Antifreeze Proteins from a Sea Ice Diatom

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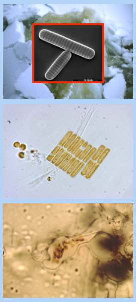
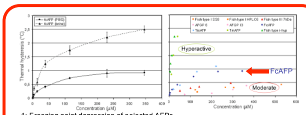
INTRODUCTION



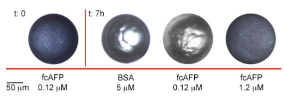
Polar sea ice is an extreme habitat with regard to its abiotic constraints as low temperatures and high brine salinities. However, several microorganisms proliferate within the ice and on its peripheries, thus creating an ecosystem of global significance.The diatom *Fragilariopsis cylindrus* is a dominant species in sea ice communities, and its recently found antifreeze proteins (AFPs) probably contribute to its success in ice. AFPs are proteins that bind to ice and influence its growth, lower the freezing point (freezing hysteresis) and inhibit recrystallization[1]. Here we present a characterization of an AFP from *F. cylindrus* (fcAFP) in a crystallographic frame and interpret our data in the context of sea ice.

ANTIFREEZE ACTIVITY

We used a fcAFP isoform shown to be active under conditions typical for sea ice and recombinantely expressed in *E. coli.* The protein showed moderate freezing hysteresis activity, further increased by salt addition (1). Moreover, FcAFP caused strong inhibition of recrystallization in shock frozen polycrystalline ice (2). The effect was monitored for 7 hours at -4°C.



1: Freezing point depression of selected AFPs.

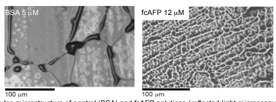


2: Protein solutions after shock freezing and after annealing for 7 hrs at -4°C.

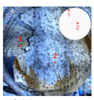
ICE MICROSTRUCTURE

Frozen fcAFP solutions showed marked influence of the protein on ice microstructure. FcAFP caused structures with oriented striations (3-4). A mushy structure, possibly with inclusions with brine and unbound proteins, may have formed due to irregularities in ice growth caused by the bound fcAFPs.

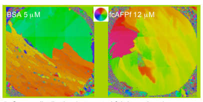
Moreover, the effect of fcAFPs caused gradual c-axes transitions and irregular grain boundaries (5).



3: Ice microstructure of control (BSA) and fcAFP solutions (reflected light microscopy).



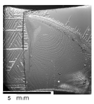
4: Striations are perpendicular to the c-axis (image through cross polarizers).



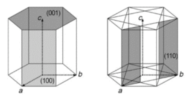
5: C-axes distribution (automated fabric analyzer).

INTERACTION WITH ICE

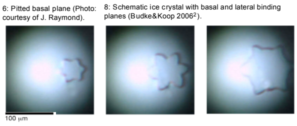
Submersion of an ice crystal into a solution of fcAFP at temperatures below the freezing point caused dense pitting formation on the basal plane (6). Crystals growing in the presence of fcAFPs were star-like (7), while control showed a disc-like habit. These results suggest that fcAFPs bind on the basal plane and the lateral faces of ice crystals (8).



6: Pitted basal plane (Photo: courtesy of J. Raymond).



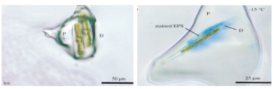
8: Schematic ice crystal with basal and lateral binding planes (Budke&Koop 2006[2]).



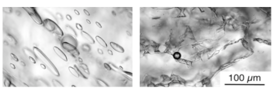
7: Ice crystal growing in a solution of fcAFP.

FcAFP IN SEA ICE

Physiological protein concentration measured by immuno-blotting in cells was too low for an effective freezing hysteresis and recrystallization inhibition.



9: Diatoms in a brine pocket surrounded by EPS (Krembs at al. 2002[3]).



10: Brine pockets in artificial sea ice without (left) and with EPS (right) (Krembs et al. 2011[4]).

FcAFPs may accumulate around cells in an envelope of exopolymeric substance (EPS), made of polysaccharides and proteins (9). The protein fraction was shown to be effective in influencing sea ice structure, increasing porosity and retaining brine (10)[4].

CONCLUSIONS

FcAFPs have strong antifreeze activity, that may be of interest for industrial applications. In sea ice, physiological concentrations are too low to have any relevant antifreeze acitivity. However, fcAFPs may accumulate in EPS and reach high concentrations. In contact with ice, fcAFPs may create a mushy structure as in picture 3, forcing the formation of brine pores and thus creating an environment where sea ice organisms can locate and survive.

REFERENCES:
1 Kristiansen, Zachariassen (2005): The mechanism by which fish antifreeze proteins cause thermal hysteresis, Cryobiology;
2 Budke, Koop (2006): Ice recrystallization inhibition and molecular recognition of ice faces by Poly(vinyl alcohol), ChemPhysChem;
3 Krembs, Eicken, Junge, Deming (2002): High concentrations of exopolymeric substances in wintertime sea ice: Implications for the polar ocean carbon cycle and cryoprotection of diatoms, Deep-Sea Res;
4 Krembs, Deming, Eicken (2011): Exopolymer alterations of physical properties of sea ice and implications for brine habitability and biogeochemistry in a warmer Arctic, PNAS.