

Study on ultrasonically accelerated aggregation phenomenon of A β peptides based on cavitation-bubble dynamics

キャビテーションバブルに着目した A β ペプチドの超音波凝集加速現象の研究

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1. Introduction

Alzheimer's disease (AD) has been a serious issue on aging society. Amyloid- β (A β) peptides deposit on cerebral cortex, and form neurotoxic aggregates for causing AD. Aggregation process of A β peptides is therefore deeply related with onset and development of AD. However, the mechanism remains unclear, and no effective treatments for AD appear.

Aggregation of A β peptides takes a very long term about 60 years, *in vivo*. This fact prevents us from understanding the detail aggregation mechanism of A β peptides. Previous studies then reconstructed A β 's aggregation with peptide concentrations much higher than the physiological concentration to accelerate the phenomenon. However, this approach will cause many various and complicated pathways from monomer to aggregates and will be difficult to reproduce the phenomenon *in vivo*. Recently, it has been found that the aggregation reaction of various peptides, including A β , can be drastically accelerated by ultrasonication for the peptide solution [1-3]. However, the aggregation acceleration mechanism caused by ultrasonication is still unclear. If this mechanism is revealed, it will be possible to form various A β 's aggregation *in vitro*, which is important for the drug discovery.

In this study, we focus on A β_{1-40} peptide, which consists of 40 amino acid residues with molecular weight of 4,331 Da. We perform ultrasonication experiments for A β_{1-40} solutions, measuring the acoustic pressure, for producing well-oriented needle-like aggregates called amyloid fibrils. Their concentration is evaluated by fluorescence dye called thioflavin-T (ThT) assay, and their structure is observed by transmission electron microscopy. These aggregates were similar to those observed in AD patient's brain [4]. Then, we propose a new accelerated aggregation model by combining the bubble-dynamics theory and

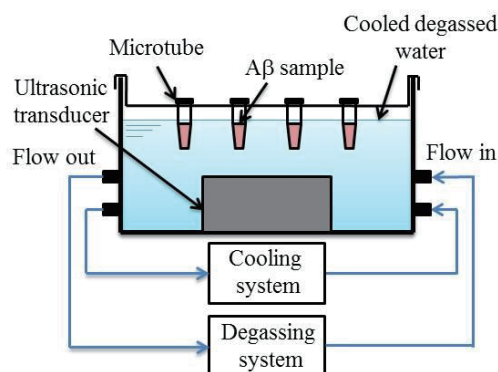


Fig.1 Schematic of experimental system we developed for ultrasonication experiments.

the two-step reaction model. This model successfully explains the drastic increase in the reaction-rate constant for nucleation.

2. Experimental Procedure

The lyophilized A β sample was diluted to 10 μ M with 20% dimethyl sulfoxide / 80% 100 mM PBS containing 100 mM NaCl (pH 7.4) mixture. This solution was poured in the microtubes (500 μ L) and we performed ultrasonication for them. A single ultrasonication sequence consisted of 1 min ultrasonication and 9 min incubation, and this 10 min sequence was repeated. For monitoring the formation of amyloid fibrils, we used the ThT assay; ThT selectively binds to cross- β -sheet structure which is amyloid fibril's unique structure, and emits high intensity fluorescence. ThT fluorescence intensity was measured every 30 min over an ultrasonication experiment. At fluorescence measurement, we used excitation light with 450 nm and scanned the detected wavelength from 450 to 500 nm, measuring the fluorescence peak intensity.

For performing ultrasonication experiments, we developed the experimental system shown in **Figure 1**. The ultrasonic transducer with fundamental frequency of 26 kHz was located in a water tank, which was filled with degassed water for avoiding loss in the acoustic power caused by bubble cloud there. The water temperature was kept under ~ 17 °C by the homebuilt cooling device.

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Several microtubes (1.5 mL vol.) containing 500 μL A β solution were set above the transducer at different locations so as to perform the ultrasonication experiment with various acoustic pressures simultaneously.

3. Model for Ultrasonically Accelerated Aggregation

Schematic of our aggregation acceleration model is shown in **Figure 2**. When A β solution is irradiated with ultrasound, cavitation bubbles are generated in the solution (**Fig. 2 (a)**). A β monomer involves highly hydrophobic amino acids, which prefer air-water interface. Thus, A β monomers are attracted to the cavitation-bubble surface (**Fig. 2 (b)**) during the bubble growth period, and they are condensed at the center of the bubble with the subsequent bubble collapse (**Fig. 2 (c)**). Near the bubble center, the concentration of A β monomers thus increases. Because the gas in the bubble is compressed by an adiabatic process, temperature of the gas increases about a few thousand kelvins (**Fig. 2 (c)**). The probability of formation of aggregation nucleus follows the Boltzmann distribution, and nuclei of amyloid fibrils are significantly promoted by temperature increase with bubble collapse.

4. Result and Discussion

We performed ultrasonication experiments to A β samples. The data obtained by these experiments were fitted by the simple nucleation-growth model as shown in **Fig. 3**:

$$[B] = [A]_0 \left\{ 1 - \frac{k_n + k_g[A]_0}{k_g[A]_0 + k_n \exp(k_n + k_g[A]_0)t} \right\}$$

Here, $[B]$, $[A]_0$, k_n and k_g denote the concentration of fibrils, initial concentration of monomers, rate constants of nucleation and fibril growth, respectively. Calculation results of k_n and k_g at different 2nd harmonic pressures are shown in **Table 1**. k_g was nearly unchanged, however, k_n was drastically increased by tens-orders of magnitude with the increase of 2nd harmonic pressure. These results indicate that ultrasonication accelerate the aggregation reaction of A β peptides by particularly promoting nucleation reaction.

Then, we explain this effect quantitatively. First, we calculate the bubble radius motion based on Keller-Miksis equation for estimating a span of influence of the local condensation effect. Second, the hot spot temperature of cavitation-bubble was calculated from radius changing by assuming adiabatic compress process described by following:

$$T = T_0 \left(\frac{R_0}{R} \right)^{3(\gamma-1)}$$

Here, T , R , and γ denote the temperature of the gas inside the bubble, bubble radius, and specific heat ratio, respectively. Subscript 0 means an initial

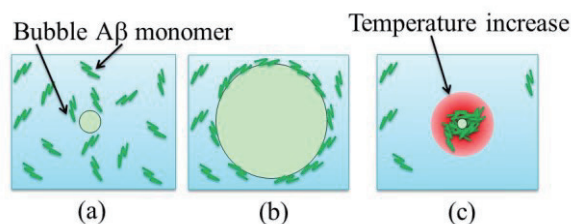


Fig.2 New aggregation acceleration model

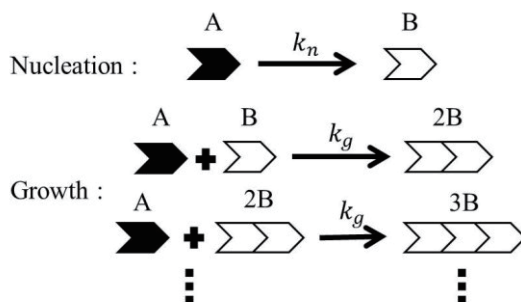


Fig.3 Schematic of the two-step reaction model

Table.1 Fitting result of aggregation experiments

	2nd harmonic pressure [kPa]			
	20.4	30.3	97.5	130.3
k_n [1/h]	2.97×10^{-17}	2.81×10^{-11}	9.30×10^{-9}	7.00×10^{-8}
k_g [1/h μM]	0.594	0.384	0.983	4.22

value. From this calculation, we obtained the maximal temperature and the minimal radius of the bubble, which are involved in the thermal diffusion equation as initial and boundary conditions. By solving the thermal diffusion equation, we calculate the probability of nucleation of A β monomers based on the Boltzmann distribution. In consequence, we succeeded in reproducing tens-orders of magnitude increased k_n even at room temperature.

In conclusion, we showed that ultrasonication for A β solution particularly promotes the nucleation of A β monomers through two effects, the local condensation and activation of chemical reaction by temperature increase with bubble collapse. We quantitatively discussed about acceleration effect of A β -peptide aggregation by cavitation bubble using proposed model to explain the drastic increase in the rate constant for nucleation.

References

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