In-situ observations of recrystallization processes of ice crystals including AFP type III

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Antifreeze proteins (AFPs) inhibit the ice crystal growth and fulfill an important role for the survival strategy of living organism in the cold region. Our interests are in learning about the fundamental mechanism of the protein functions by studying the ice crystal growth kinetics as well as the protein kinetics at the ice interface. Furthermore it is well known that AFPs improve the shelf life of frozen foods and so on [1]. In order to clarify its mechanism, we carried out in-situ observations of recrystallization processes affected by the existence of AFPs. In this research we used AFP type III labeled with fluorescein isothiocyanate (FITC) and carried out measurements of the grain size changes and the distributions of AFP molecules in the frozen polycrystalline ice.

To observe the recrystallization process, we prepared a thin ice sheet composed of small ice grains. The thin ice sheet was formed on a cover glass by the dropping method. A small water droplet including AFPs labeled by FITC was dropped on a cover glass chilled previously by liquid nitrogen, and then it was spread to make the thin water film on the glass and then quickly freeze. Controlling the falling height and the droplet size, we can obtain uniformly-flat ice sheet composed of small ice grains with the nearly same size and random orientations. The sample made by this method was put in a chamber maintained at a constant temperature just below the melting point and used as an initial state for the recrystallization processes of ice grains, and in-situ observations were carried out by switching the phase contrast and fluorescent microscopes.

Fig.1 shows the pictures of ice grains taken by phase contrast microscope ((a) and (c)) and fluorescent microscope ((b) and (d)). The concentration of AFP III in the droplet was 0.01mg/ml and the temperature of chamber was -1^{o}C. Pictures (a) and (b) were taken in 66 min after the initial state, and pictures (c) and (d) in 121 min. Average grain sizes were 13.0μm and 14.7 μm for both times, respectively and the recrystallization was strongly constrained. Fluorescent pictures clearly show that the protein molecules are found along the triple junctions of ice grain boundaries. Using this system, we try to analyze the recrystallization process in three dimensions. Analysis results will be given at the poster presentation.

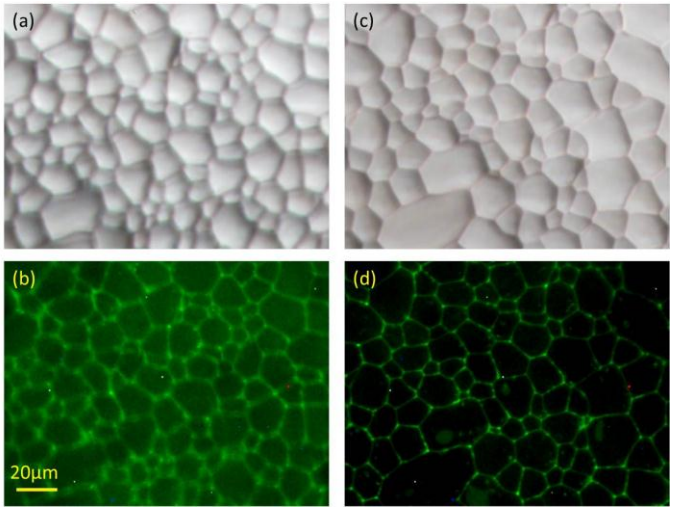

Fig. 1 Pictures of recrystallization processes of ice grains. In (b) and (d), the networks of triple junctions are clearly observed.

References

[1] Yin Yeh, R. Feeney: Chemical Reviews, 96, 601(1996)