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Energies and latent heats of water in plant tissue interfaces for analysis of freeze stress[1]

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Abstract

Water transitions that stress freezing plants, and literature regarding intermolecular bonding of the liquid, have led to the development of a concept that distinguished forms of freeze stress energy. Parameters that characterize equilibrium and nonequilibrium freezing are calculated. The derivation, used previously to identify adhesion as a form of freeze stress, is extended for quantitative biophysical analysis of specific forms of the freeze stresses that alter genetic expression and elicit protective plant responses.

Keywords: Freeze Stress; Water; Energy; Latent heat

1. Introduction

Of all the types of stress that affect plants, those associated with freezing are the most susceptible to systematic analysis. Growth, hardening, severe stress, and injury tend to occur in rather distinct and manageable sequences, and severe freeze stresses involve definable physical events rather than metaphysical mysteries. Causes of disruptive effects can be analyzed; many have been, and in various ways. Each biophysical approach to freeze stress analysis requires simplification of a complex living system to the essential features of a suspected cause of injury. The approach discussed here developed from a study of freeze-induced water redistribution patterns in crowns and leaves of winter barley plants [1]. Ice in these tissues contributed to an equilibrium freeze stress which injured the plants at a higher temperature than that required to

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cause injury by freeze dehydration. Consideration of the water transitions that stress plants and the literature regarding the structure of ice and liquid water [2,3], and especially the dynamic nature of water systems [4,5], has led to development of an idealized concept of interfacial water molecules as resonating oscillators [6]. The parameters derived from this concept provided a means of estimating rates of stress development that accompany different forms of equilibration as plant tissues freeze [7]. Natural systems that would retard the rate of stress development with decreasing temperature by a suspected form of energy were sought as components of hardiness, especially those that might affect the normal lethal temperature range of field plants [1].

2. Statistics and methods

When tissues freeze, ice is a new phase that establishes the chemical potential of water at equilibrium distribution between all of its states and associations. If the displacement from equilibrium with ice is great enough, the free energy for crystal growth can cause injury from stresses of nonequilibrium freezing [1]. This category of stress can be avoided by slow freezing of an ice-nucleated system. Then the form of energy that causes injury depends on the means by which water distribution equilibrates.

The colligative freezing effect is the most commonly recognized means of equilibration. The free energy at equilibrium is a function of the mole fraction of liquid water in the solution. The freeze point depression is generally estimated by the Clausius–Clapeyron equation and is often expressed as osmolality (Table 1).

However, because water molecules have exchangeable kinetic energy which has normal distribution and because hydrogen bond energy fluctuates, ice as well as other water associations are in a dynamic state. They continuously break down and rebuild at interfaces [4,5]. Overall changes are determined by differences in the number of water molecules escaping compared with the number being trapped in ice and each association with other plant substances. The tendency of water molecules to be held in an association depends on the potential energy exceeding the translational kinetic

Table 1
Statistics for liquid water and ice as a function of temperature [2]

Temperature/K	Vapor pressure/mm Hg		ΔG^{a}/cal mol^{-1}	ΔH/cal mol^{-1}
	Liquid	ice		
273	4.579	4.579	0.0	1435
268	3.163	3.013	−25.6	1324
263	2.149	1.950	−51.0	1221
258	1.436	1.241	−74.9	1122
253	0.943	0.776	−94.9	1036
248	0.607	0.476	−119.2	939

[a] $\Delta G = RT \ln P_{ice}/P_{liquid}$.

energy of the molecule at the interface. The difference between associated and free liquid water is characterized by the latent heat of transition. Subtle recrystallization phenomena in plants prompted partition of chemical potential into freezing, melting, and hydration components for interfaces between ice, liquid, and hydrophilic plant substances. This permitted calculation of activation energies for each transition. The rationale is based on resonance between oscillating interfacial water molecules, which avoids the conceptual chaos of molecular interaction based on elastic collision.

3. Results

The activity of water with respect to ice crystal growth is a function of temperature which is the modulus of energy distribution as described by Gibbs [8]. Thermal, energy is the energy that is exchangeable between molecules in elastic collision as described by Maxwell [9]. The simple translational component for water was assumed to be the same as that of a monatomic gas, with which it would have a balanced exchange at an isothermal interface [6]. An interfacial molecule was considered to oscillate, somewhat like a pendulum, between maximum translational energy as it passes a lattice site, which then would be exchanged for potential energy as it moved away from this center of electrostatic attraction (Fig. 1). Only molecules with translational energy greater than the activation energy of melting would escape from the ice lattice. If the molecule did not escape from the interface into the higher potential energy state of

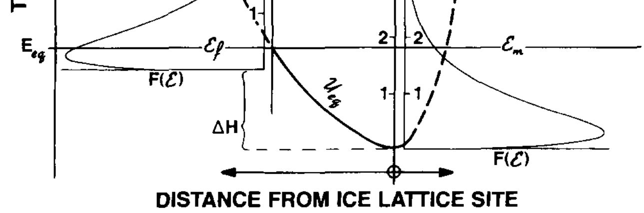

Fig. 1. Energy distribution for ice and liquid water at 0°C: ξ_f, activation energy of freezing; ξ_m, activation energy of melting; $F(\xi)$ frequency of water molecules with translational kinetic energy labeled in kcal mol^{-1}; U, potential energy of interfacial water molecules labeled in kcal mol^{-1}; U_{eq}, potential energy curve of an interfacial water molecule at equilibrium with ice and liquid water.

liquid before its kinetic energy was expended, potential energy would accelerate the molecule back toward the lattice site again passing at its maximum translational energy.

The asymmetry of the interfacial potential energy curve would affect the pattern of oscillation. High frequency within the ice lattice would focus translational energy of interfacial molecules into the lower frequency extending toward liquid. The electrostatic attraction of the lattice site not only would affect the translational energy but also would orient the polar vectors of the water molecule to match those of the ice lattice. Rotational energy would be acquired by the interfacial molecules as they approached liquid. This tumbling disorientation (libration) would reduce the distinction between liquid and ice, so the latent heat of these molecules would be decreased by the shift of their frequency function as liquid toward that of ice, conserving translational energy of the molecule. The rotational energy would be redistributed as the molecule was reoriented by vector forces in its return to ice. Reversible libration of interfacial water would pump heat from liquid to ice. This effect would contribute to limitation of size for stable ice nuclei and to recrystallization of small irregular crystals to large crystals with plane interfaces. Also, pumping heat from liquid water at 0°C would limit the size of a stable liquid water droplet in ice under adiabatic conditions.

The activation energy of freezing would then be the translational energy which a liquid molecule at the interface could not initially exceed without being accelerated in its approach to the ice lattice site to a translational energy greater than the activation energy for melting. A molecule of water in liquid with translational energy equal to the activation energy for freezing would have the same total energy (translational kinetic plus potential) as a molecule in ice at the activation energy of melting. This also is the mean energy of interfacial water molecules when the system is at equilibrium . The frequencey of oscillation would be determined by the rotational energy acquired in approach to liquid and released in return to ice, as well as by the bond energy of the ice lattice site. These parallel oscillating water molecules would resonate into the lower energy state of ice, having simple translational oscillation, and the higher energy state of liquid with its added rotational degrees of freedom. These patterns of energy distribution for ice and liquid water at 0°C are diagrammed in Fig. 1.

The chemical potential for a molecule at an interfacial lattice site to melt was determined by its translational energy in excess of the activation limit, and that for a molecule in liquid at the interface to freeze, by its translational energy deficiency [6]. The free energy of melting (ΔG_m) equals

$$\int_{\xi_m}^{\infty} (\xi_m - \xi) F(\xi) \mathrm{d}\xi$$

and the free energy of freezing (ΔG_f) equals

$$\int_{0}^{\xi_f} (\xi - \xi_f) F(\xi) \mathrm{d}\xi$$

Fig. 2 is a graph of integrals for calculation of activation limits. The latent heat is the difference between the activation limits. At 0°C the free energies of melting and freezing

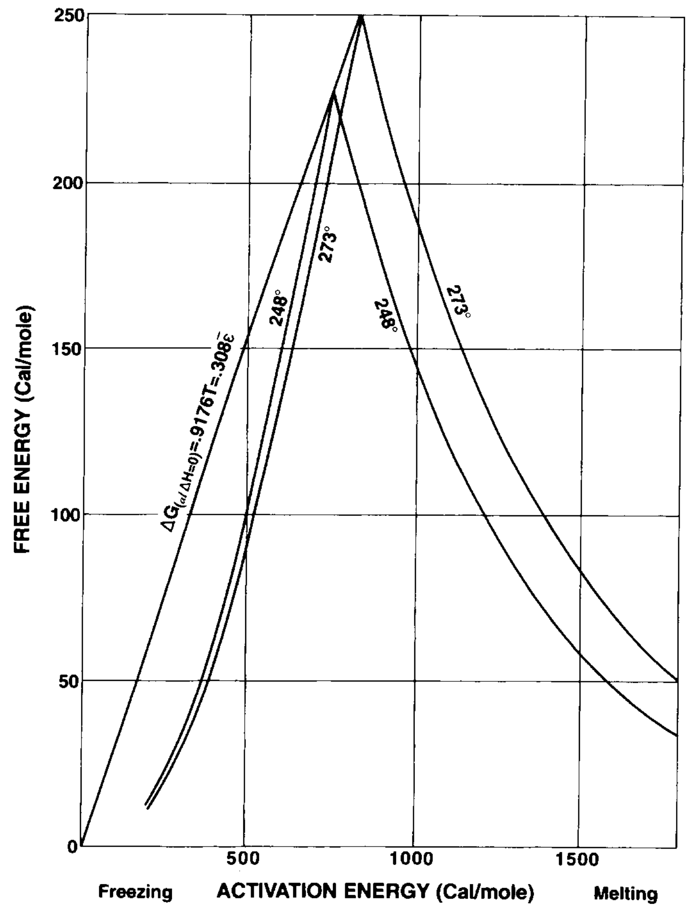

Fig. 2. Free energy of freezing and of melting as functions of activation energy and temperature.

are equal. At lower temperatures, the activation limits for nonequilibrium transition are determined by simultaneously satisfying the free energy and latent heat parameters. The equilibrium values are determined by a proportional reduction in the activation energy of melting and of freezing to free energy equilibrium as a result of competitive interaction (Table 2).

Competitive interaction between ice and hydrophilic substances for interfacial water occurs as an ice crystal grows through pure liquid water toward a hydrated substance and the latent heat of freezing is opposed by the heat of hydration. Equilibration of the free energy of freezing with those of melting and hydration affects the state of interfacial water. The interaction of the potential energies decreases the minimum energy required for melting ice, but the melt is only partial. Molecules with energy less than that of free supercooled liquid can then escape from an ice lattice site at the interface into the

Table 2
Derived parameters for analysis of freeze stress in plants

T	ΔG_f	ΔG_m	ΔG_{eq}	ξ_f		ξ_m		ΔH_{eq}	U_{adh}
				nq	eq	nq	eq		
273	49	49	49	375	375	1810	1810	1435	0
268	76	50	61	440	405	1774	1645	1240	129
263	99	48	72	510	440	1735	1510	1065	225
258	123	48	82	560	460	1680	1400	940	280
253	146	48	89	600	480	1636	1310	830	326
248	170	51	98	640	495	1580	1215	720	365

Key: ΔG_f, free energy of freezing; ΔG_m, free energy of melting; ΔG_{eq}, free energy of freezing and melting of ice at equilibrium with hydration of a hydrophilic substance; ξ_f, activation energy for freezing; ξ_m, activation energy for melting; ΔH_{eq}, latent heat of freezing water at equilibrium with hydration of a hydrophilic substance; U_{adh}, energy of adhesion through interfacial liquid water; eq, equilibrium; nq, nonequilibrium.

hydration liquid of the polymer. This decrease in total energy requirement is equal to the shift in the activation energy of melting as ice competes with a hydrated substance for interfacial liquid. A corresponding decrease in translational energy conserved by libration causes a proportional decrease in the activation energy of freezing. The difference between the energy required for transfer from ice to the hydration liquid versus transfer to free liquid must be supplied if ice were to be separated from the hydrated substance and, therefore, is a component of adhesion at subfreezing temperature.

The shift in activation energies that maintains equilibrium as the temperature decreases causes a decrease in the latent heat of freezing when freezing equilibrates with hydration. This differs from colligative equilibration which results from reduction of water density in liquid as freezing increases solute concentration. Either effect will satisfy calculation of freeze point depression.

4. Discussion

The freeze dehydration kill temperature is near −30°C for the hardiest winter cereal cultivars, although these plants are often killed at crown temperatures from −10°C to −15°C in fields during winter. Minus 30°C corresponds with freeze desiccation of 140 cal mol^{-1} (Fig. 3). If intercellular liquid were pure water, this level of adhesive freeze stress could be generated by −6°C.

Ice initially forms in the intercellular water. At a steady sub-freezing temperature, intracellular water diffuses to and freezes in the intercellular space. The cell wall presssure is relieved and the osmotic pressure of the intracellular solution is determined by the vapor pressure of ice. Ice outside of the plant such as snow, or ice crystals in a solution of intercellular solutes acts as a passive water accumulator. However, the

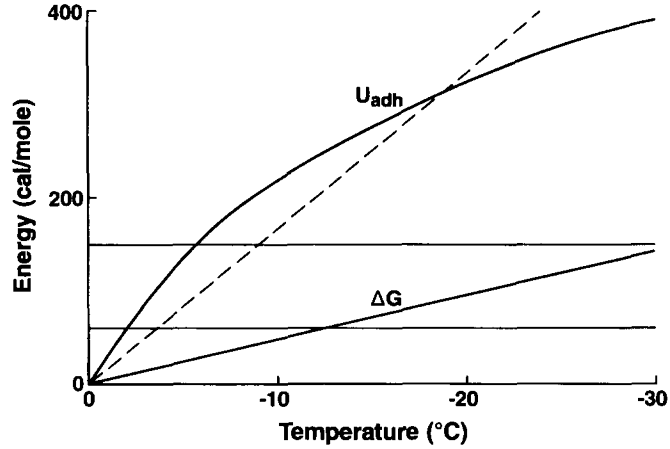

Fig. 3. Energies of adhesion and desiccation as functions of decreasing temperature: U_{adh} adhesion energy generated in a pure water interface between ice and a hydrophilic substance (from Table 2); the broken line is a previously published estimate (Thermochim. Acta, 154 (1989) 379–380); ΔG, freeze desiccation energy (from Table 1). Horizontal lines: range of maximum stress-generating energy tolerated by cell membranes based on analysis of adhesion between phospholipid vesicles [11] and survival of winter cereal tissues [8].

intercellular space of hardy plants contains only a small volume of dilute solution in which the solute-water ratio is further reduced by the large volume of water that diffuses from protoplasts as freezing progresses. The intercellular water freezes as irregular crystals with extensive interface. Freezing of pure water in the intercellular space would result in very strong adhesions between ice crystals, between ice and hydrated plant substances, and between hydrated plant substances such as cell wall and membrane.

The initial small volume of dilute intercellular liquid contains too little solute to prevent adhesion as freezing progresses when only pure water diffuses from protoplasts. In response to freezing, fructan in vesicles of hardy winter cereal protoplasts is hydrolyzed to sugar, some of which in secreted into the periplasmic space. The secreted sugar melts ice which increases the interfacial liquid and relieves adhesions between the membrane and cell wall [10]. Although massive leakage is an index of injury, controlled secretion of protective substances is an adaptive mechanism. Rapid resorption of intercellular sugars by protoplasts as the tissue thaws cleans the intercellular spaces and helps prevent disease.

When plants of a cultivar are killed in the −10 to −15°C temperature range, a plant breeder must know whether the deficiency is in the genetic system required for modification of stress or in that required for tolerance. Thermal analysis helps define the problem and identify appropriate genetic sources for improvement of winter hardiness.

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