Maddalena Bayer-Giraldi, Ilka Weikusat, Cornelia Isert, Sepp Kipfstuhl

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

Ice growth in the presence of an antifreeze protein

Introduction

Antifreeze proteins (AFPs) are widely distributed in nature in cold-tolerant and cold-adapted organisms as polar fishes, cold-tolerant plants and fungi, sea-ice microorganisms. One of the main characteristics of AFP activity is their inhibition of ice recrystallization, i.e. of the growth of large crystals at the expenses of small grains. This effect suggests that the biological function of AFPs is to prevent mechanical damage of cells caused by large ice crystals.

The control of ice grain size by AFPs is of interest for potential applications of the proteins, that span from the medical sector to food industry and the design of technical, ice-free surfaces. Understanding the effect of AFPs on ice grain growth is of crucial relevance. Here we present a study of ice growth with microstructure observations in the presence of AFPs. We analyzed the effect of the protein from the polar diatom *Fragilariopsis cylindrus*, a dominant species within sea ice.

Protein isolation

We transformed one AFP isoform from *F. cylindrus* into *E. coli* for recombinant protein production. The protein was expressed as a fusion product with a His-tag and further tags to enhance protein solubility. We purified the AFPs with 2 chromatographic steps, separated by a digestion to remove the tags.

Fast freezing

Shock-freezing produced small crystals and AFPs from *F. cylindrus* clearly inhibited ice recrystallization. Frozen samples were annealed and observed by optical microscopy (Fig. 1) and using the Otago optical recrystallometer (fig. 2). In the presence of AFPs ice grains did not recrystallize but maintained their original, small dimensions.

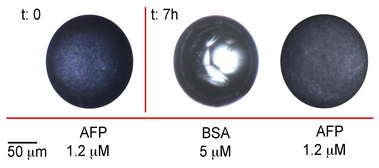
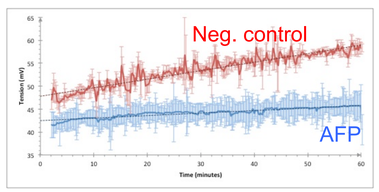


Fig. 1: Frozen sample drops of an AFP solution and a negative control (BSA) at optical microscopy. After shock-freezing the samples appeared optic thick. After 7 h at −4°C the negative control was clear due to recrystallization, whereas the AFP sample appeared still thick. Modified after Bayer-Giraldi et al. (2011)[1].

Fig. 2: Recrystallization measured by an optical recrystallometer. The measured tension is an expression for light transmitted through the sample, which increases with recrystallization.



Slow freezing

Slow freezing did not cause small crystals. Solutions of AFP and negative controls were frozen at −5°C in a Petri dish. Ice nucleation was induced by introducing small ice crytsals into the solutions as soon as the freezing point was reached, in order to avoid supercooling. Ice microstructure was observed through crossed polarizers and by optical microscopy.

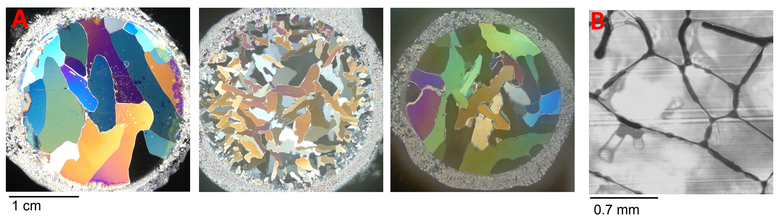


Fig. 3: Frozen solutions without AFPs, observed through crossed polarizers (A) and at optical microscopy (B). Samples were cut with a band saw and microtomed to obtain polished surfaces.

Ice grains without AFPs appeared delimited by clearly defined and smoothly curved boundaries, and homogeneous in size distribution within samples. Variations between samples were dependent on uncontrollable fluctuation in the freezing conditions.

In the presence of AFPs grains showed characteristic features:

1. one or few dominant large crystal (fig. 4A);
2. gradual transition in c-axis orientation within single grains (fig. 4A), and sometimes blurred grain boundaries;
3. smaller grains often needle-like, with sharply edged boundaries (fig. 4A, B);
4. Complex sublimation etching features (fig. 4B), typically delimited by grain boundaries indicating a crystal lattice dependence of etching, possibly due to incorporation of AFPs.

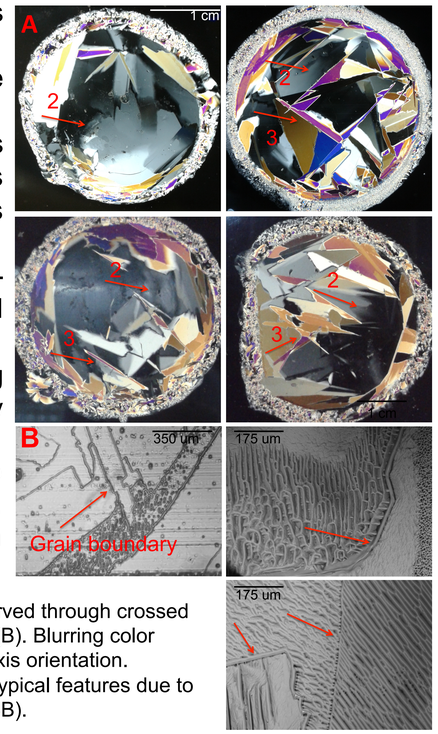


Fig. 4: Frozen solutions with AFPs, observed through crossed polarizers (A) and at optical microscopy (B). Blurring color values indicate gradual transitions of c-axis orientation. Numbered red arrows indicate selected typical features due to AFPs (fig. 4A) and grain boudaries (fig. 4B).

➡ AFPs from *F. cylindrus* are strong inhibitors of recrystallization, but do not cause small ice crystals at low freezing rates under the conditions applied here. Further analyses relating sublimation patterns caused by AFPs to crystal orientation may help to understand the mechanism of attachment of AFPs with respect to ice crystallography.

References: 1 Bayer-Giraldi et al. (2011) Characterization of an antifreeze protein from the polar *diatom Fragilariopsis cylindrus* and its relevance in sea ice, *Cryobiology* 63.

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ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG
HELMHOLTZ GEMEINSCHAFT

BREMERHAVEN
Am Handelshafen 12
27570 Bremerhaven
Telefon 0471 4831-0
www.awi.de