Enhanced crystallization of proteins induced by laser trapping at the air/water interface

Hokkaido Univ. [1], Chem. Sci. Eng., Hokkaido Univ. [2], ○Atsushi Miura[1,2], Kyoko Ueda[2], Izumi Yoshimatsu[2], Noboru Kitamura[1,2]

E-mail: atsushi.miura@sci.hokudai.ac.jp

Laser trapping is a potential approach to make an assembly of small molecules and particles with spatial and temporal controllability. It was reported that tight focusing of a continuous-wave (CW) and/or pulsed near-infrared (NIR) trapping laser to the air/solution interface of amino acid/D_2O solution induces crystallization rapidly.[1,2] Although crystallization of small biological molecules such as amino acid is possible, protein crystallization is still difficult.

Recently we have reported that laser trapping with CW NIR laser at the air/solution interface of a hen egg-white lysozyme (HEWL)/D_2O solution ([HEWL] = 40 mg/mL, NaCl = 2.0 wt%, pD = 5.1) induces assembly formation, many micrometer-sized liquid droplet formation and, eventually, enhanced crystallization after liquid droplet formation.[3] Raman microspectroscopy of formed liquid droplets indicates the concentration of HEWL is elevated nearly ten-times higher than that of applied HEWL solution; i.e. droplets are highly saturated. However real-time crystallization under laser trapping has not been realized yet.

In contrast to the HEWL, we have found that cage-shaped supramolecular protein, ferritin (Fer) can be crystallized under laser trapping. Focusing of CW NIR trapping laser to the air/solution interface of the ferritin/D_2O solution ([Fer] = 10 mg/mL, 0.5M $(NH_4)SO_4$, $CdCl_2$ = 0.2 wt%, pD = 7.0) induced the crystal formation from the focal spot within a few tens of minutes (Fig. 1a). Furthermore, laser-induced crystals grew much faster than that formed by spontaneous nucleation without laser irradiation (Fig. 1b). It suggests that Fer crystals were emerged in locally-induced high concentration region at the focal spot since crystal growth speed is affected by protein concentration. We carry out Raman and polarized Raman microspectroscopy during laser trapping-induced crystallization to clarify the origin of different crystallization behaviors of HEWL and Fer. Polarized Raman results of HEWL indicate protein molecules are aligned along the polarization direction of trapping laser beam. These results indicate that real-time laser trapping crystallization of protein will be possible by controlling the supersaturation and molecular orientation.

[1] Sugiyama, T. *et al.*, Acc. Chem. Res. 2012, 45, 1946. [2] Miura, A. *et al.*, Appl. Phys. B 2013, 112, 473. [3] Yoshimatsu, I. *et al.*, 2015 JSAP autumn meeting, Nagoya.

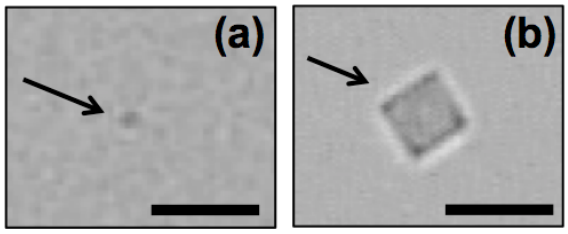

Figure 1 (a) Fer crystal formed by 25 min laser irradiation and (b) the same crystal grown with 36 min. Scale bar; 10 μm.