

## CARBOHYDRATES

## All dried up

The sugar trehalose helps microorganisms to withstand drought conditions, but the mechanism by which this occurs is poorly understood. An investigation of the holes in the different structural forms of the sugar could provide clues as to how this bioprotection is possible.

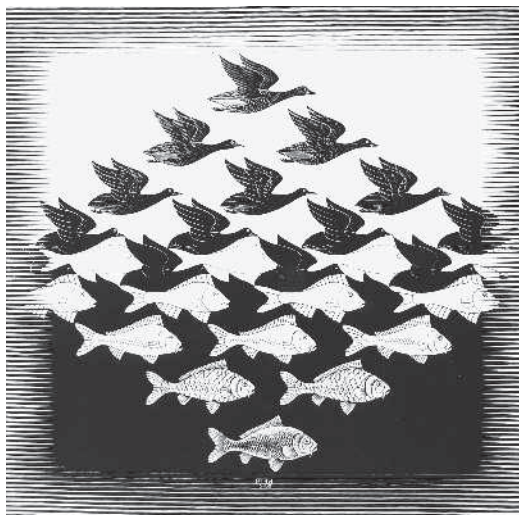
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**A**lmost all living cells die if they lose more than 20–30% of their water content, unless the biomolecules are able to maintain their functional conformation as water is removed. Trehalose — a disaccharide sugar formed from two glucose units — is used by some plant and animal cells to survive under extreme conditions of dehydration. Drought-adapted organisms are able to induce the production of trehalose as they desiccate<sup>1</sup>, and the dried organisms survive in a dormant state and subsequently resuscitate when environmental humidity permeates the cells, restoring the original moisture conditions. Several working hypotheses have been advanced as to the role of trehalose in this process, but no clear-cut explanation of its action has yet been formulated<sup>2,3</sup>. In this issue, Kilburn *et al.*<sup>4</sup> address this problem by studying the dehydration of trehalose and its transformation to anhydrous and amorphous phases. They have pictured these structural transitions via changes in intermolecular hole radii, as a consequence of water escaping from the crystalline cage, and subsequent structural rearrangements. Their measurements provide the first direct real-time observations of holes created by water escaping from trehalose polymorphs, taking us a step closer to a full understanding of this fundamental process.

The measurements of Kilburn *et al.*<sup>4</sup> were made possible by the application of positron annihilation lifetime spectroscopy (PALS) to address the problem of trehalose dehydration. This technique was originally introduced to the polymer field to elucidate the size and density of intermolecular cavities, or 'free volume', in non-metallic condensed matter at the nanometre length scale<sup>5</sup>. This is possible as positrons — the antimatter counterpart of electrons with positive instead of negative charge — have a high



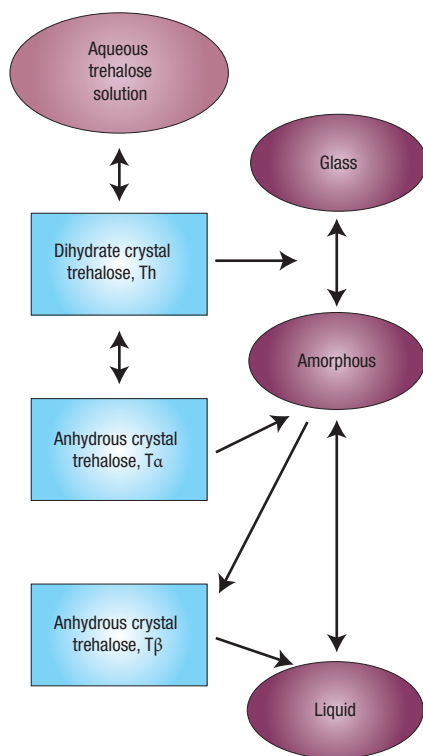
**Figure 1** Continuous transformation. Dihydrate trehalose crystals during dehydration can be depicted by Escher's *Sky and Water I* illustration, with water molecules — the bird figures in various shades — escaping from the relaxing condensed phase, represented by fish.

probability of annihilation on collision with electron surfaces within the cavity where they are generated, emitting gamma radiation as they do so. The lifetime and the intensity of gamma rays emitted in the annihilation process respectively correlate with the void size and number, thus revealing the free-volume of the material.

Trehalose has received considerable attention not only because of this role in nature, but also for its potential use as a highly efficient natural preservative<sup>6</sup>. Although some microorganisms generate their own trehalose when they begin to desiccate, the protective capabilities of the sugar can also be induced through its artificial addition. Indeed, paramount importance is given to the possibility of using trehalose or other semi-synthetic sugars as a means of preserving valuable and delicate proteins, or biological systems such as living cells at ambient temperature, without the need for freeze drying.

The slowing down of biomolecules encapsulated in the sugar matrix is by far the most accepted definition of the role of trehalose in the

**Figure 2** Transformations of trehalose polymorphs. From dehydration of an aqueous solution, a dihydrate trehalose, Th, is formed. The crystalline form, T $\alpha$ , and the amorphous phase can then be obtained, depending on the temperature and rate of water evaporation. T $\beta$  is the most stable anhydrous form. Among all the transformations, the reversible hydration/dehydration between Th and T $\alpha$  is the key process studied by Kilburn *et al.*



bioprotection process — it encases molecules and membranes the way amber traps insects. However, the way water plays its role in the process is also cryptic, as adding water to sugar glasses normally increases their mobility, lowering their glass-transition temperature, whereas a water molecule can be immobilized in solid dihydrated trehalose and maintain the same hydrogen bonding network as in the solvated trehalose<sup>7</sup>.

Given that water immobilization is the final goal of preservation under low-moisture conditions, the hydration–dehydration of trehalose has been widely studied with experimental and theoretical means<sup>8–10</sup>. The continuous dehydration and ensuing structural relaxation process inspires an artistic connection with an Escher illustration (Fig. 1). In general, the transformations of hydrated polymorphs of sugars are tuned by the rates of temperature change and water removal. In this respect, trehalose, with its three crystalline polymorphs — one dihydrate form, Th, and two crystalline anhydrous forms, T $\alpha$  and T $\beta$  (see Fig. 2) — and the fact that its glassy state has the highest transition temperature of all disaccharides, is unexpectedly different from the homologous disaccharides maltose and sucrose, with lower

glass-transition temperatures and less polymorphs. Although the addition of water to an amorphous phase generally facilitates an increase in mobility and therefore a drop in the glass-transition temperature, it has been postulated that the reason the glass transition of amorphous trehalose is still high, even in the presence of residual water, is that Th dihydrate crystallites are locally formed within the amorphous matrix. The ability of amorphous trehalose to capture moisture into the cages of Th crystallites, coupled with the reversible transition between the dihydrate form Th and the anhydrous form T $\alpha$  achieved by extraction of water from the Th form, provides the perfect platform for water immobilization during dehydration.

Kilburn *et al.* show via measurements of the free volume of trehalose that in the amorphous phase, water inclusion simply increases the average intermolecular hole size. In the crystalline dihydrate, Th, however, the water is confined as a one-dimensional fluid in channels of fixed diameter. These channels allow diffusion of water in and out of the crystallites, and can therefore act as both a sink and source of water in low-moisture systems. Thus, trehalose polymorphism and the formation of a water-immobilizing crystalline-glassy nanocomposite phase of Th crystallites in the amorphous matrix seem to provide a reasonable basis for the survival of biological functions by dehydration.

The experiments reported by Kilburn *et al.*<sup>4</sup> show that the movement of water can be directly correlated to the structures and free volumes present in the trehalose under bioprotectant conditions. The link that is still missing is the coupling of the static picture of hole size and distribution with measurements of the temperature dependence of local structure dynamics (for example, from inelastic scattering experiments). It is important that such experiments are carried out in the same controlled conditions to enable firm conclusions to be drawn. Using these results in conjunction with computer simulation should eventually tailor the correct and final mechanism of bioprotection.

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