Mechanical Characterization of Prion Fibrils using Coarse-grained Modeling Approach *in Silico*

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ABSTRACT

Prions are self-replicating proteins composed of β-sheet secondary structures that can cause neurodegenerative disorders such as Bovine Spongiform Encephalopathy(also known as mad cow disease), and they also can be found in various kinds of disease such as Alzheimer's diseases and type II diabetes [1]. At the molecular level, prions propagate an amyloid fibril by forming β-sheet structures that are oriented perpendicularly to the fibril axis, and connected through a dense hydrogen-bonding network which makes them stable [1]. Because of the mechanical toughness, understanding the mechanical characteristics of prion protein could play a key role to the development of the pathological treatment for those diseases caused by prion. One of the well-known prion proteins is HET-s protein which appears to have specific biological function in the filamentous fungus Podospora anserine. In this study, we elongated the HET-s(218-289) and characterized the mechanical properties of HET-s. Normal Mode Analysis(NMA) with Elastic Network Model(ENM) are used to predict the possible elastic deformation mode of the HET-s(218-289). ANM(Anisotropic Network Model) and continuous beam model are also used to make the result.

Keywords: prion, mechanical characterization, elastic network model, coarse-grained model, normal mode analysis, Euler-Bernoulli beam theory

1 INTRODUCTION

In recent days, many researchers are trying to apply mechanical method into the field of biology, especially in the field of study of protein [2, 3]. There are two types of protein – globular protein and fibrilar protein which makes a fibrilar form composed of aggregation of β-sheet second structure. Fibrilar proteins are called amyloid fibril, and prion protein exists among the amyloid proteins. Prion has self-replicating characteristics. It means prions make them longer by themselves, and if prions collapse during their growing process, each piece of collapsed prion starts self-replicating. Also, prion is extremely resistive in high temperature and chemically severe environment due to its strong hydrogen bonding network.

Because of this characterics, prions are considered as a cause of some diseases such as variant Creutzfeldt-Jakob Disease (vCJD). In nature, both normal prion proteins (PrP) and misfolded prion proteins (PrP^{sc}) are exist. PrP is more likely to globular protein, and PrP^{sc} has a fibrilar structure composed of β-sheet structures. If PrP^{sc} enters into human brain, PrP^{sc} starts contaminating normal PrP to PrP^{sc} structure. PrP^{sc} also have a characteristic of repeating self-replicating, so if PrP^{sc} are broken inside of the brain, piece of PrP^{sc} starts growing. Because of those mechanical stability and infectious characteristics of PrP^{sc}, it becomes hard to remove PrP^{sc} in human brain. PrP^{sc} continuously increases in brain tissue and alternates normal cell, eventually causes the malfunctioning of the brain tissue.

Amyloid fibrils also has a strong mechanical toughness, so developing tool for analysing structure and characterizing the mechanical properties of protein can be utilized in the application of biological materials for engineering such as synthesising of new materials. Because of reasons above, method development for structural analysis of protein may useful [3].

In spite of the importance of the mechanical properties for protein and bio-materials, achieving mechanical properties of protein by experiments is still hard work, because experimental environment control and sample preparation is relatively delicate job and it could give bad effect to the results if sample is spoiled. For these reason, Molecular Dynamics (MD) simulation is introduced to observe the protein behaviour. We can see time-dependent behaviour of entire molecular structure through MD simulation, but MD simulation has limitation to apply for a large molecular structure because MD simulation requires a lot of computational resources for relatively heavy molecules.

To describe mechanical characteristics of protein structure effectively, coarse-grained models are developed. One of those coarse-grained models is Elastic Network Model (ENM), which was first suggested by Tirion[4] and later by several researchers[2, 5]. The key feature of ENM is to describe the protein structure based on C^{α} atoms in such ways that Cα atom within the neighbourhood are connected by an elastic spring with an identical force

constant. Despite its simplicity, ENM is very robust in predicting the conformational dynamics of protein structures. There are several research that applied ENM for studying mechanical properties of protein fibrils such as Yoon *et al* [2].

Although the structure of PrP^{sc} is not revealed because of lack of appropriate sample, Prions have also been described in yeast and filamentous fungi. The infectous form of prions has been characterized as a β sheet-rich molecular aggregate termed by amyloid fibril [1]. One of the well-known prion proteins is HET-s(218-289) protein which appears to have specific biological function in the filamentous fungus Podospora anserine, so we decided to study mechanical properties of prion protein in fibrilar form by using filamentous fungi prion HET-s(218-289). Structure of HET-s(218-289) are already revealed based on the research of Wasmer *et al* [1], and we can find it easily at the Protein Data Bank (PDB ID: 2RNM). Also, HET-s(218-289) shows obvious β-sheet structure and fibrilar form, so it is appropriate for this study.The molecular structure of HET-s(218-289) is shown at Fig.1.

In this study, we have elongated the HET-s(218-289) and characterized the mechanical properties of the virtual model of HET-s. Elastic Network Model (ENM) and Normal Mode Analysis(NMA) are used to observe the possible elastic deformation mode of the HET-s(218-289). ANM (Anisotropic Network Model) and continuous beam model are also used to make the result.

2 METHOD

2.1 Model protein

As we mentioned above, we selected HET-s(218-289) as a base protein for our study. HET-s(218-289) is a fungi prion, and its structure is already defined. Also HET-s(218-289) is expected to have a similar structure and characteristics of PrP^{sc}.

The overall organization of a HET-s(218-289) fibril is a left-handed β solenoid with two windings per molecule. It has total 79 residues, and the core of the fibril is defined by three β strands per winding (six β strands per molecule, β1a~β3a, β1b~β3b) that form continuous in-register parallel β sheets. The cross-sectional area of fibril is shaped as triangle. The degree of arc between β1b and β2a (β3b and β4a) is 150°, and the arc between β2a and β2b (β4a and β4b) is 90°. The twisting angle between the monomer is 1.8°, and it means that the twisting angle between the one unit fibril (composed of two windings) are 3.6°. [1] Also, the length between monomer is 0.47 nm, meaning that the length between the unit fibril is 0.94 nm [6].

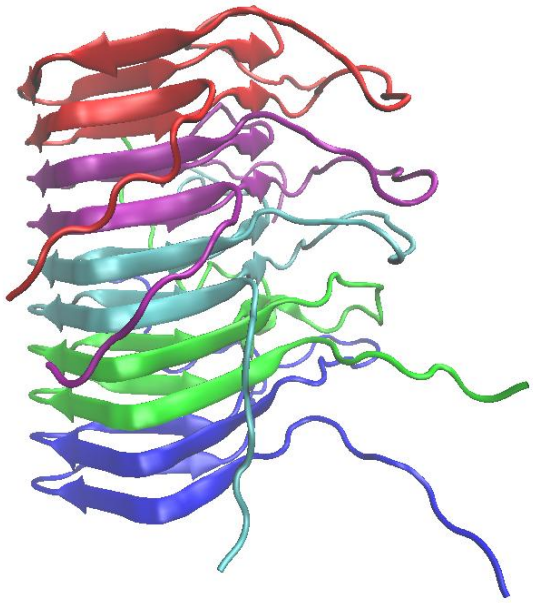

Fig.1. Molecular structure of prion fibril Het-s(218-289) plotted by VMD. (pdb id: 2rnm)

2.2 Constructing fibril structure

The structure of HET-s(218-289) protein can be easily found on the Protein Data Bank(PDB ID: 2RNM) that was revealed by solid-state NMR. We have built the three times elongated virtual protein model of HET-s fibril in direction of fibrilar axis.

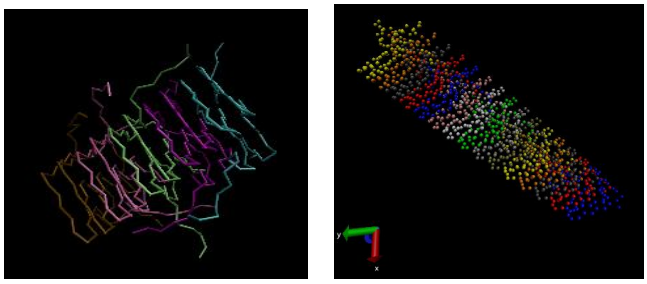

Fig. 2. The original structure of HET-s(218-289) is at left, and the constructed structure is at right.

2.3 Elastic Network model

Because protein molecules are a set of numerous atoms and their molecular mass is relatively high, it is hard to analyze entire structure through MD simulation work. However, in spite of complicated structure of protein, only a few but heavy atoms give meaningful effect to the mechanical properties of entire protein. Those atoms are mainly Cα atom.

Therefore, simulation for Cα structure from original protein structure could show similar results compared to the simulation for the entire structure, and it is called Elastic Network Model (ENM). By extracting Cα network from the

protein, we can build ENM effectively. Figure.3 briefly shows the concept of ENM. In that picture, spheres represent Cα atom, and linear springs represent hydrogen bond. For the analysis, stiffness matrix K is derived from the original protein model. There are two methods named Gaussian Network Model (GNM) and Anisotropic Network Model (ANM). [7] By GNM, only the magnitude could be derived, but ANM can derive 3-directional movement from potential function. In this research, stiffness matrix is obtained by using ANM. As a data, 12.01g/mol for atomic mass of Cα and 0.5 kcal/mol·Å^2 for spring constant are used [2].

2.4 Normal mode analysis

We can find the unique value of structure with lumped mass and elastic spring in terms of stiffness and mass. The method to find the unique value called eigenvalue and eigenvector is named Normal Mode Analysis (NMA). By substituting the stiffness matrix and mass matrix of given structure into the eigenvalue problem equation as $\mathbf{K}\boldsymbol{v} = \lambda\boldsymbol{v}$, we can get eigenvalue (λ) and eigenvector ($\boldsymbol{v}$) that is key to derive the natural frequency and mode shape.

3 RESULT

3.1 Natural frequencies

As a result of NMA, the natural frequencies and mode shapes of HET-s for each primary mode are obtained. We compared this result of natural frequency to the other simulation data from similar protein (hIAPP). hIAPP is an amyloid fibril composed of amyloidogenic core with β strands that has relation to type II diabetes. and the comparison is like the table below.

Mode	Natural Frequency (HET-s)	Natural Frequency (hIAPP)
1st Bending	0.3923 THz	~0.1 THz
2nd Bending	0.5289 THz	-
3rd Bending	0.8790 THz	~0.1 THz
Twisting	0.9739 THz	~0.5 THz
Axial	6.8064 THz	~1.25 THz

Table.1 Comparison for the simulation results of HET-s and hIAPP.

The comparison between HET-s and hIAPP fibril is similar in point of view of frequency unit (THz), but because of its fundamental difference such as structure, length and molecular weight, all frequencies of HET-s are bigger than those of hIAPPs. That's because of need of more energy that is needed for the deformation of HET-s structure.

3.2 Mode shape

We also got the mode shape for five primary modes. 1st to 6th modes were rigid body mode. Three bending mode(7th, 8th and 10th mode), one twisting modes(9th mode) and one axial modes(17th mode) are observed. There are gap between mode index of 1st twisting mode(9th) and 1st axial mode(17th), and between that two modes, second bending and twisting modes or coupled modes are found.

It is interesting that the existence of three bending mode, and it is because HET-s(218-289) has a triangular cross-section.

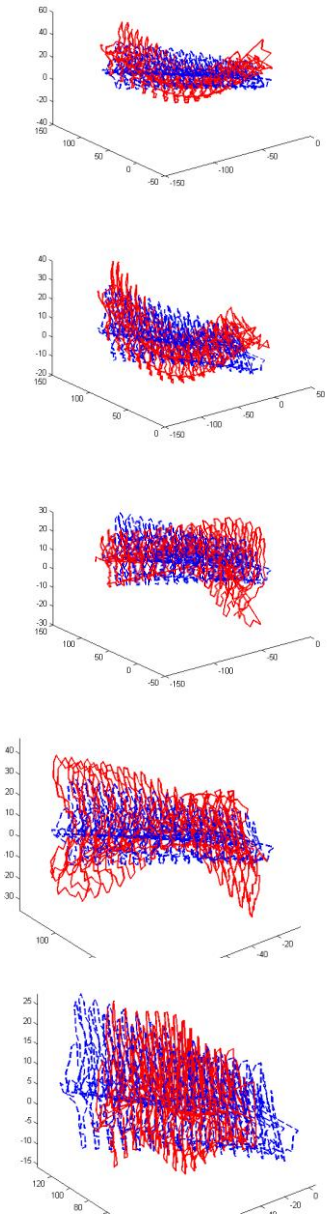

Fig.3. Deformation modes of prion fibril Het-s(218-289). 1st, 2nd, 3rd bending modes, twisting mode, and stretching and/or compression mode(from up to down). The deformed structure is red and non-deformed structure is blue.

4 CONCLUSION

In this research, we have modelled and simulated the mechanical characteristics of HET-s fibrilar protein. We obtained the virtual model of HET-s protein prion by using data from Protein Data Bank, and observed the mechanical characteristics by using NMA, the structural dynamical method.

By NMA analysis we got five primary mode and natural frequency. Interestingly there were three bending modes, and the possible reason for three bending mode is that the cross-section of HET-s fibril is triangular shaped.

Moreover, by this research, we developed the method for how to build and control the length of the Elastic Network Model for fibrilar protein in direction of fibrilar axis. This method would be helpful to the future research and valuation on length-dependent mechanical characteristics for HET-s protein.

As a future work, we are going to focus on the research for the material properties of HET-s protein with respect to the fibril length by applying Euler-Bernoulli Beam Model.

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