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A Biological Model for Controlling Interface Growth and Morphology

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Materials and Process Modeling and Computation

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Abstract

Biological systems create proteins that perform tasks more efficiently and precisely than conventional chemicals. For example, many plants and animals produce proteins to control the freezing of water. Biological antifreeze proteins (AFPs) inhibit the solidification process, even below the freezing point. These molecules bond to specific sites at the ice/water interface and are theorized to suppress solidification chemically or geometrically. In this project, we investigated the theoretical and experimental data on AFPs and performed analyses to understand the unique physics of AFPs. The experimental literature was analyzed to determine chemical mechanisms and effects of protein bonding at ice surfaces, specifically thermodynamic freezing point depression, suppression of ice nucleation, decrease in dendrite growth kinetics, solute drag on the moving solid/liquid interface, and stearic pinning of the ice interface. Stearic pinning was found to be the most likely candidate to explain experimental results, including freezing point depression, growth morphologies, and thermal hysteresis. A new stearic pinning model was developed and applied to AFPs, with excellent quantitative results. Understanding biological antifreeze mechanisms could enable important medical and engineering applications, but considerable future work will be necessary.

Contents

Introduction

Biology and technology often deal with similar problems. For example, controlling the morphology of a solidifying surface is important in biological systems that freeze, like frost-tolerant plants, and in technological systems that freeze, like die-casting. But where technological solutions are often brute-force (e.g. mechanical mixing), biological solutions are usually elegant, specific, and robust (e.g. tailored surfactant proteins). Because of this, technological solutions are subject to detrimental side effects, such as mixing-induced inhomogeneities, that biological systems avoid. In this project, we develop a preliminary understanding of biological strategies for regulating freezing in order to inform the technology of solidification.

Biological antifreeze proteins (AFPs) are found in coldwater fish, overwintering frogs, frost-tolerant plants, insects, etc. AFPs take many shapes – linear, helical, or equiaxed – but they share a few features in common. They are of high molecular weight, comprised mainly of hydrophobic groups, and they include a small, hydrophilic region. Because AFPs bond to specific sites on the ice surface, they are effective in very small concentrations. AFPs influence freezing in several important ways. First, they enable ice and water to co-exist at temperatures below the melting temperature. How they achieve this is not known, but it is not by conventional melting-point depression, as they are present in far too small a concentration for solution effects. Moreover, when freezing does occur, AFPs slow and alter the ice growth mode, usually causing *c*-axis (basal plane) growth facets. AFP-mediated ice growth is gentler to cell walls than natural ice growth, causing minimal residual damage upon melting. Finally, the AFP effect is not reversible. While water containing AFP freezes below its normal melting temperature, ice containing AFP melts at its normal melting temperature; that is, AFP causes thermal hysteresis [1,2].

Is it feasible to apply the AFP model to control solidification in engineered systems? Due to economics and high temperature instability, we cannot hope to synthesize protein molecules to control metal casting. However, there is some evidence that the effects of these molecules are not predicated on their protein nature, but on more general chemical features, such as their size and chemical bonding characteristics. To examine these aspects of the AFP phenomenon, we applied our knowledge of the thermodynamics and phenomenology of solidification to understand biological solutions for mediating ice crystal formation and growth. The efforts in this one-year project focused around a search of the biological antifreeze protein (AFP) literature, and an analysis of current data and models using materials science tools. We studied five proposed AFP mechanisms: thermodynamic freezing point depression, suppression of ice nucleation, decrease of dendrite growth velocities, solute drag on the moving solid/liquid interface, and stearic pinning of the ice interface. While much critical information is lacking, we were able to eliminate some hypotheses and corroborate others, improving our understanding of these complex systems.

Freezing Point Depression and Nucleation Suppression

A literature search conclusively revealed that thermodynamic freezing point depression and/or suppression of ice nucleation are insufficient to explain the dramatic effects of AFPs on freezing (c.f. [1,3]). The undercoolings observed (up to 1.9°C in polar fish, for example) are far too large for solution thermodynamics to account for. Furthermore, at these undercoolings, the critical nucleus size of ice particles is small, and numerous heterogeneous nucleation sites exist, so ice crystal nucleation will be substantial, even if AFPs inhibit a particular nucleation mode. Both of these mechanisms were eliminated from further study

Dendrite Growth

Solidification in an ice crystal proceeds by the advance of a six fold symmetric array of dendrites where the growth directions of the dendrite tips lie in the basal plane of the hexagonal unit cell (i.e. a snowflake). The generally accepted physical picture of dendrite growth is the so-called microscopic solvability theory [4,5]. Microscopic solvability predicts that the operating point of a dendrite, that is its growth velocity and tip radius, is determined by the anisotropy in the solid-liquid interfacial energy, γ. The crystallographic orientation dependence of γ can be written as: $\gamma = \gamma_o\left[1 + \varepsilon_m \cos(m\theta)\right]$ where γ_o is the interfacial energy averaged over all orientations and ε represents the typically small anisotropy. If the angle θ is measured within the basal plane, then m=6, reflecting the six-fold symmetry, and Koo et al. [6] have measured the ice-water anisotropy parameter ε_6 to be quite small, approximately 0.002. If θ is measured normal to the basal plane, then m=2 and $\varepsilon_6 \approx 0.3$. A detailed summary of dendrite growth theory is beyond the scope of this report; however, for the purposes of the present discussion, the important conclusion from microscopic solvability theory is the fact that the dendrite growth rate is very sensitive to the small anisotropy, and the lower the value of ε the lower is the growth rate.

Microscopic solvability theory offers an intriguing possibility as to the mechanism of AFPs. If the proteins segregate preferentially to certain crystallographic planes on the ice-water interface, then, by the Gibbs adsorption theorem, the interfacial free energies of those planes will be decreased. A decrease of γ on some crystal planes leads to the possibility of a decrease in the anisotropy and hence a suppressed dendritic growth velocity. There exists some evidence that AFPs segregate more strongly to certain planes. Haymet et al. [7] have shown that the winter flounder peptide "HPLC6" segregates most strongly to the (2,0,-2,1) plane of the hexagonal structure, which would lower the ε_2 parameter. Moreover, an alanine mutant was found to accumulate on the (2,-1,-1,0) plane, which would suggest a lowering of the ε_6 anisotropy.

The suppression of ice growth rate via the suppression of anisotropy is consistent with general observation of AFP behavior. The mechanism acts on the growth rate and does not affect, to a large extent, the nucleation kinetics. Furthermore, the anisotropy explanation is irreversible. On melting, where dendrites do not form, the solid-liquid interface velocity is no longer slowed by the AFP accumulation. Although dendrite growth theory offers a plausible explanation for the action of AFPs, much more research is required to adequately test the idea. Atomistic simulations to compute γ and its anisotropy, as well as detailed phase field simulations to model the solidification behavior in ice-water, are necessary.

Solute Drag

It is now well established that AFPs act to significantly slow the growth of ice crystals within the blood stream [3]. Thus, we investigated whether the growth rate suppression can be explained by the phenomenon of solute drag. It is well known within the metallurgical literature that concentrations of impurities as low as the parts per million level can significantly reduce the rate of grain boundary migration in alloys.

A generally accepted theory of solute drag was proposed by Cahn [8] and his formula for the velocity of the solid-liquid interface (V) in the presence of a solute drag effect is given by:

$$P = \lambda V + \frac{\alpha C_0 V}{1 + \beta^2 V^2} \tag{1}$$

The driving force P is the chemical potential difference between solid and liquid, □□, which can be converted into an undercooling ΔT via:

$$P \equiv \Delta\mu = (L/T)\Delta T \tag{2}$$

where L is the latent heat per unit volume and T is the temperature. Thus, the Cahn expression becomes:

$$(L/T)\Delta T = \lambda V + \frac{\alpha C_0 V}{1 + \beta^2 V^2} \tag{3}$$

The parameter λ is a mobility in the absence of solute drag. For this mobility we have used a theory due to Mikheev and Chernov [9], which has been shown to be an accurate description of molecular dynamics results for the kinetic coefficient in simple metal systems [10]. In this theory, λ is given by:

$$\lambda = 1.6 N_V (k_B T)\sqrt{m/k_B T} \tag{4}$$

where N_v is the number of atoms per unit volume, k_B is the Boltzmann constant, and m is the atomic mass. Note that the square root term is the reciprocal of the thermal velocity.
The term α in equation (1) is:

(5)

where the integral is over the liquid side only because we assume no diffusion in the solid. Furthermore, the diffusion coefficient $D(x)$ is a constant, and for typical proteins in water D is about 1×10^{-6} cm^2/s. For the interaction energy, we chose the same linear dependence as Cahn:

$$E(x) = E^0 - (E^0/b)x \tag{6}$$

where $b = 10 \times 10^{-8}$cm. For the ice-water system, Haymet et al. [7] comment on the E^0 term as follows: ". . . its magnitude can be confidently estimated to be small, at most a few tenths of a kcal/mole." In the results to follow we have used -1 kcal/mole.

The term β in equation (1) is defined as:

$$\frac{\alpha}{\beta^2} = \frac{N_v}{k_B T}\int_0^\infty \left(\frac{dE}{dx}\right)^2 D(x)dx = \frac{N_v D}{k_B T}\frac{(E^0)^2}{b} \tag{7}$$

Finally, C_o in equation (1) is the concentration of AFP in solution and was taken as $C_o = 1\text{x}10^{-6}$.

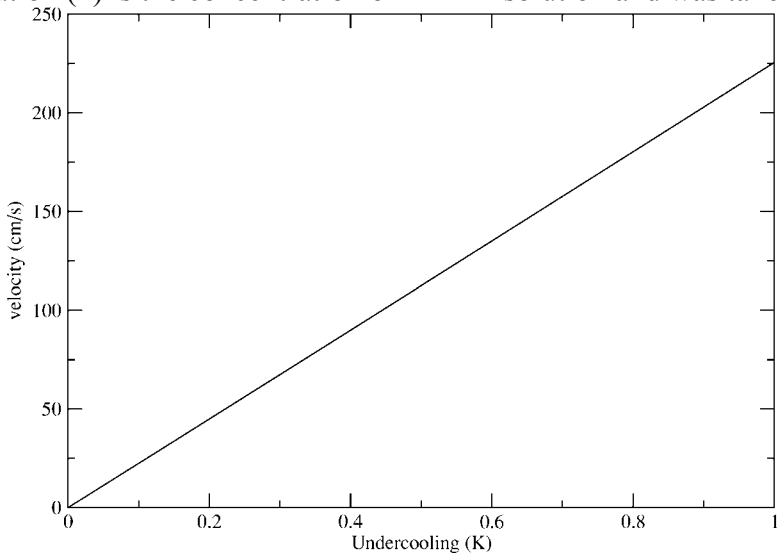

Figure 1. Interface velocity versus undercooling for an AFP/water system, as computed using the Cahn theory of solute drag. The linear relationship across the undercooling range indicates little, if any, effect of solute drag on the solid/liquid interface velocity.

Inserting these results into equation (1), we calculate the velocity of the solid interface as a function of the undercooling, as shown in Figure 1. Because the linear relation between velocity and driving force is reproduced even for very small undercoolings, there is no evidence of solute drag as an antifreeze mechanism. In fact, at undercoolings approaching those observed experimentally (≥ 1 K), the solidification velocity is very large (>200 cm/s). Even when the parameters are varied to their reasonable limits, little drag effect is observed. For example, Figure 2 shows velocity versus undercooling when the interaction energy is increased to a substantial -5 kcal/mole. While there is some drag effect, it occurs at extremely low undercooling (note the change in the *x* axis dimensions).

These results reveal that solute drag cannot explain the action of AFP. The shift of the velocity vs. undercooling relationship with solute drag relative to the intrinsic rate is very small, much less than 0.1 C. It appears the concentration is too low and/or the binding energy too small to explain the very slow ice growth rates observed in nature. This absence of solute drag in solidification is consistent with the current thinking in metal systems, for example the experiments of Aziz [11].

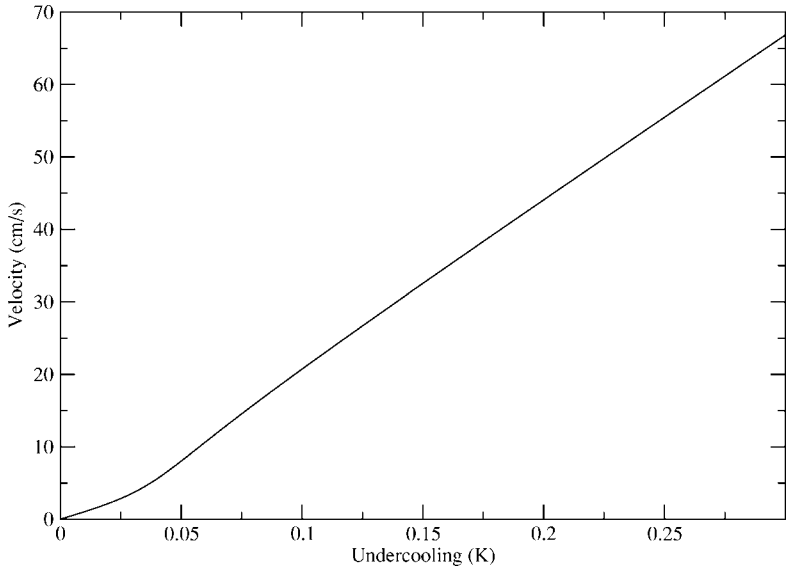

Figure 2. Interface velocity versus undercooling for an AFP/water system, computed using a very high interaction energy in the Cahn theory of solute drag. While non-linearity indicates some solute drag at very small undercoolings, the effect is quite small.

Stearic Pinning

Some biologists propose that AFPs inhibit solidification due to their size rather than their chemistry [1]. To move past the array of large proteins, the solidifying surface must increase in area, which costs energy. Materials scientists are familiar with such 'particle pinning' in a variety of systems. However, AFP systems are different from most metallurgical solidification situations [c.f. 12], since the AFPs chemically bond with the ice surface, and are not pushed ahead or rejected by the ice. In this case, it is the energetic cost of engulfing the AFPs, and not the kinetic inhibition of pushing particles, that limits solidification. Therefore, we apply metallurgical theory for a boundary moving past a rigid particle, rather than the theory of particle pushing, to this problem.

Developing an analog of the Smith-Zener theory for particle pinning in solids [13], we analyzed geometric pinning by AFPs as a function of molecular size and concentration, for two extremes in the surface properties of the AFPs. The driving pressure for solidification is given by equation (3). Following Smith and Zener [13], we note that pinning force arises from the decrease in solid-liquid interfacial area at the AFP intersection. Assuming that each particle on the boundary exerts maximum pinning force on that boundary, the total pinning pressure per unit volume is

$$P_{pin} = N_A \pi \gamma r \tag{8}$$

where N_A is the number of AFP molecules per unit interfacial area, γ is the surface energy of the solid-liquid interface per unit area, and r is the AFP radius of gyration. By balancing the driving force with the pinning force, we conclude that the interface stagnates when

$$\frac{L}{T}\Delta T = N_A \pi \gamma r \tag{9}$$

To examine the weakest pinning situation, we assume AFPs contact the interface at random. While we do assume that AFPs 'stick' upon contact with the interface (and are not pushed ahead of it), we do not suppose that AFPs segregate preferentially to the interface. In this case, again following Smith and Zener, we may write

$$N_A = \frac{3}{2}\frac{f_v}{\pi r^2} \tag{10}$$

where f_v is the volume fraction of AFPs in solution. In the dilute limit, where the AFPs occupy negligible volume per mole of water, we find

$$f_v = \frac{4\pi}{3} C_o A r^3 \tag{11}$$

where A is Avogadro's number. (The correction for non-dilute systems is straightforward to calculate.) Combining equations (9), (10) and (11) and solving for undercooling, we find

$$\Delta T = \frac{2\pi A T \gamma}{L} C_o r^2 \tag{12}$$

Note that the predicted undercooling at which the ice/water interface stagnates increases with C_o as observed in experiment [1] and with the AFP size. Using L=333 MJ/m^3, T=273 K, and γ=33 mJ/m^2, we calculate undercooling as a function of AFP concentration and protein size as shown in Figure 3. Even when the AFP particles are only randomly correlated with the interface, their presence is sufficient to provide substantial undercooling at small (parts per million) levels. This is the first quantitative analysis of AFP mechanism to show realistic undercooling, and it does so with a minimum of physical assumptions.

To consider the opposite regime, we assume site saturation of AFPs on the ice surface. In site saturation, AFPs occupy all possible bonding sites at all times; this may occur due to preferential bonding or at AFP concentrations large enough to fill all surface sites. In that case, the number of AFPs per unit area N_A becomes the number of bonding sites per unit area on the ice surface, which is a constant, B. Then, equation (12) becomes

$$\Delta T = \frac{\pi B T \gamma}{L} r \tag{13}$$

The undercooling is now independent of the AFP concentration and is linearly dependent on protein radius. An approximate calculation of B assumes that one site in 400 ice unit cells can accommodate an AFP (reasonable to avoid protein overlap at these radii). Since AFPs bond to the prismatic facets of ice, the unit cell area is given by the product of the basal lattice constant, a=4.54Å, and the prismatic lattice constant, c=7.32Å. Using these values, we find B=7.5x10^{15} m^{-2}. Figure 4 shows undercoolings for typical protein radii; these undercoolings are rather large compared to experimental observations. Of course, adjusting the site occupancy up or down can modify these values proportionally.

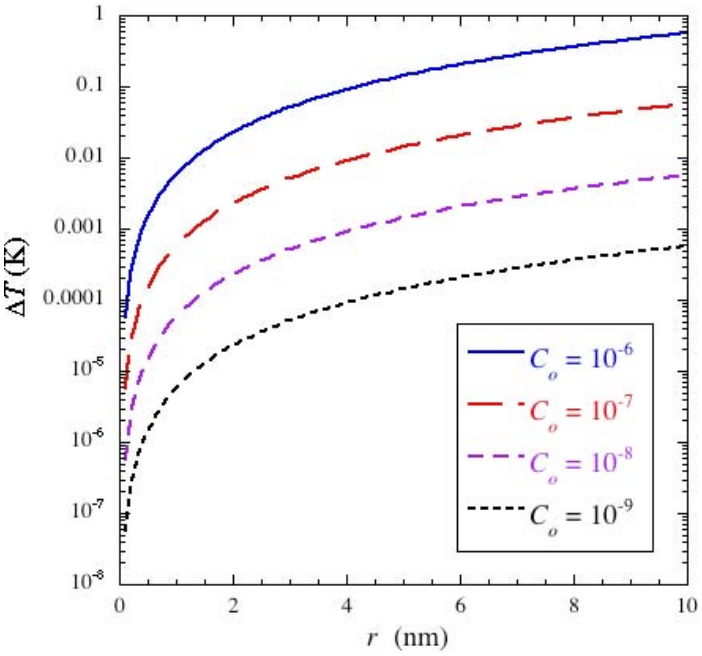

Figure 3. Undercooling as a function of AFP concentration C_o *and radius* r *for stearic pinning by random AFPs. Attainable undercooling increases with* C_o *and* r. *Note that the undercooling values are consistent with experiment.*

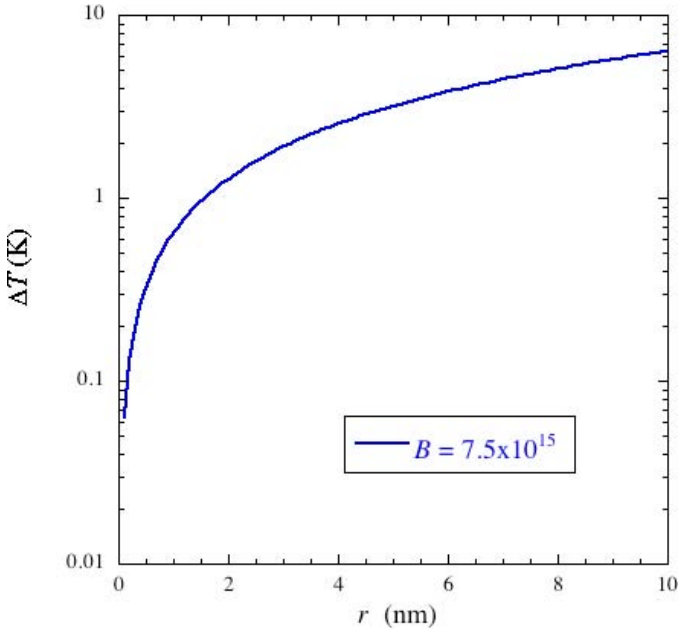

Figure 4. Undercooling as a function of AFP radius r *for stearic pinning by site-saturated AFPs. Attainable undercooling increases with* r. *Note that the undercooling values are higher than observed in experiments.*

Comparison of the random and site saturated models would suggest that for typical AFP systems, the AFPs do not occupy all possible surface sites. In that case, the random model applies and undercooling depends on both AFP concentration and size as observed in experiments. However,

at high AFP concentrations or for particularly chemically active AFPs, the site saturated model provides an upper limit for attainable undercooling.
It is commonly observed in AFP systems that growth is inhibited only to a certain undercooling; at lower temperatures, needles of ice grow rapidly. The stearic pinning model explains that result, since once the driving force (i.e. the undercooling) becomes large enough, the advancing ice surface engulfs its AFP particles and is able to grow unconstrained. Note that at large undercoolings, unconstrained growth will be very fast indeed, as shown in Figure 1.

Thermal hysteresis also follows naturally from the stearic pinning model. During freezing, the advancing ice front must increase its surface area (and thus its energy) in order to engulf the attached AFP particles, so the temperature must decrease to provide an additional driving force. However, upon melting, the front reduces its energy by ejecting the AFP molecules and so can melt normally.

Finally, we note that two aspects of the chemical nature of the AFP molecule are important to this theory. First, the hydrophilic portion of the molecule allows it to bond to the ice surface, which enables pinning by preventing pushing during solidification. Second, the hydrophobic portion of the molecule must have a similar surface tension with respect to ice and liquid water. If the molecule has a low surface tension with ice, it will be engulfed easily during solidification. Conversely, if it has a low surface tension with liquid, it will detach from the ice surface in order to remain in solution. Experimental studies indicate the AFPs typically meet both of these chemical requirements [1].

Conclusions

Although AFPs were discovered 30 years ago, they remain poorly understood. This stems, in part, from a focus on local chemistry rather than on system properties. In this project, we applied thermodynamic and geometric concepts from materials science to the problem of AFP-mediated freezing for the first time. Using this materials science approach, we provide new, physical insight into freezing point depression, growth regulation, and thermal hysteresis. Specifically
:

- We confirmed via a literature search that thermodynamic freezing point depression and/or suppression of ice nucleation are insufficient to explain the dramatic effects of AFPs on freezing.
- We have speculated that AFPs act to lower the anisotropy in the ice-water interfacial free energy, thereby lower the growth rate of dendrites. More research is needed to validate this model.
- We applied the Cahn solute drag model to prove that growth rate suppression cannot be explained by the phenomenon of solute drag.
- We developed a stearic pinning model, based on the Smith-Zener theory, to show that geometric effects may cause the experimentally observed freezing point depression, growth morphologies, and thermal hysteresis.
-

Future Work

For both medical and food applications, it is important to be able to freeze biological tissues gently, with minimal damage to cell walls. This requires controlling the solidification process to

maintain certain growth rates and facets, as AFPs do. Improving the biological freezing process could enable longer storage life for blood (currently 45 days), freezing organs for transport or storage (organs cannot currently be frozen), and refining the texture of frozen foods.
If the biological model can be applied to technological systems, a variety of technologies will be impacted as well. Casting of metals could be improved, with greater process control and product homogeneity. Detrimental impurities used to control growth morphology could be eliminated from electrodeposition processes. Vapor deposition processes could yield smoother, denser thin films. Sandia has a vested interest in all of these areas, for conventional weapons components like nose cones (casting), microsystems devices (LIGA electrodeposition), and electronic components (film deposition).

As materials science informs biology, so biology can inform materials science. If we can understand how biology controls freezing so elegantly, we can hope to apply our insight to technological systems. From a biological perspective, this will require more experimental data on AFP size and structure, on site occupancy on ice surfaces, and on freezing kinetics and morphology. From a materials science perspective, insight into how protein molecules bond to the ice interface may aid in the development of new solidification modifiers.

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