloid [5]. These amyloid prions aggregate into fibrillar forms with structural features similar to the structural features of amyloid fibrils [6]. In addition to structural features, the prion growth mechanism is similar to the amyloid accumulation mechanism in that it self-proliferates once the seed

is placed. Studies on the mechanical properties of prion fibrils are as important as studying the properties of amyloids, since the structural features of prions are like those of amyloid in that they both contain abundant β-sheets. Thus, measuring the mechanical properties of prion fibrils is necessary to understand the mechanism of proliferation and aggregation of prion fibrils in detail.

Among prions, a prion protein of a filamentous fungus *Podospora anserina* called 'HET-s' is known to control a genetic proofing process called heterokaryon incompatibility, a programmed cell death phenomenon when a prion-containing cell and a non-prion-containing cell fuse into a single cell [7]. In 2012, Mizuno et al. announced the structural dependence of HET-s amyloids on the pH condition of the external environment [5]. When the pH exceeds 3, the HET-s fibrils that are initially in a singlet form tend to self-aggregate into an amyloid-like nanosheet form—or the form of "angled-layer aggregates"—that resembles a fishing net structure. In addition, as the infectivity increases with the pH level, it is also known that lattices formed at pH 4 are infectious for seeds encountering the external environment.

In this study, the Molecular Dynamics (MD) method was effectively used to reconstruct the coarse-grained model of the HET-s nanosheet structure, and vibrational characteristics of the elastic network model (ENM) was measured using the normal mode analysis (NMA). We investigate the frequency variation per different lattice sizes and the corresponding flexural rigidities and bending elastic moduli. We report the mechanical characteristics of the amyloid-like HET-s nanosheets, which are expected to serve as a template for future prion-related studies.

2. Material and Methods

2.1 Material

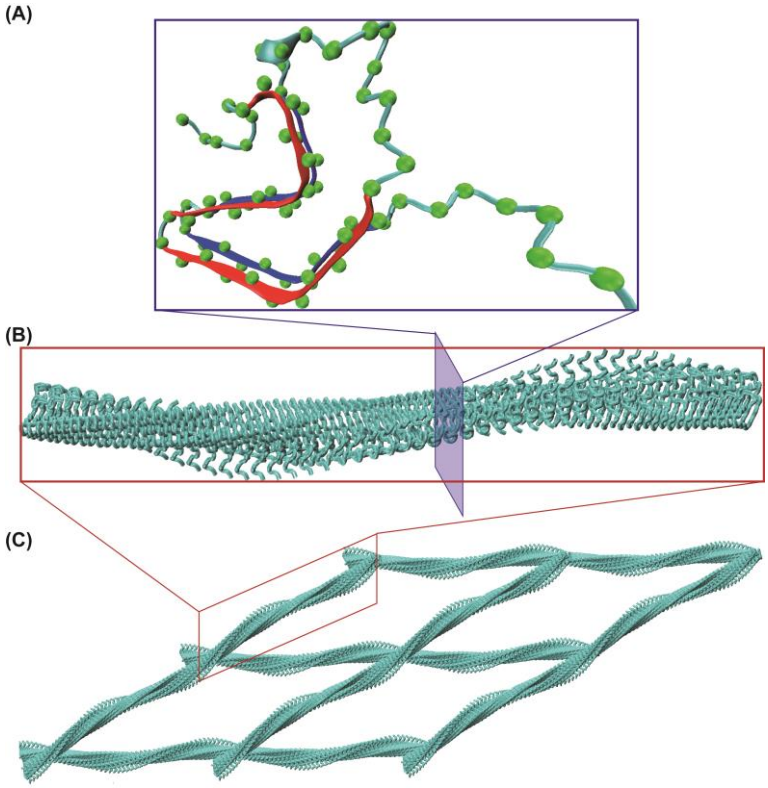

Figure 1: Hierarchical formation of reconstructed conformation of amyloid-like HET-s nanosheet structure (C). HET-s nanosheet is composed of a pitch length fibril (B), each layer of which is composed of HET-s 218-289 protein segment (A), (PDB ID: 2KJ3).

From Protein Data Bank (PDB), we used the structural model proposed by Melckebeke et al. (PDB ID: 2KJ3) [8]. From 2KJ3 HET-s structure, we extracted the 218–289 residues from residues 217–295 as shown in Fig. 1(A). It is said that a singlet HET-s fibril has an approximate pitch length of 41

nm and a circumference of 4.6 nm. Also, each HET-s base structure was equally spaced with gaps of 0.94 nm with a circular arc length of 0.146 nm [5]. Using this information, we calculated the number of stacking base structures, radius of the fibril, and twisting angle of each base structure to be approximately 44 base structures, 0.7321 nm, and 8.296°, respectively. Moreover, we found that the fibrillar axis position to be near residue Arg238.

Based on the work of Mizuno et al. we constructed the nanosheet structure after reconstructing the HET-s fibril. We set the crossing angle between two HET-s fibrils as 30° [5], and for the contacting region we have conducted an equilibrated MD simulation. Using two HET-s fibril protein segments, we discovered that the contacting region of the two HET-s fibrils to be residue 230–235 and residue 266–271 with a distance of ~1.2 nm. From the geometrical information obtained from MD simulation, we created a pH 4 Het-s nanosheet structure. Using fibrils with various lengths, we created nanosheets having widths from 1 pitch length up to 10 pitch length as Fig. 1(C).

2.2 Methods

To determine the mechanical properties of the HET-s nanosheet structures, we applied a coarse-grained model called the ENM [9]. ENM considers only the C_α atom of amino acids and simplifies the protein structure by considering interactions between atoms as harmonics. Despite its simplicity, ENM can describe the motion and major deformation modes of the protein structure with robustness, with lower computational cost than required by MD simulation.

The key to the simplification of the ENM is the interaction between atoms, which is considered equal within the cut-off distance and therefore limits the interaction range. The potential energy for ENM is defined for the interaction between atoms within the cut-off range, expressed as:

$$V = \frac{\gamma}{2}\sum_{i=1}^{N-1}\sum_{j=i+1}^{N}\left(\Delta\mathbf{R}_j - \Delta\mathbf{R}_i\right)^2 \cdot h\left(r_c - \mathbf{R}_{ij}\right). \tag{1}$$

where γ is a force constant of a harmonic string held between two atoms i and j; N is the number of C_α atoms, which is equivalent to the number of residues, within the protein structure; $\Delta\mathbf{R}_i$ is the fluctuation of the i-th residue's position vector $\mathbf{R}_i$; $\mathbf{R}_{ij}$ is the distance between two atoms i and j; r_c is the cut-off distance defined by the interaction range between atoms as 1.2 nm; and h is a Heaviside unit step function. The potential energy can also be expressed in matrices notation:

$$V = \frac{1}{2}\Delta\mathbf{R}^{\mathrm{T}}\mathbf{H}\Delta\mathbf{R}. \tag{2}$$

where $\Delta\mathbf{R}$ is a $3N$-dimensional vector of the position fluctuation of all residues; $\Delta\mathbf{R}^{\mathrm{T}}$ is its transpose; and $\mathbf{H}$ is the ($3N\times3N$) second derivative of the potential energy-also referred as the Hessian matrix. Calculating the eigenvalues and eigenvectors of the Hessian matrix allows us to determine the deformation modes and the corresponding natural frequencies ω using the following relation:

$$\omega_i = \sqrt{\frac{k_B T}{\zeta_i M_C}}. \tag{3}$$

where k_B, T, ζ_i, ω_i, and M_C are the Boltzmann constant, temperature set as 300 K, i-th eigenvalue, natural frequency corresponding to the eigenvalue, and molecular weight of the C_α atom, respectively.

3. Conclusion

In this research, we investigated the vibrational characteristics and the corresponding mechanical properties of coarse-grained, amyloid-like HET-s nanosheet structure at the atomic scale via NMA analysis on the ENM. From the reconstructed coarse-grained HET-s nanosheet structure, where the contact region of the singlet fibril was verified using all-atom MD simulations, the natural frequencies

and their corresponding mode shapes were obtained. We compared our results with previously reported 2D nanomaterials. We have verified that the HET-s nanosheet structure has similar vibrational characteristics as those of graphene sheets, and other film-coating materials. We hope this vibrational study of HET-s nanosheet structures may provide information for future prion-related studies.

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