Adsorption Rate of Antifreeze Proteins Determines Their Ice Growth Inhibition Activity

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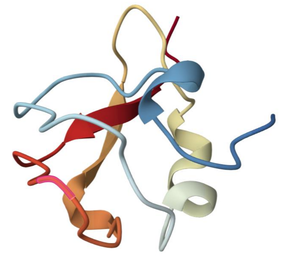
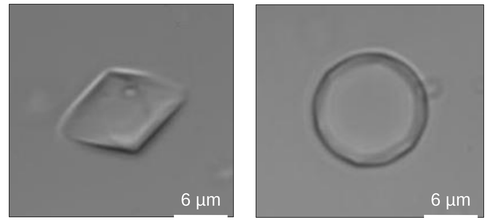
ABSTRACT

As a cold-variation mechanism, antifreeze proteins/peptides (AFPs) or antifreeze glycopeptides (AFGPs) are produced by various organisms for protection against freezing injury. This study delves into the unique properties of AFPs and AFGPs and their impact on the nonequilibrium freezing point, specifically exploring the phenomenon of thermal hysteresis (TH) as a metric for the specific activity of these proteins. TH measurements were conducted using a nanoliter osmometer to elucidate the concentration-exposure time-activity relationship. Antifreeze Proteins (AF(G)Ps) inhibit ice growth via an adsorption-inhibition mechanism that assumes irreversible binding of AFGPs to embryonic ice crystals and the inhibition of further growth. Using fluorescence microscopy to measure the adsorption rate of the AFGP, this study reveals an experimental link between TH activity and adsorption rate.



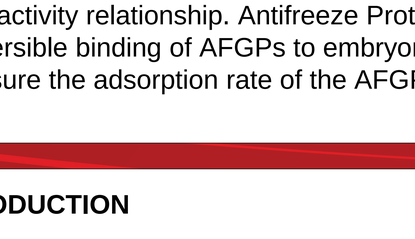
Type III Antifreeze Protein



Ice Crystals Growing in AFP Solutions

INTRODUCTION

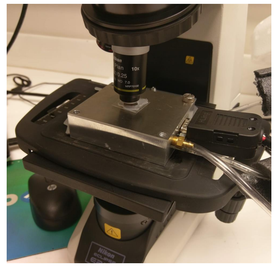
- AFPs are specific proteins, glycopeptides, and peptides made by different organisms to allow cells to survive in sub-zero conditions (Berger et al., 2019).
- Binding of AFP to ice results in a separation between melting and freezing points of ice crystals (thermal hysteresis, TH) (Drori et al., 2014).
- Concentration of AFP, ambient temperature and exposure time may affect the activity of AFP, resulting in changes in TH (Deng et al., 2020).
- The rate of AFP accumulation on ice is determined by an interplay between AFP diffusion from the bulk solution to the ice−water interface and the subsequent adsorption of AFPs to the interface (Thosar et al., 2023).



- Absorption rate is the slope of the linear curve of the AFP concentration vs. the inverse of the time constant τ. Concentration of AFP is 22.5 mg/mL. The data was fitted using eq 1. Scale bar = 10 μm (Meister et al., 2018).

METHODOLOGY

TH measurements were performed using a nanoliter osmometer (below).



TH activity is the difference between the melting point and freezing point (burst point).

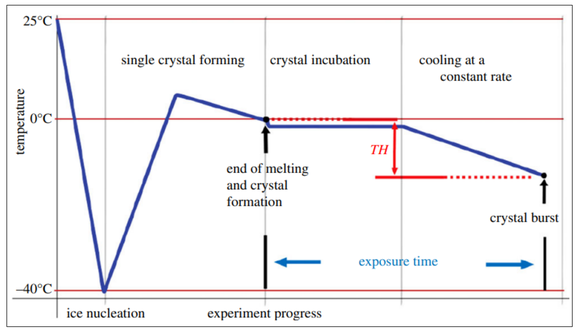


Figure 1. Stages of the measurement are indicated by text. The sample temperature in the nanoliter osmometer is represented by the bold line. TH is indicated by the double-headed vertical arrow.

$$I = I_{\max}\left(1 - e^{-\left(\frac{t}{\tau}\right)}\right)$$

Equation 1. Used for non-linear fitting in determining adsorption rates. *I* is the fluorescence intensity at time t, *I*max is the maximum intensity obtained, and τ is a time constant, $\tau = k_{on}C^{-1}$, k_{on} is the adsorption rate, and C is the AFP concentration.

FINDINGS

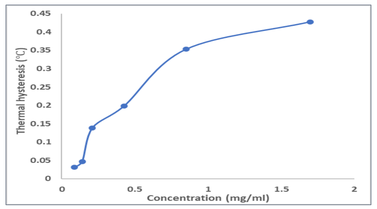


Figure 2. Concentration-Activity (TH)
TH in AFPIII-dimer decreases as the solution was diluted.

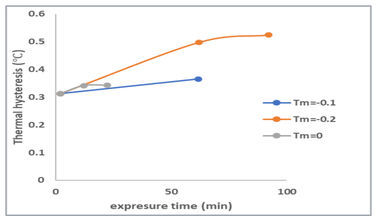


Figure 3. Exposure time-Activity (TH)
TH increases with increasing exposure time.

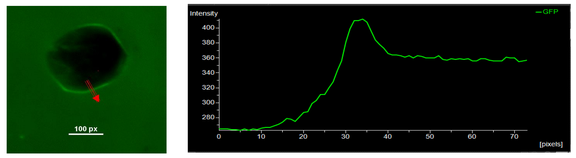


Figure 4. The fluorescence intensity across the ice surface was measured (red line) and plotted.

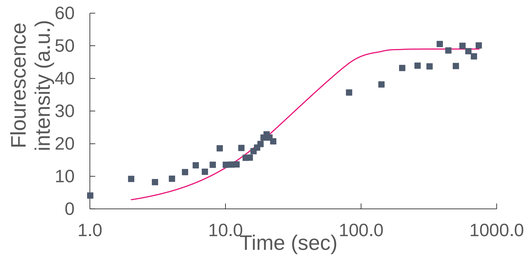


Figure 5. Accumulation of AFP on ice surface during the experiment is presented (maximum value of each frame) and fitted using Eq.1.

CONCLUSIONS & RECOMMENDATIONS

- Through the corresponding function diagram, it can be found that increasing the concentration and prolonging the exposure time can help increase the activity of ice-binding proteins.
- The scope of the study will be expanded in future studies by measuring other types of AFP samples and extending the concentration-exposure time-activity relationship analysis to mixed AFP samples.
- In the process of measuring the adsorption rate, it is necessary to strictly control the influence of irrelevant factors, such as background noise and accurate focus.

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