In-cell crystal engineering for the development of solid biomaterials

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Protein crystals, which have a highly ordered arrangement of protein molecules, have a great potential for applications in molecular storage, separations, and catalysis.[1] Protein crystals formed in living cells are known to have various natural functions such as virus and toxin storage, immune activation, and solid catalysts. However, the crystallization process in living cells, which is the key to the functions, is still unclear. We have previously reported in-cell crystal engineering to construct solid catalyst-containing enzymes and prepare the protein assemblies using protein crystals formed in insect cells.[2] In addition, we have found that polyhedra crystal (PhC), which is thought to crystallize only in insect cells, is also formed in E.coli. In this study, we attempt to elucidate the mechanism of in-cell protein crystallization using crystallization systems in E.coli for the design of solid materials (Figure 1).

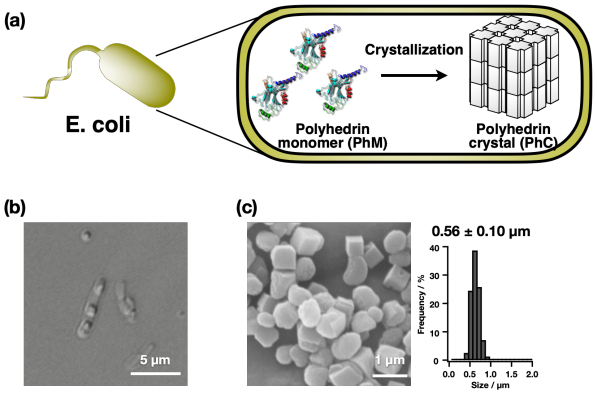

Figure 1. Crystallization of PhMs in E. coli. (a) Schematic representation of crystallization of PhM, (b) Phase-contrast image and (c) SEM image and a size histogram of recombinant PhCs purified from E. coli.

We elucidated the temperature- and time-dependent crystallization of polyhedrin monomers. The crystal formation was confirmed by scanning electron microscopy (SEM), small angle X-ray scattering (SAXS), and X-ray diffraction. SEM image of isolated PhC revealed the cubic crystals with an average size of 0.56 μm which is similar morphology to PhC formed in insect cells (Figure 1).. Time-dependent crystallization indicates that the cubic crystals were formed within 1h after IPTG induction, and crystal size was increased up to 6h. X-ray diffraction experiments showed that PhC grown for 4 and 6 h were analyzed, although PhC grown for 1 and 2 h could not be analyzed because of the small number of indexed images. Now, we are investigating the crystallinity of the PhC, especially, the initial stage of crystallization using SAXS.

1) M. Kojima et al, *Biomaterials Sci.* **2022**, *in press*.

2) T. Nguyen *et al. ACS Appl. Nano Mater*. **2021**, 4, 1672. S. Abe *et al. Angew. Chem. Int. Ed.* **2021**, 60, 12341.