Fabrication of Amyloid Fibrils of Cytochrome *c* Disulfide Dimers by Optical Trapping

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Our group has successfully demonstrated optical trapping-induced amyloid fibril formation of a domain-swapped dimer of cytochrome *c* (cyt *c*) [1]. Optical trapping with a focused laser beam increases the local concentration of cyt *c* at the laser focus, where the conformation of cyt *c* is deformed, achieving nucleation of amyloid fibrils. Here, we apply this optical trapping method to various disulfide dimers of cyt *c* mutants (G45C, T58C, A83C, and E104C) to fabricate amyloid fibrils and discuss different morphologies of their amyloid fibrils, depending on the direction of laser polarization.

The cysteine-introduced cyt *c* mutants were produced with an *E. coli* expression system, and the purified cyt *c* mutants were incubated to form disulfide dimers. The disulfide dimers were purified by gel filtration chromatography in 50 mM potassium phosphate buffer, pH 7.0, and then the solvent was exchanged to D_2O in order to suppress temperature elevation by 1064-nm laser irradiation. 10 μL of each dimer solution was added into a container, which was put on a stage of a microscope. A continuous-wave laser beam of 1064 nm was employed as a trapping light source and tightly focused at a position of 20 μm above the container bottom with a 100X objective lens (NA = 1.4). The laser power throughout the objective lens was fixed to be 300 mW. Thioflavin T (ThT) was added into the solution as a fluorescent indicator for amyloid fibrils. The structures of amyloid fibrils fabricated by optical trapping were confirmed by TEM.

When the trapping laser was irradiated into the solutions of the disulfide dimers, a few-micrometer sized aggregate was formed after ~15 minutes. Further laser irradiation into the aggregate caused its sudden and rapid growth, and at the same time, the fluorescence intensity from ThT started to be tremendously enhanced. These results support that the nucleation of amyloid fibrils is achieved at this time. TEM observation was carried out for amyloid fibrils of each disulfide dimers formed by optical trapping, and we found that the fibril structures depended on the position of the disulfide bond of cyt *c* dimers. In the presentation, we will also present that laser polarization-dependent morphology changes in their amyloid fibrils and discuss the dynamics and mechanism from the viewpoint of alignment of fibril structures affected by the direction of laser polarization.

References

[1] K. Yuyama *et al*., *Angew. Chem. Int. Ed.*, 56, 6739 (2017).