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Ultrasonication-triggered Amyloid Fibrillation of Proteins

超音波による蛋白質のアミロイド線維形成

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1. Protein Folding and Amyloid Fibrillation

Proteins fold to their unique 3D structures to exhibit their functions (**Fig. 1**). Recently, it has become clear that the denatured proteins or their fragments often misfold to form amyloid fibrils, ordered aggregates with a linear and rigid morphology. Because amyloid fibrils are associated with various critical diseases such as Alzheimer's disease and dialysis related amyloidosis, understanding the mechanism of amyloid fibrillation in comparison with protein folding has been one of the most important topics of protein sciences.

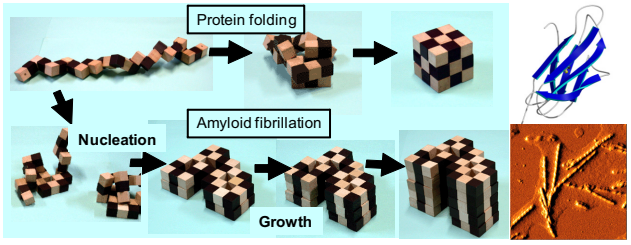

Fig. 1. Comparison of protein folding and amyloid fibrillation.

Amyloid fibrils form through nucleation and growth, similar to the crystallization of substances. Because of the high free energy barrier of nucleation, the spontaneous formation of amyloid fibrils occurs only after a long lag phase. Once the nucleus formed, subsequent elongation is rapid. Thus, the amyloid fibrillation is accelerated by the addition of preformed fibrils (i.e. seeds). In the seeding experiments, ultrasonication has been often used to make seeds by breaking down preformed long fibrils into short fibrils.

To accelerate the nucleation and thus the amyloid fibrillation, the protein solution has been agitated by stirring or shaking. We expected that ultrasonication, a strong agitator of the solution, is useful for nucleation. Indeed, spontaneous fibrillation was effectively promoted by applying ultrasonic pulses (1). Taking advantage of unique properties of ultrasonication, we propose several approaches for examining the amyloidogenicity of proteins (2-4). Moreover, we suggest that ultrasonication will be useful for clarifying the roles of supersaturation in life.

2. Ultrasonication-dependent fibrillation (1)

Acid-denatured β2-microglobulin (β2m), a protein responsible for dialysis-related amyloidosis, is monomeric at 0.3 mg/ml and pH 2.5 for several days under quiescent conditions. We examined the effects of ultrasonic pulses on the acid-denatured monomeric β2m. The ultrasonicator used was a water-bath-type model with the ultrasonication frequency of 20 kHz, and the power output of 350 watts. Reaction mixtures were ultrasonicated from three directions (i.e. two sides and bottom) for 1 min and then incubated for 9 min without sonication, a process that was repeated during incubation at 37ºC.

Upon ultrasonication, amyloid specific thioflavin T fluorescence increased markedly after a lag time of 1– 2 h with a simultaneous increase of light scattering (**Fig. 2**). Atomic force microscopy images showed the formation of short fibrils. When the sonication-induced fibrils were used as seeds in the seeding experiment, a rapid formation of long fibrils was observed demonstrating seed-dependent fibril growth. No serious change in chemical properties of β2m was detected.

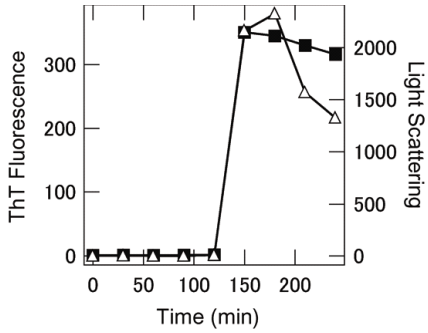

Fig. 2. Ultrasonication-dependent amyloid fibrillation of β2m at pH 2.5.

The results indicate that the acid-denatured β2m solution is supersaturated and ultrasonication breaks the supersaturation, producing amyloid fibrils. Because spontaneous fibrillation rarely occurs under quiescent conditions or takes a long lag-time even under agitation with stirring, ultrasonication is an efficient method to accelerate the fibril formation.

3. Ultrasonication-dependent Production and Breakdown Lead to Minimum-sized Fibrils (2)

As shown above, ultrasonication can induce monomeric β2m to form amyloid fibrils. On the other hand ultrasonication can break down preformed fibrils into shorter fibrils. Combining these two opposing effects, we showed that ultrasonication pulses are useful for preparing monodispersed amyloid fibrils of minimal size. The small homogeneous fibrils will be of use for characterizing the structure and dynamics of amyloid fibrils, advancing molecular understanding of amyloidosis.

4. Combined Use of Ultrasonication and Plate Reader: HANABI (3)

Ultrasonication is useful for inducing amyloid nucleation and thus the formation of fibrils, while the use of a microplate reader with amyloid specific thioflavin T fluorescence is suitable for detecting fibrils in many samples. Combining the use of ultrasonication and a microplate reader, we propose an efficient approach to studying the amyloidogenicity of proteins (**Fig. 3**).

A combined system "HANABI (HANdai Amyloid Burst Inducer)" for inducing and detecting amyloid fibrils automatically was made with Elekon Co. (Chiba) and Corona Electric Co. (Tokyo) (**Fig. 4**). HANABI will be useful for a high-throughput assay of the amyloidogenicity of proteins. Moreover, HANABI can be used for accelerating and detecting crystallization of various substances including native proteins.

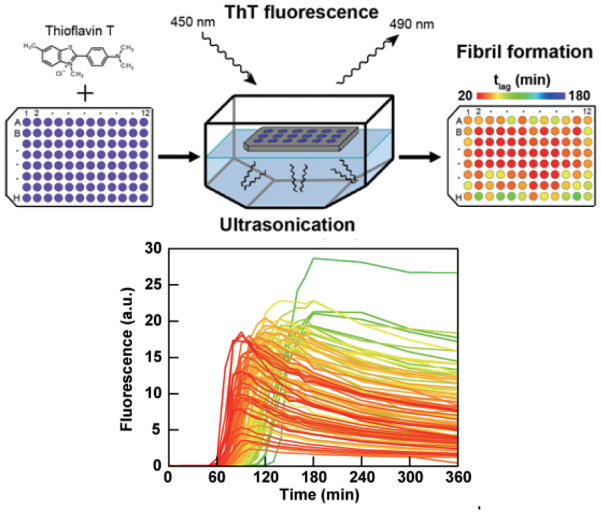

Fig. 3. Ultrasonication-dependent acceleration of amyloid fibrils.

Fig. 4. Amyloid-burst inducer HANABI. Left: outside view; middle: ultrasonicator; right: microplate.

5. Distinguishing Amyloid Fibrils and Amorphous Aggregates (4)

Amorphous aggregates are another types of aberrant aggregates associated with protein misfolding diseases. Although amorphous aggregates and amyloid fibrils differ in morphology, the two forms are often difficult to distinguish. We showed that, on the basis of the effects of ultrasonic pulses on the kinetics of formation, crystal-like amyloid fibrils and glass-like amorphous aggregates can be distinguished.

6. Conclusions

In the studies of amyloidosis, ultrasonication has been used to break down preformed fibrils into shorter fibrils. We showed that ultrasonication can induce the monomeric solution of amyloidogenic proteins to form amyloid fibrils. We propose that ultrasonication is one of the best means of accelerating amyloid nucleation and thus the formation of fibrils. Solubility and supersaturation will be key factors for further understanding the aggregation of proteins. Ultrasonication-triggered release of supersaturation and consequent precipitation of solutes will be common to various substances and open the new field of supersaturation-limited life science.

Acknowledgment

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