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Understanding freeze stress in biological tissues: Thermodynamics of interfacial water

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Abstract

A thermodynamic approach to distinguish forms of freeze energy that injure plants as the temperature decreases is developed. The pattern resulting from this analysis dictated the sequence of thermal requirements for water to exist as an independent state. Improvement of freezing tolerance in biological systems depends on identification of a specific form of stress, just as control of a disease depends on identification of the pathogen causing the disease. The forms of energy that stress hydrated systems as temperature decreases begin with disruption of biological function from chill injury that occurs above freezing. Initiation of non-equilibrium freezing with sufficient free energy to drive disruptive effects can occur in a supercooled system. As the temperature continues to decrease and freezing occurs in an equilibrium manner, adhesion at hydrated interfaces contributes to disruptive effects as protoplasts contract by freeze-dehydration. If protective systems are able to prevent injury from direct interactions with ice, passive effects of freeze-dehydration may cause injury at lower temperatures. The temperature range in which an injury occurs is an indicator of the form of energy causing stress. The form of energy is thus a primary guide for selection of a protective mechanism. An interatomic force model whose response to temperature change corresponds with the enthalpy pattern might help define freeze stress from a unique perspective.

Keywords: Freezing stress; Ice adhesion; Activation energy; Latent heat; Equilibrium freezing; Non-equilibrium freezing

1. Introduction

Several different forms of stress, each causing a symptomatic injury, were identified from an analysis of freezing in plants [1]. This analysis was based on monitoring the content, distribution, and phase of water at successively lower temperatures. When plants are suddenly frozen while in a supercooled condition the rate of freezing can be used to characterize non-equilibrium processes that injure plants in the initial stages of freezing [1]. Processes distinct from those characterized by kinetics are driven by thermodynamics as the plant gradually cools after the initial freezing event [2].

Improvement of freezing tolerance in biological systems depends on identification of a specific form of stress, just as control of a disease depends on identification of the pathogen causing the disease. The forms of energy that stress hydrated systems as temperature decreases begin with disruption of biological function from chill injury that occurs above freezing. Initiation of non-equilibrium freezing with sufficient free energy to drive disruptive effects can occur in a supercooled system. As the temperature continues to decrease and freezing occurs in an equilibrium manner, adhesion at hydrated interfaces contributes to disruptive effects as protoplasts contract by freeze-dehydration. If protective systems are able to prevent injury from direct interactions with ice, passive effects of freeze-dehydration may cause injury at lower temperatures. The temperature range in which an injury occurs is an indicator of the form of energy causing stress. The form of energy is thus a primary guide for selection of a protective mechanism.

A thermodynamic approach has been used to distinguish a form of stress called adhesion from dehydration [3]. Dehydration is a more passive stress than adhesion and causes less tissue distortion than the disruption caused by partial adhesion of cells to ice crystals. As freezing progresses, other stresses within the

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water interface between hydrophilic plant substances and ice, cause ice crystals to become a major new histological feature in winter hardy plants.

Stresses that develop in interfacial water can be analyzed by thermodynamics. The simplest model for interfacial water is based on the interaction of liquid water with ice. A steady state of freezing and melting (recrystallization) occurs in pure water under adiabatic conditions at 273 K resulting in a change of ice crystal size and shape while a constant proportion of ice to liquid is maintained [4]. When the temperature is lowered, freezing predominates until competitive interaction occurs between ice crystals with non-matching lattices, which modifies the interfacial water and restores balance to the system.

Although energy transitions do not define the forces that stress living systems, realistic molecular stress dynamics have to be consistent with thermodynamic responses to temperature change [5]. This study is a thermodynamic analysis of interfacial water as freezing progresses in living tissue. It is intended to provide a new starting point for an investigation of freezing processes in living tissue using statistical mechanics analyses developed by Gibbs [6,7].

2. Methods and results

At 273 K when ice and liquid water are in a steady state of melting and freezing, latent heat is the result of a “hot” ice molecule leaving the ice lattice, exchanging kinetic energy for potential energy and becoming a “cold” liquid molecule. The difference between these two energies is the latent heat and the upper and lower limits of latent heat can be defined as activation energies [8] (Fig. 1). Ice molecules at the liquid interface that have translational kinetic energy greater than the activation energy of melting (upper limit of latent heat) are the only ones able to make the transition from ice to liquid, and only liquid water molecules with translational kinetic energy less than the activation energy for freezing (lower limit of latent heat) can make the transition from liquid and become ice [8]. Activation energies of freezing and melting are also the translational kinetic energies of chemical resonance between interfacial water and ice (Fig. 2). Free energy drives the system toward this resonance which produces the most stable state.

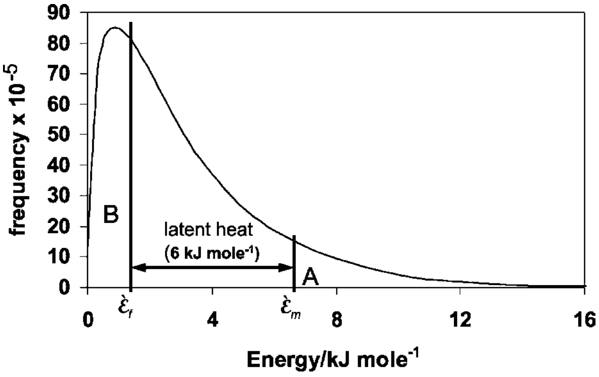

Fig. 1. Maxwell–Boltzman distribution of the translational kinetic energy of water molecules at 273 K (adapted from [8]). The region “A” represents ice molecules with enough energy to escape an ice lattice (melt) and “B” represents liquid molecules with energy so low that they will add to the lattice (freeze). During equilibrium freezing the energy represented by the two integral moments A and B will be equal. The vertical line at “E_m” is the minimum translational kinetic energy that ice molecules must have to escape the lattice (activation energy of melting) and “E_f” is the maximum energy a liquid molecule can have and still add to the lattice (activation energy of freezing). Latent heat is the distance between E_m and E_f and is the total energy which must be acquired by a molecule to escape the ice lattice, or the amount of energy it gives up as it becomes part of the lattice.

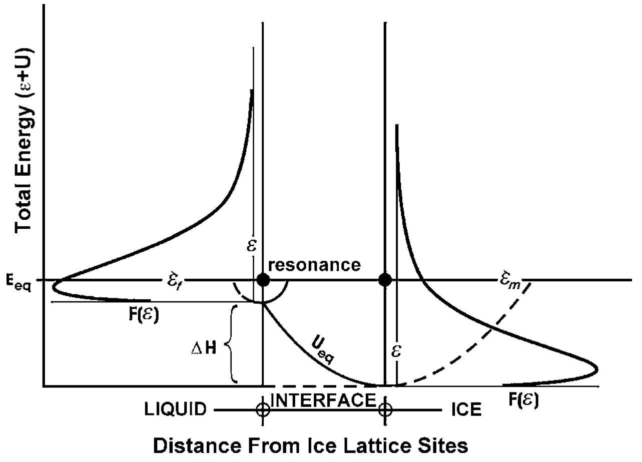

Fig. 2. Resonance of a perfect water molecule. This is a cartoon of an ice–liquid water interface as a simplistic abstraction of resonance for a Gibbs microcanonical distribution of interfacial molecules [6,7]. Under these conditions a perfect water molecule will have the same total translational kinetic and potential energy at the equilibrium transitional energy of the canonical distribution. This figure is essentially a separation of frequencies of kinetic energy for freezing and melting that was illustrated by a single distribution in Fig. 1. Resonance is illustrated by a perfect water molecule located at either of two opposing sites (black dots) in the interfacial force field of latent heat. This molecule translationally oscillates by exchange of kinetic energy for potential energy, and then back to kinetic energy. The amount of energy represented by the resonating molecule remains constant, at E_eq, no matter which of the two sites it is located. However, the form (potential vs. kinetic) of energy changes and is illustrated by the small and large semi-circles. The bottom of the semi-circle represents the perfect water molecule when it has maximum kinetic and zero exchangeable potential energy. The top of the semi-circle, at the intersection of the E_eq line, is the point where the molecule has maximum potential and zero kinetic energy. The large semi-circle represents the potential vs. kinetic energy exchange of a molecule on the ice side of the diagram and the small semi-circle represent this same exchange but of a molecule on the liquid side. U_eq represents the potential energy that is exchanged for kinetic energy. $F(\varepsilon)$ represents the Maxwell–Boltzman distribution of translational kinetic energy, E_f is the activation energy for freezing (see Fig. 1A). E_m is the activation energy for melting. ε is the translational kinetic energy of a water molecule at its interfacial site. ΔH is latent heat.

As the temperature is reduced below 273 K, freezing exceeds melting, free water freezes and an interface develops between crystals of non-matching lattices. This freezing has been characterized empirically in terms of latent heat and activation energies down to 248 K (−25 °C) (Fig. 3) [9]. As the temperature is reduced further the thickness of the ice–liquid interface diminishes until freezing and melting again come to a balanced state. The diminishing interface results in a shift in activation energy and latent heat (Fig. 3).

Activation energies from 273 to 248 K (0 to −25 °C, the temperature range of empirical heat capacity data) were calculated for both interfacial and free water under equilibrium and non-equilibrium conditions to evaluate adhesion energy (Table 1)

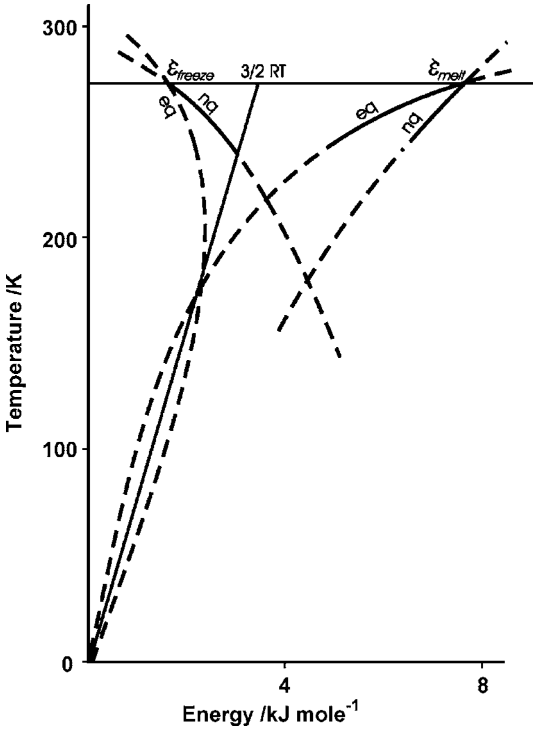

Fig. 3. Kinetic activation energies of non-equilibrium and equilibrium freezing and melting calculated (solid lines) from 273 to 248 K and extrapolated (broken lines) to 0 K by spline technique. The solid line labeled 3/2 RT is the mean translational kinetic energy from 273 to 0 K. At 273 K, latent heat, which is the difference between E_{freeze} and E_{melt} is at a maximum. Note that latent heat, which is the difference between the line labeled nq for freezing (left side) and the nq line for melting (right side), becomes smaller as the temperature is reduced. At approximately 170 K latent heat become zero; at this temperature adhesion energy is at a maximum (Table 1). Below 170 K activation energies are reversed and latent heat becomes negative. Above 273 K activation energies for the equilibrium and non-equilibrium lines are also reversed. The difference between latent heat for non-equilibrium (nq) and equilibrium (eq) freezing is shown in Fig. 4.

[9]. These activation energies were extended to 0 K (Fig. 3) by spline technique [10] to observe the response of activation energies to temperatures between 0 and 248 K. The enthalpy diagram (Fig. 4) was then derived from empirical data for ice and extrapolation of Washburn's equation for the vapor pressure of ice [4]. Because the intercepts of the activation energies for freezing and melting (Fig. 3) occur at about the same temperature (approx. 170 K) as the intercept of the latent heat of ice and interfacial water (calculated from Washburn's equation), Washburn's equation seemed to be an acceptable approximation to determine the heat capacity to 0 K.

Decreasing the temperature of an ice–liquid system from 273 K shifts the energy distribution causing free energy of freezing to predominate (Table 1). The shift in energy distribution was calculated from the vapor pressure of ice and liquid water as a measure of displacement from the balanced condition ($\Delta G_{\text{frz}} - \Delta G_{\text{mlt}}$, Table 1). As free water freezes, opposing interfaces are drawn together and become enantiomorphic (ice–interface–liquid–interface–ice). Further decreases in temperature draws the remaining interfacial water into a stronger interaction with ice, resulting in increased adhesion. Adhesion increases at the expense of kinetic freedom, and causes a decrease in latent heat. When all the free water is frozen the enantiomorph becomes a simpler system of ice–interface–ice.

The adhesion energy between ice crystals in the temperature range of latent heat (between 273 and 170 K, Fig. 3) was calculated to be greater than the net free energy of freezing (Table 1). If this equilibrium could be reversed without altering the temperature, then adhering ice crystals could be separated; a partial reversal could be accomplished by introduction of a solute into the interface. This has in fact been observed in several winter cereal crops during a mild freeze. In an extended period of freezing at 270 K, fructan reserves were hydrolyzed in rye, barley [11] and oat [12] and a concomitant increase in hexose sugars in apoplastic fluid was observed.

Free energies of freezing and melting involve only translational kinetic energy that is beyond the activation energies (Fig. 1). A reduction of the translational kinetic energy of the liquid side of the interfacial water is required to balance the free energies. The system becomes balanced as freezing diminishes the interfacial water. Balancing involves a proportional change in the magnitude of both the free energy of freezing and of melting so that the free energy of melting

Table 1
Estimates of thermodynamic distinctions between ice and interfacial water as a function of temperature

T (K)	Non-equilibrium			Equilibrium		
	ΔH	ΔG_{freeze}	ΔG_{melt}	ΔH	$\Delta G_{\text{freeze\&melt}}$	Adhesion
			Deca Joules mole^{-1}			
273	601	21	21	601	21	0
263	513	41	21	460	29	87
248	390	71	21	322	39	137
233	293	107	20	224	47	176
198	82	199	17	78	59	205
173	0	267	16	0	65	216

Non-equilibrium (nq) ΔH = latent heat of freezing in normal liquid water (273–248 K empirical, 233–173 K extrapolated) $\Delta G_{\text{frz}} = \int_{\text{o}}^{\dot{\varepsilon}_{\text{frz}}} (\varepsilon - \dot{\varepsilon}_{\text{frz}}) F(\varepsilon)\, \mathrm{d}\varepsilon$, $\Delta G_{\text{mlt}} = \int_{\dot{\varepsilon}_{\text{mlt}}}^{\propto} (\dot{\varepsilon}_{\text{mlt}} - \varepsilon) F(\varepsilon)\, \mathrm{d}E$, ε = translational kinetic energy of a water molecule at its interfacial site. Equilibrium (eq) ΔH = latent heat of freezing interfacial liquid water calculated from the free energy balance. Adhesion = the reduction in transitional energy $(E_{\text{nq}} - E_{\text{eq}}) = \dot{\varepsilon}_{\text{mlt}_{\text{nq}}} - \dot{\varepsilon}_{\text{mlt}_{\text{eq}}} = (\Delta H_{\text{nq}} + \dot{\varepsilon}_{\text{frz}_{\text{nq}}}) - (\Delta H_{\text{eq}} + \dot{\varepsilon}_{\text{frz}_{\text{eq}}})$.

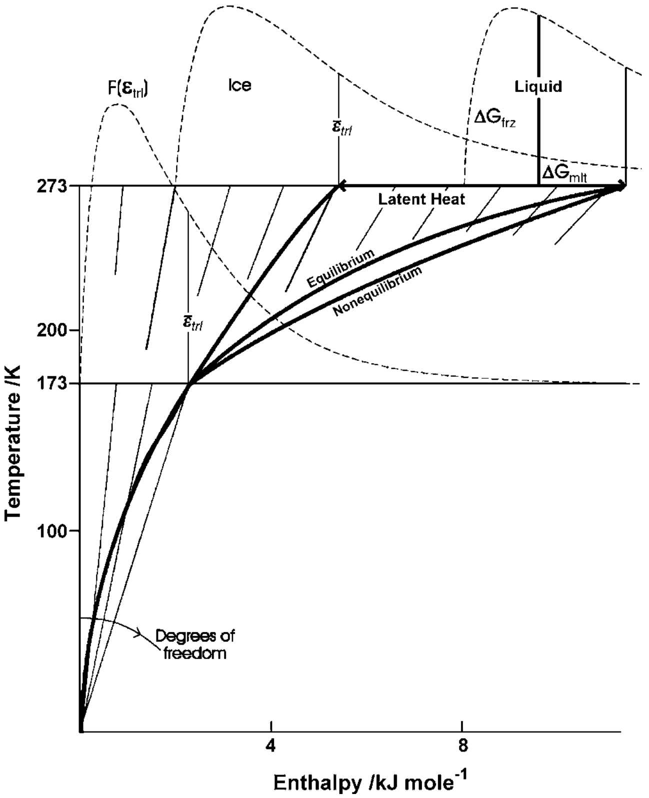

Fig. 4. Enthalpy of ice from 0 to 273 K, liquid water at 273 K, and empirical latent heat from 248 to 273 K from Dorsey [4]. Energy of liquid water from 173 to 248 K was estimated by integration of the specific heat, which was calculated with an equation derived by Washburn from the vapor pressure of ice [4]. Empirical values for heat capacity of ice were used to calculate the energy from 0 to 173 K. The work function, which is the predominant cause of stress kinetics during the onset of freezing, is not included in the graph because it is superseded by more subtle thermodynamic causes as freezing progresses. The Maxwell–Boltzmann frequency diagrams (broken lines) are for three degrees of freedom for a normal distribution of translational kinetic energy expressed in polar coordinates. The curve labeled "$F(\varepsilon_{\mathrm{trl}})$" is the frequency distribution of kinetic energy at 173 K. The line labeled $\bar{\varepsilon}_{\mathrm{trl}}$ is mean translational kinetic energy of this distribution. The lines indicating degrees of freedom (DF) are shifted to the right between 173 and 273 K because of the acquisition of nearly two additional unspecified DF. These additional DF are shown on the left side of the darker DF line. Additional DF are acquired by water as it melts. Note the convergence at 173 K of: (1) the energy resulting from 3 degrees of freedom, (2) the integration of specific heat capacity and (3) the latent heat for equilibrium and non-equilibrium freezing.

increases by the same factor (α) that reduces the free energy of freezing ($\Delta G_{\mathrm{eq}} = \alpha \Delta G_{\mathrm{mlt}} = \alpha^{-1} \Delta G_{\mathrm{frz}}$). So at equilibrium $\Delta G_{\mathrm{eq}} (\Delta G_{\mathrm{mlt}})^{-1} = \Delta G_{\mathrm{frz}} (\Delta G_{\mathrm{eq}})^{-1}$ since both ratios $= \alpha$. Thus, $\Delta G_{\mathrm{eq}} = (\Delta G_{\mathrm{frz}} \times \Delta G_{\mathrm{mlt}})^{1/2}$.

3. Discussion

3.1. Adhesion and recrystallization

In this study the energy of adhesion is considered to be the energy required to liberate interfacial water to a free state. This is the energy necessary to separate opposing ice crystals.

When the temperature decreases from the freezing point, the activation energies of freezing and melting converge with the latent heat as the interface diminishes to zero near 170 K (Figs. 3 and 4). At this temperature the energy of adhesion equals the mean translational kinetic energy of ice. This means that there would be no thermal distinction between ice and interfacial water and migratory recrystallization [4] would cease.

Successful freeze-preservation of growing tissue requires high rates of ice nucleation during the early stages of freezing so that only very small ice crystals are formed [13]. Prevention of migratory recrystallization that results in development of larger disruptive crystals thus requires storage below 170 K.

3.2. Degrees of freedom

The enthalpy of ice from 0 to 273 K (and interfacial water above 170 K) shows the pattern with which degrees of freedom are acquired. The first 3 d.f. freedom for ice are acquired from 0 up to 170 K and then nearly two more are acquired as the system reaches 273 K. At this point, ice formation terminates and the equilibrium and non-equilibrium activation energies intersect (Fig. 4). At 273 K interfacial water has acquired approximately twice as many degrees of freedom as ice has (the heat capacity of ice is 1/2 that of liquid water) and now liquid water can exist in a free state. A molecular dynamics model of the interatomic forces involved in water structure that provides a sequence of acquisition of degrees of freedom as a function of temperature might help define forms of freeze stress in biological system as well as the mechanisms required for protection from them.

3.3. Reversal of latent heat

In the temperature response pattern ascending from 0 K that demonstrates a reversal of latent heat, 273 K is also one of two intercepts of activation energies. The first region, below 170 K, where latent heat becomes negative (Fig. 3), is a temperature range where no interfacial water exists. The second intercept of activation energies at the freezing point (Fig. 3) is a reversal of latent heat between equilibrium and non-equilibrium freezing. It is interesting that this is also the beginning of a range, above which no ice can exist.

3.4. Alteration of interfacial water

A clear understanding of fluid dynamics of liquid water involves considering it to consist of points which represent small volumes with dimensions greater than the mean free path of individual molecules [14] or elements of extension-in-phase that may change in shape but within which many molecules act as a unit for statistical mechanics analysis [3,4]. The physical properties of interfacial water may be affected when the interface is diminished by freezing. For example, the free energy of freezing and melting impact the liquid interface perpendicularly, rather than in parallel and may polarize a thermal distribution by extending 1 d.f. freedom at the expense of the other two. This polarization could accentuate the disruptive forces of adhesive stress.

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