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ATP-dependent amyloid fibrillization of α-synuclein under the ultrasonic irradiation

超音波照射下における ATP 依存的αシヌクレインのアミロイド線維形成

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1. Introduction

Although adenosine triphosphate (ATP) is one of the most important molecules and serves as an energy carrier in living cells, ATP has been reported to play a role as hydrotrope which dissolves the hydrophobic molecules in aquaous solution.[1)] However, we recently found that polyposhosphate (polyP), consisting of several dozen of covalently linked phosphate grous, significantly accerelated the amyloid fibrillization.[2)] Thus ATP containing triphosphate and adenosine may also have a potential to induce the amyloid fibrillization.

Amyloid fibrils, which are aberrant protein aggregates with several μm in a length and ~10 nm in a diameter, cause more than 25 disorders, including neurodegenerative diseases such as Parkinson's disease (PD). α-Synuclein (αSyn) is the causative protein of PD and is an intrinsically disordered protein consisting of 140 amino acid residues. αSyn forms oligomers as well as amyloid fibrils, and these aggregates are able to interact with ATP synthase and open the permeability transition pore in PD.[3)]

Ultrasonic irradiation has been employed for triggering the nucleation of amyloid fibrils so far, and we developed a Handai amyloid burst inducer (HANABI) (Fig. 1),[4)] with which amyloid fibrillization was promoted and automatically measured. Cavitation bubbles generated during the ultrasonic irradiation efficiently induce the nucleation and fragmentation of amyloid fibrils. In this study, we examined the amyloid fibrillization of αSyn at varying concentrations of ATP under the ultrasonic irradiation using HANABI instrument. We found that the amyloid fibrillization of αSyn was remarkably induced by several millimolar concentrations of ATP at the neutral conditions. We also examined amyloid fibrillization by using ADP and AMP. These reactions also changed depending on pH of the solution through a mechanism similar to that observed in polyP.

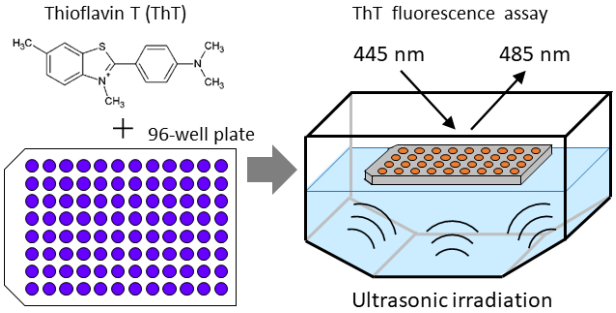

Fig. 1 Schematic image of HANABI instrument. HANABI combines a water bath-type ultrasonic irradiator and a fluorescence microplate reader.

2. Materials and Methods

αSyn was expressed using *Escherichia coli* and purified through several steps using anion exchange column chromatographies, as previously described.[5)] Thioflavin T (ThT) and the other reagents were purchased from Wako Pure Chemical Industries Ltd. and Nacalai Tesque, respectively.

Amyloid fibrillization was performed at 0.3 mg/ml αSyn, 20 mM NaPi pH 7.5, 5 μM ThT in the presence of varying concentrations of ATP, ADP or AMP. Amyloid experiments were also performed at pH 4.5 and 2. Ultrasonic wave was applied to reaction mixtures in 96-well plates from three directions (i.e., two sides and bottom) for 1 min and then incubated for 4 min without ultrasonic irradiation using a HANABI instrument (Fig. 1). Ultrasonic irradiation was repeated at 37 °C, and the 96-well plate was moved throughout the reaction. The frequency of the instrument was 17–20 kHz and the power output was set to 90% of a maximum of 550 W.

In addition, to develop the high-throughput screening device for the assay of amyloidogenic components in human sample, we employed the thermal cycler to induce the amyloid fibrillization upon heating the reaction solution up to ~95 °C.

3. Results and Discussion

Under the ultrasonic irradiation, amyloid fibrillization of αSyn was accelerated at equal to or more than 5 mM ATP (Fig. 2), which was comparable with the concentration of ATP inside cells. CD spectra showed that the amyloid fibrils were rich in β-sheet conformation, and EM observation showed that αSyn formed rigid rod-like fibrils depending on the concentrations of ATP (Fig. 3). By comparing with ATP, ADP and AMP, the longer the phosphate groups of them, the more efficiently amyloid fibrils were formed.

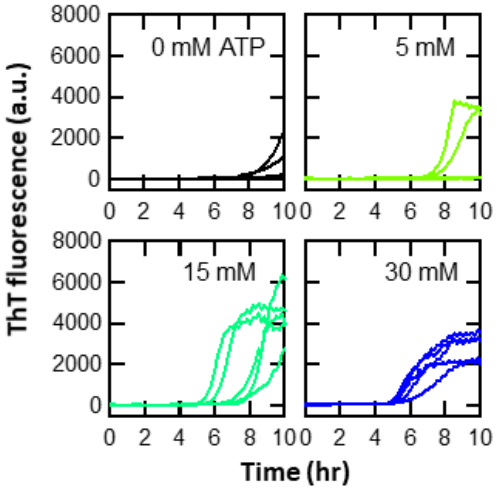

Fig. 2 Amyloid fibrillization of αSyn at varying concentrations of ATP at the neutral pH under the ultrasonic irradiation.

In addition, αSyn formed not only the amyloid fibrils but also amorphous aggregates depending on pH of the solution. Amyloid fibrils could be formed by the preferential hydration of ATP at the neutral pH, but amorphous aggregates were formed by the charge-charge interaction between negatively charged ATP and positively charged αSyn at the acidic conditions. In general, amyloid fibrils were formed above its solubility limit and further increases in driving forces, leading to the formation of glassy amorphous aggregates by the rapid breakdown of supersaturation. Interestingly, at around isoelectric point (pI) of αSyn (pI=4.7), amyloid fibrils were significantly formed without ATP by the pI precipitation and amyloid fibrillization was suppressed in the presence of ATP. These aggregation mechanisms were similar to those between αSyn and polyPs including inorganic phosphate.[6)]

Although αSyn forms unfolded conformation at the neutral pH, the preferential hydration stabilizes more compact conformation such as the partially folded conformation and amyloid fibrils by the intramolecular and intermolecular interactions, respectively. In addition, the charge-charge interaction may interfere the pre-existed interactions that aggregate proteins and can prevent the aggregation. Thus, ATP will have two faces; one is the property as hydrotrope and the other is the property as kosmotropic salt in Hofmeister series.

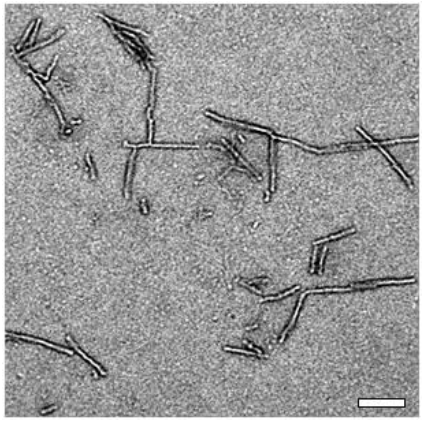

Fig. 3 EM image of αSyn amyloid fibrils at 5 mM ATP. The scale bar is 200 nm.

We now also develop the high-throughput screening device for the assay of amyloidogenic components in human sample by using the thermal cycler. The protein aggregation can be induced by the hydrophobic interaction between molecules upon heating. We refer the recent results of amyloid fibrillization using the thermal cycler.

4. Conclusion

ATP induced the amyloid fibrillization of αSyn at the several millimolar concentrations under the ultrasonic irradiation. Thus, ATP consisting of triphosphate and adenosine has a property as kosmotropic salt and has a potential to induce the protein aggregation *in vivo*.

Acknowledgments

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