

**Communication**

# Glass Formation and Desiccation Tolerance in Seeds<sup>1</sup>

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## ABSTRACT

The formation of intracellular glass may help protect embryos from damage due to desiccation. Soluble sugars similar to those found in desiccation tolerant embryos were studied with differential scanning calorimetry. Those sugars from desiccation tolerant embryos can form glasses at ambient temperatures, whereas those from embryos that do not tolerate desiccation only form glasses at subzero temperatures. It is concluded that tolerant embryo cells probably contain sugar glasses at storage temperatures and water contents, but intolerant embryo cells probably do not.

The ability to survive the desiccated state is the result of adaptations that prevent cellular destruction during the withdrawal of water. As water leaves a cell that does not tolerate desiccation, many events occur: solutes become more concentrated, possibly increasing the rate of destructive chemical reactions; some solutes may crystallize, changing the ionic strength and pH of the intracellular solution; proteins become denatured, many irreversibly; and membranes become disrupted, leading to the loss of compartmentation. It has been suggested that the presence of large amounts of soluble sugars within a cell can prevent the damaging effects of desiccation (2). Soluble sugars are known to form hydrogen bonds and thus may substitute for water in maintaining hydrophilic structures in their hydrated orientation, even when water is no longer present (2). Water replacement by soluble sugars has been demonstrated in model systems, where soluble sugars were able to preserve the functional integrity, measured as Ca<sup>2+</sup>-ATPase activity, of desiccated microsomes through a dehydration/rehydration cycle (2).

Another mechanism by which sugars may act to protect the cell during desiccation is by the formation of an intracellular glass (5). As a solution becomes concentrated during drying, the solutes may crystallize, or the solution may become supersaturated with an accompanying increase in viscosity. When the viscosity reaches the point at which the diffusion of water is precluded (the diffusion rate equals 10<sup>-6</sup> nm s<sup>-1</sup> in a simple aqueous solution), the solution assumes the mechanical properties of a plastic solid (3). In this state,

the solution is called a glass. A glass is essentially an undercooled liquid; therefore, its existence is temperature dependent. A solution that exists as a glass at one temperature will melt at a higher temperature, giving rise to a liquid and the possibility of crystallization (3).

The benefits of glass formation, or vitrification, to an organism undergoing water loss are many. Glasses preclude chemical reactions requiring diffusion, thus ensuring stability during a period of dormancy; they fill space, and thus by sheer bulk may prevent cellular collapse; an amorphous glass may trap chaotropic solutes, preventing their becoming concentrated; and glasses may permit the continuance of hydrogen bonding at the interface between the glass and hydrophilic surfaces in the cell (1). These properties would help ensure the survival of an organism during a period of desiccation.

Using DSC<sup>3</sup>, Williams and Leopold (10) found evidence of a glass-like state in desiccated corn embryos. Transitions similar to those made by glasses were detected in both the lipid and nonlipid portions of the embryo, and it was hypothesized that the soluble sugar component of the embryo was responsible for the nonlipid glass transition (10). Soluble sugars comprise 20% of the dry weight of corn embryos (4) and are known to form glasses readily (6). In the present study, the dependence of glass formation on hydration and temperature was tested for sugar mixes similar to those found *in vivo*. The formation of glass at room temperature by sugar mixes similar to those found in desiccation tolerant seed axes was taken as evidence supporting the important role of sugars in desiccation tolerance.

## MATERIALS AND METHODS

To test the glass forming properties of sugar mixes, combinations of sugars similar to those described for seed axes tolerant and nontolerant of desiccation were mixed in aqueous solutions. On a dry weight basis, mature corn embryos contained 17% sucrose and 3% raffinose; when they had germinated beyond the point at which desiccation tolerance is lost, they contained no raffinose, and monosaccharides had become the predominant sugars (4). A mix representative of DT axes consisted of 85% (w/w) sucrose and 15% (w/w) raffinose, whereas a mix representing NDT consisted of 75% (w/w) glucose and 25% (w/w) sucrose (4). In addition, pure

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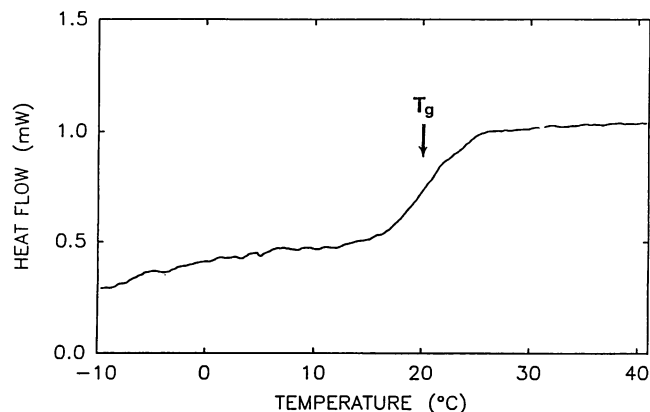
<sup>3</sup>Abbreviations: DSC, differential scanning calorimetry; DT, sugars from desiccation tolerant embryos; NDT, sugars from non-desiccation tolerant embryos; T<sub>g</sub>, midpoint temperature of the glass transition.

sucrose and pure sorbitol were tested. All sugars were obtained from Sigma Chemical Company. Sugars were dissolved in distilled water at a concentration of 100 mg/mL and were loaded into preweighed sample pans (1–5 mg sugar/pan). Samples were allowed to equilibrate for at least 3 d at 35°C over saturated salt solutions having the following water activities: KNO<sub>3</sub>, 0.90; KCl, 0.84; NaCl, 0.75; NH<sub>4</sub>NO<sub>3</sub>, 0.60; MgCl<sub>2</sub>, 0.33; and LiCl, 0.12. Water activity is a measure of the thermodynamic activity of water molecules and can be calculated as the RH divided by 100. Values for the RHs at the specified temperature were taken from published values (8). Water contents of the samples remained constant after 3 d of equilibration.

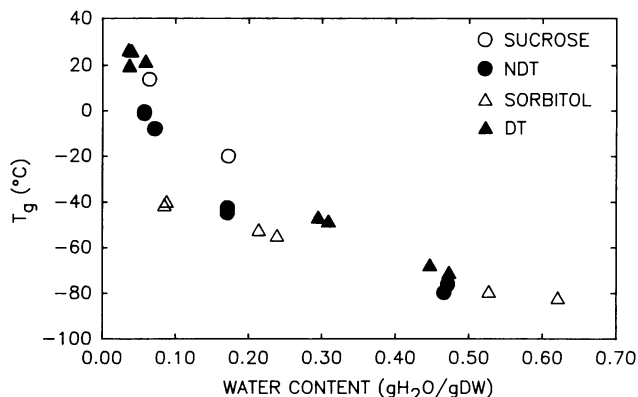
DSC (Perkin-Elmer) was used to detect the presence of glass in samples. As a glass was heated through its melting point, an abrupt increase in its heat capacity occurred, as compared to the empty reference pan. This was seen as an endothermic shift in the thermogram and was called the glass transition temperature (Fig. 1). Glass transitions could be distinguished from ice melting, which formed endothermic peaks rather than shifts in the baseline. Following equilibration over saturated salt solutions, sample pans were sealed, and DSC was performed at a scanning rate of 20°C/min. This scanning rate produced prominent glass transitions. Although the transition temperatures determined are possibly less accurate than those determined by a slower scanning rate (10), comparisons among samples are valid if scanned in the same manner.  $T_g$  represents the glass transition temperature for the sample, determined from the midpoint of the temperature range over which the change in specific heat occurred. Dry weights were obtained after calorimetry by puncturing the sample pans and drying the samples *in vacuo* over fresh P<sub>2</sub>O<sub>5</sub> at 70°C for at least 16 h. This drying protocol was sufficient to dry the samples completely.

## RESULTS AND DISCUSSION

Glass transitions were observed in most sugar samples containing less than 0.7 g H<sub>2</sub>O/g dry weight, and  $T_g$  increased



**Figure 1.** A typical glass transition measured by DSC. The deflection in the baseline centered at 20°C indicates endothermic heat flow associated with the melting of the vitreous state. The sample shown is the DT sugar mix after equilibration at a water activity of 0.33.



**Figure 2.** Effect of hydration on glass transition temperatures of sugars. Each point represents the midpoint temperature of the glass transition obtained by heating the sample at 20°C/min. After DSC, the water content of each sample was determined gravimetrically by measuring the water lost by drying over P<sub>2</sub>O<sub>5</sub> *in vacuo* at 70°C.

as the water content of the samples decreased (Fig. 2). The dependence of  $T_g$  on the solute concentration is well documented for aqueous glasses (1, 3); the question remained as to whether  $T_g$  could occur at a temperature relevant to a desiccated seed axis. If a vitreous state is to be important to the survival of a desiccated organism, it is crucial that the glass form at ambient temperatures, *i.e.* in the temperature range at which dehydration generally occurs. This requirement is met by the DT mixture, representative of the sugar composition of desiccation tolerant corn embryos, and by sucrose. These two samples vitrified at ambient temperatures when they had dried to low water contents similar to those at which corn is stored (generally less than 0.13 g H<sub>2</sub>O/g dry weight [10]).

Differences in the glass forming tendencies of the sugars were most evident at the lowest water contents, especially when compared on the basis of water activity. At a water activity of 0.33, the glass transition temperature of sorbitol was -40°C, as compared to  $T_g$ s of -8°C, 14°C, and 21°C for NDT, sucrose, and DT sugars, respectively (Fig. 3). These differences are not due to variable water contents among the samples, as can be seen in Figure 4. Samples equilibrated at a water activity of 0.33 that formed glass had water contents of 0.04 to 0.08 g H<sub>2</sub>O/g dry weight, yet the range of  $T_g$ s exceeds 60°C. For a pure substance,  $T_g$  is a function of its mol wt (3); thus, the trend for higher  $T_g$ s for the DT sugar mix can be explained in part by the presence of higher mol wt sugars, namely raffinose and sucrose.

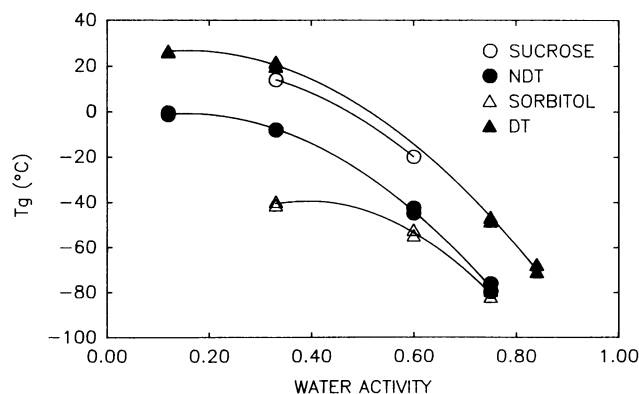
The difference between the  $T_g$ s for DT and NDT sugars supports the contention that the ability to tolerate desiccation may, in part, depend upon the ability to vitrify at ambient temperatures. This difference means that at low water contents, the aqueous compartments of the desiccation tolerant cell are essentially an amorphous solid. At the same low water contents, the aqueous compartments of the non-desiccation tolerant cell are a very viscous liquid, with diffusion rates that do not preclude the crystallization of cell components, or, possibly, chemical reactions (1).

Raffinose is a crucial component of the DT mix. It was

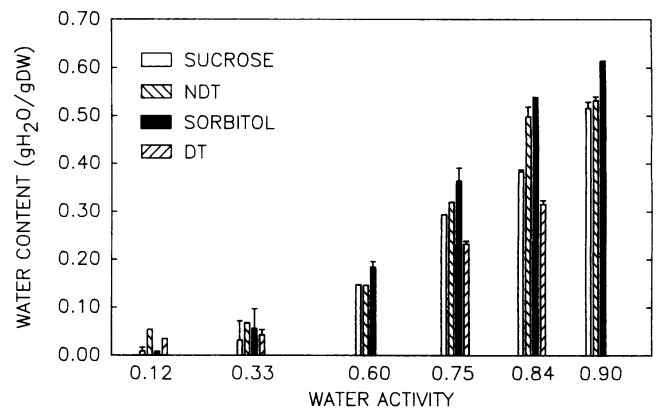
previously determined that the ability of a seed axis to tolerate desiccation correlated with the presence of sucrose plus oligosaccharides, *i.e.* raffinose and stachyose (4). It was hypothesized that the oligosaccharides might prevent the crystallization of sucrose in the drying axis, thus promoting the formation of a glass (4, 5). This hypothesis is supported by indications that samples of sucrose and sorbitol equilibrated at a water activity of 0.12 crystallized, as did two of three samples of sucrose at a water activity of 0.33. When these samples were scanned by DSC, no transitions were detected, and the water contents of less than 0.01 g H<sub>2</sub>O/g dry weight indicate that water had been excluded from the crystalline matrix (Fig. 4) (7). Although no direct measurements of crystallization were made, the absence of transitions and the low water contents of the pure sucrose and sorbitol samples at the lowest water potentials are suggestive of the presence of crystals rather than a glass. Samples containing a mixture of sugars did not crystallize at any of the water potentials studied.

It is acknowledged that the cells of a seed axis contain much more than soluble sugars and membranes. The effects of other components, *e.g.* soluble proteins and salts, on vitrification were not tested; however, Smythe (9) found that the crystallization of sucrose was not impeded significantly by organic acids and salts; the most effective inhibitors of sucrose crystallization were oligosaccharides. This implies that not all cell components can interrupt the crystalline matrix of sucrose, and that raffinose may be necessary to ensure the formation of a glass rather than a crystal during drying.

The results of this study reaffirm the importance of soluble sugars to desiccation tolerance in seed axes. Sugar mixes similar to those found in desiccation tolerant axes are capable of forming glasses at temperatures greater than zero, whereas sugar mixes similar to those found in axes that do not tolerate desiccation only form glasses at subzero temperatures. Sucrose alone can also form a glass at ambient temperatures; however, its tendency to crystallize might interfere with its ability to



**Figure 3.** Glass transitions of sugars equilibrated at various water activities. Each point represents the midpoint temperature of the glass transition obtained by heating the sample at 20°C/min. Lines were drawn as a visual aid. No glass transitions were obtained for sucrose and sorbitol at a water activity of 0.12.



**Figure 4.** Water contents of sugars equilibrated at various water activities. Bars represent the hydration, measured as the weight of water per dry weight of sample after equilibration at the desired water activity. Each bar represents the mean water content of at least two samples.

prevent damage due to desiccation. As soluble sugars comprise up to 20% of the dry weight of some embryos (4), it is conceivable that the glass-forming properties of the sugars may dominate the behavior of the embryo during desiccation and may play a role in desiccation tolerance.

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#### LITERATURE CITED

- Burke MJ (1986) The glassy state and survival of anhydrous biological systems. In AC Leopold, ed, *Membranes, Metabolism and Dry Organisms*. Cornell University Press, Ithaca, NY, pp 358–364
- Crowe JH, Crowe LM, Carpenter JF, Rudolph AS, Wistrom CA, Spargo BJ, Anchordoguy TJ (1988) Interactions of sugars with membranes. *Biochim Biophys Acta* **947**: 367–384
- Franks F (1985) *Biophysics and Biochemistry at Low Temperatures*. Cambridge University Press, Cambridge
- Koster KL, Leopold AC (1988) Sugars and desiccation tolerance in seeds. *Plant Physiol* **88**: 829–832
- Leopold AC, Vertucci CW (1986) Physical attributes of desiccated seeds. In AC Leopold, ed, *Membranes, Metabolism and Dry Organisms*. Cornell University Press, Ithaca, NY, pp 22–34
- Luyet B, Rasmussen DH (1968) Study by differential thermal analysis of the temperatures of instability of rapidly cooled solutions of glycerol, ethylene glycol, sucrose and glucose. *Biodynamica* **10**: 167–191
- Makower B, Dye WB (1956) Equilibrium moisture content and crystallization of amorphous sucrose and glucose. *J Agric Food Chem* **4**: 72–81
- Rockland LB (1960) Saturated salt solutions for static control of relative humidity between 5° and 40°C. *Anal Chem* **32**: 1375–1376
- Smythe BM (1967) Sucrose crystal growth II. Rate of crystal growth in the presence of impurities. *Aust J Chem* **20**: 1097–1114
- Williams RJ, Leopold AC (1989) The glassy state in corn embryos. *Plant Physiol* **89**: 977–981