ICE CRYSTAL HABIT IN THE PRESENCE OF AN ANTIFREEZE PROTEIN FROM A SEA ICE DIATOM

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Antifreeze proteins (AFPs) from polar and cold-tolerant organisms are able to control ice growth as a result of their adsorption on the ice crystal surface. The proteins bind to selected crystallographic planes, which are characteristic for each kind of AFP. As a consequence of the adsorption, the freezing point of the solution is locally lowered below the melting point, following the Gibbs-Thomson equation. Within the difference between the melting and the freezing point (hysteresis gap), the macroscopic growth of the crystals is arrested. Below the freezing point, crystals grow with a burst of determined shapes. Their habit is dominated by the crystallographic planes inhibited by the proteins, due to the geometric adsorption selectivity as well as to the adsorption rate of the proteins. Most of the well-studied fish AFPs do not bind to the basal plane, therefore crystals burst as needles parallel to the c-axis. At strong supercoolings the protein adsorption rate is not fast enough to balance radial growth due to heat dissipation, and crystals develop dendrites. However, data about the shape of the crystals after the burst, and well below the freezing point, are still scarce, resulting in an incomplete understanding of the growth inhibition mechanisms by the different AFP types.

Here we present ice growth experiments in the presence of AFPs from the polar diatom *Fragilariopsis cylindrus*, a dominant microalga in sea ice assemblages. The protein was recombinantely expressed and purified from *E. coli*. Ice grain habit was observed at light microscopy in a dish, from the hysteresis gap until growth into a solid sample of polycrystalline ice. Crystals grew with an expansion of the basal plane area, but a suppressed development parallel to the c-axis compared to negative controls, resulting in thin ice “sheets”. A slow three-dimensional growth was revealed by the formation of characteristic features as pits and groves. Dendritic growth was limited, presumably among others an effect of the rapid heat dissipation through the thin sheet. The effect of the AFPs was still evident hours after the initial burst, as shown by analyses of the fully frozen polycrystalline block. Microtomed ice observed through crossed polarizers revealed marked microstructural features, which confirm a freezing history as a process of sheet after sheet freezing.

Our results suggest that proteins bind, among others, to the basal plane of crystals, as also proposed in previous studies. Analyses of the habit showed a crystal shape relatively stable over time, and explain the microstructural features in the polycrystalline sample. Furthermore, the studies about this kind of AFP contribute to complete the puzzle of growth inhibition by AFPs.