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Accelerating amyloid fibril formation by multi-channel ultrasonic chemical reactor

多チャンネル超音波化学反応装置を用いたアミロイド線維の形成促進の研究

Kentaro Noi[1†], Kichitaro Nakajima[2], Keiichi Yamaguchi[2], Masatomo So[3], Kensuke Ikenaka[4], Hideki Mochizuki[4], Yuji Goto[2], and Hirotugu Ogi[5] (Inst. NanoSci. Design, Osaka Univ.; [2]Global Center for Med. Eng. Info., Osaka Univ.; [3]Inst. Protein Res. Osaka Univ.; [4]Grad School Med., Osaka Univ.; [5]Grad. Sch. Eng., Osaka Univ.)

野井健太郎 [1†], 中島吉太郎 [2], 山口圭一 [2], 宗正智 [3], 池中建介 [4], 望月秀樹 [4], 後藤祐児 [2], 荻博次 [5] ([1] 阪大　ナノセンター, [2] 阪大　国際医工, [3] 阪大　蛋白研, [4] 阪大院　医, [5] 阪大院　工）

1. Introduction

Amyloid fibrils, known as misfolding aggregates of particular proteins, are often reported to be involved in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. A common feature of amyloid fibrils formed from various amyloidogenic protein is that they are composed of highly ordered β-sheet-rich structure. One of problems in the study of amyloid fibrils is that formation of amyloid fibrils takes a long time. Ultrasonic irradiation to an amyloidogenic-protein solution is a useful method for promoting amyloid fibril formation. As a conventional ultrasonic irradiation method, it has been developed to irradiate microplates and microtubes with ultrasonic waves through water in a water tank [1, 2]. However, this method has the problem that the sound pressure of ultrasonic waves, which is an important factor to promote amyloid fibril formation, cannot be controlled because the ultrasonic cavitation generated in the water tank scatters the ultrasonic waves.

To solve this problem, we have developed a multi-channel ultrasonic chemical reactor, which can remove the water tank and uniformly irradiate the microplate with ultrasonic waves. Figure 1 shows schematic of the developed multi-channel ultrasonic chemical reactor. One ultrasonic vibrator is installed for one well of the microplate to drive each ultrasonic vibrator independently. Therefore, it is possible to change the sound pressure, frequency, and irradiation time for each well in one microplate. Recently, we have successfully detected a very small amount of seeds, which are initial structures for amyloid fibril formation, using the multi-channel ultrasonic chemical reactor [3].

In this study, we investigate the applicability of our developed multi-channel ultrasonic chemical reactor to the amyloid fibril formation on various amyloidogenic proteins, including amyloid β (Aβ) peptides and α-synuclein, and confirm the usefulness of this reactor by performing the amyloid-formation assays under various irradiation conditions in the same microplate.

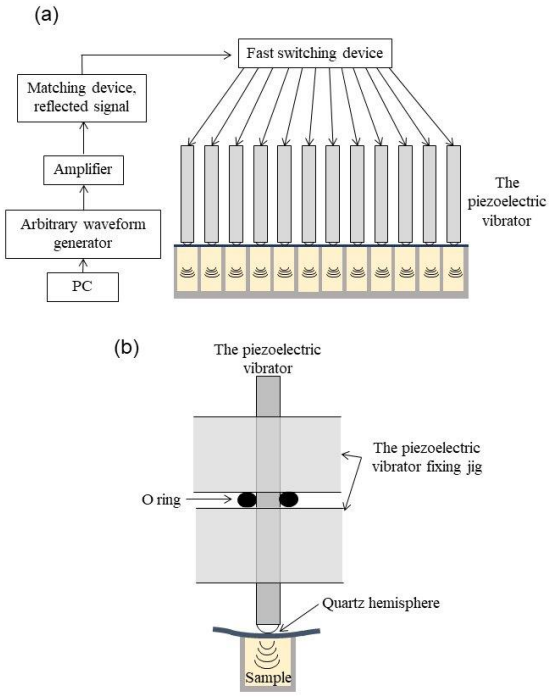

Fig. 1. (a) Schematic of the developed multi-channel ultrasonic chemical reactor. (b) Cross section view of the piezoelectric vibrator and a well.

2. Experiment Procedure

Lyophilized-powder $A\beta_{1\text{-}40}$ peptide was purchased from Peptide Institute. Recombinant human α-synuclein were expressed in *Escherichia coli* and purified as described[4]. Lyophilized-powder $A\beta_{1\text{-}40}$ was dissolved by DMSO. The $A\beta_{1\text{-}40}$ solution was diluted to 5 μM by 100 mM PBS solution with pH 7.4 containing 100

E-mail: ogi@prec.eng.osaka-u.ac.jp

mM NaCl and 5 μM thioflavin-T (ThT). The volume fraction of DMSO and the PBS solution is 1:4. Lyophilized-powder α-synuclein (7.0 μM) was dissolved by 0.8 M phosphate buffer pH 6.9 containing 5 μM ThT.

The sample solution was put in 96-well microplate. Each well was filled with the sample solution to keep air out. The 96-well microplate was then set at the multi-channel ultrasonic chemical reactor. The 96-well microplate was ultrasonicated in a cycle of 0.1 ms irradiation pules and 8 s quiescence. The power and output of the sonication were set to 28-30 kHz and various sound pressure levels. In the control measurement, the 96-well microplate was shaken in a cycle of 50 s shaking and 10 min quiescence. The amyloid fibril formation was detected using fluorescence intensity measurement of ThT, which emits fluorescence to bind specifically to amyloid fibrils. The ThT fluorescence intensity measurement took every 10 min.

3. Results and Discussion

We investigated the amyloid fibril formation reaction for $A\beta_{1\text{-}40}$ peptide using the developed reactor. Time-course curves of ThT fluorescence intensity are shown Fig. 2(a). The result indicates that the amyloid fibril formation of $A\beta_{1\text{-}40}$ peptide begins 2 hours from the start of the experiment. The sample after the measurement formed typical amyloid fibrils (Fig.2(d)). For comparison, measurement of amyloid fibril formation under mixing condition and static conditions were performed (Figs. 2(b) and (c)). The average start time of amyloid-fibril formation under each condition was evaluated based on the relative value of the fluorescence intensity of 0.1. The average times of the developed reactor, mixing condition, and static condition are 1.65, 5.09, and 23.3 hours, respectively. From there results, the developed reactor can significantly promote amyloid fibril formation of $A\beta_{1\text{-}40}$ peptide.

Next, we performed the same measurement for α-synuclein. Figure 3 shows time-course curves of ThT fluorescence intensity for α-synuclein. In the mixing condition, some wells did not change fluorescence intensity within 300 hours, and it took 150 hours even in the wells where the fluorescence intensity increased. On the other hands, when the developed reactor was used, amyloid fibril formation of α-synuclein began within 12 hours, and the reaction was completed within 20 hours. Furthermore, we investigated the effectiveness of our reactor for β2-microglobulin and lysozyme, and confirmed that the amyloid fibrosis reaction was promoted about 10 times for them. These results indicate that the developed

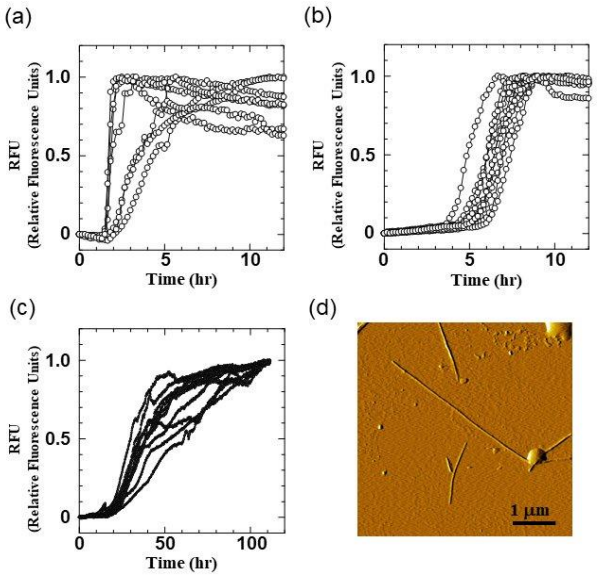

Fig. 2. Time-course curve of ThT fluorescence intensity for $A\beta_{1\text{-}40}$ peptide by (a) multi-channel ultrasonic chemical reactor, (b) mixing condition, (c) static condition. (d) An AFM image after the (a) measurement.

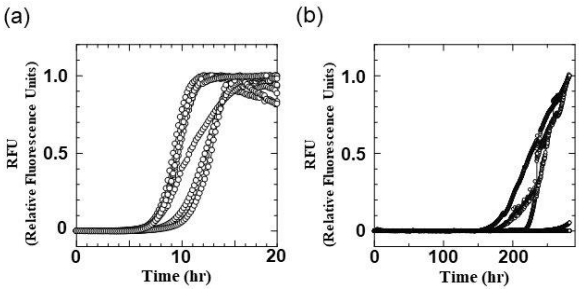

Fig. 3. Time-course curve of ThT fluorescence intensity for α synuclein by (a) multi-channel ultrasonic chemical reactor, (b) mixing condition.

multi-channel ultrasonic chemical reactor can significantly shorten the amyloid fibrosis reaction, regardless of the type of amyloidogenic protein. Also, in the developed multi-channel ultrasonic chemical reactor measurement gives highly reproducible results between each measurement and each well. Therefore, the developed multi-channel ultrasonic chemical reactor is a very useful tool for solving the time-consuming problem of amyloid fibril formation.

References

1. M. So *et al.*: J. Mol. Biol. **412** (2011) pp. 568-577.
2. K. Nakajima *et al.*: Sci. Rep. **6** (2016) 22015.
3. K. Nakajima *et al.*: Ultrasonics Sonochemistry, **73** (2021) 105508.
4. H. Yagi *et al.*: J. Biol. Chem. **280** (2005) pp. 38609-38616.