

Direct visualization of effects of antifreeze protein by advanced optical microscopy: step bunching and oscillatory growth

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Effects of antifreeze protein (AFP) on the growth of ice crystals have been so far studied by mainly measuring normal growth rates of ice crystal faces (changes in the sizes of outer shapes of ice crystals). However, very recently, “elementary steps” (the growing ends of ubiquitous molecular layers with the minimum height) of ice crystals (0.37 nm in thickness) and their dynamic behavior could be visualized directly on ice crystal surfaces grown from vapor [1], by utilizing laser confocal microscopy combined with differential interference contrast microscopy (LCM-DIM) [2]. In this study, we further improved LCM-DIM, and tried to reveal the effects of AFP on the growth of ice crystals by directly observing dynamic behavior of “steps” that appeared on ice crystal surfaces grown from supercooled water.

Figure 1 shows a typical example of the in-situ observation of ice crystal surfaces growing in an aqueous solution of 0.01 mg/ml type III AFP. As the ice crystal surface grew, bunched steps that could not be observed at the beginning appeared (Fig. 1). This result demonstrates that AFP molecules adsorbed on the ice crystal surface prevented the lateral growth of steps and hence the steps were bunched.

Zepeda and coworkers recently reported that AFP promotes oscillatory growth of ice crystals [3]. On the ice crystal surface at which bunched steps appeared and then the growth of steps stopped, we could observe that two-dimensional nucleation occurred and then steps started to regrow again.

This kind of in-situ observation can become effective means to study antifreeze mechanisms.

1) G. Sazaki, et al., *PNAS*, **107**, 19702-19707 (2010).

2) G. Sazaki, et al., *J. Crystal Growth*, **262**, 536-542 (2004).

3) S. Zepeda, et al., *Cryst. Growth Des.*, **8**, 3666-3672 (2008).

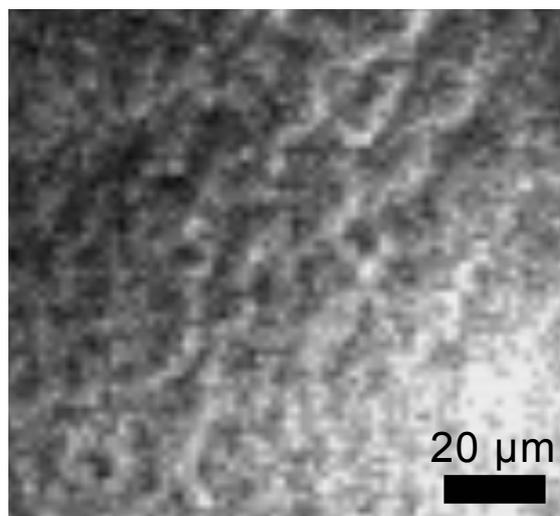


Fig. 1. Bunched steps formed by AFP on an ice crystal surface.