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Preservation of Membranes in Anhydrobiotic Organisms: The Role of Trehalose

Abstract. *Trehalose is a nonreducing disaccharide of glucose commonly found at high concentrations in anhydrobiotic organisms. In the presence of trehalose, dry dipalmitoyl phosphatidylcholine (DPPC) had a transition temperature similar to that of the fully hydrated lipid, whereas DPPC dried without trehalose had a transition temperature about 30 degrees Kelvin higher. Results obtained with infrared spectroscopy indicate that trehalose and DPPC interact by hydrogen bonding between the OH groups in the carbohydrate and the polar head groups of DPPC. These and previous results show that this hydrogen bonding alters the spacing of the polar head groups and may thereby decrease van der Waals interactions in the hydrocarbon chains of the DPPC. This interaction between trehalose and DPPC is specific to trehalose. Hence this specificity may be an important factor in the ability of this molecule to stabilize dry membranes in anhydrobiotic organisms.*

Trehalose, a nonreducing disaccharide of glucose, is found at particularly high concentrations (as much as 20 percent of dry weight) in many organisms capable of surviving complete dehydration (the so-called anhydrobiotic organisms) (*1–8*). These organisms include spores of certain fungi (*1*), macrocysts of the slime mold *Dictyostelium* (*2*), dry active baker's yeast (*3*), brine shrimp cysts (dry gastrulae of *Artemia salina*) (*4*), and the dry larvae and adults of several species of nematodes (*5*). They can persist in the anhydrobiotic state for many years, but when they come in contact with water they rapidly swell and resume metabolic activities. Survival of dehydration by some of these organisms is correlated with synthesis of trehalose during dehydration (*5, 9*) or its degradation following rehydration (*10*). We report that this molecule alters the physical properties of membrane phospholipids in the dry state in ways that may explain the remarkable stability of membranes in anhydrobiotic organisms.

Dehydration of a biological membrane usually results in such massive upheavals that the structural and functional integrity of the membrane are irreversibly damaged. For example, when vesicles of calcium-transporting membranes are dehydrated, the phospholipids form complex crystalline phases, some of which are nonbilayer structures. Intramembrane particles that represent the calcium-dependent adenosine triphosphatase in these membranes are excluded from these crystals (*11*). Upon rehydration, vesicles show evidence of morphological damage—including fusion between vesicles and redistribution of intramembrane particles—and calcium transport activity is lost (*11*). By contrast, when the membranes are dried in the presence of trehalose at concentrations of at least 20 percent of the dry weight of the membranes (concentrations similar to those found in anhydrobiotic organisms), no evidence of phase transitions is seen during dehydration, and upon rehydration, vesicles are similar morphologically and functionally to freshly prepared ones (*12*). At physiological concentrations, this stabilizing effect is a property of trehalose not shared by other carbohydrates (*13*).

Polar head groups of phospholipids normally are hydrated to some extent, and the head groups are separated from each other by these water molecules (*14*). Thus, when phospholipids are dehydrated, the packing density of the head groups would be expected to increase, thereby increasing opportunities for van der Waals interactions among the hydrocarbon chains. As a result, dehydrated lipids would be expected to enter gel phase at temperatures at which hydrated lipids are in the liquid crystalline phase. Indeed, relative to the hydrated lipid, dry phosphatidylcholine (PC), a phospholipid which is a major constituent of many membranes, has an elevated transition temperature (T_c, the temperature at which the hydrocarbon chains undergo a change from gel to liquid crystalline state) (*15*). Thus, when a membrane is dehydrated at a temperature at which it would normally be in a liquid crystalline state, PC would be expected to enter a gel phase as dehydration progresses. When PC enters a gel phase because of reduced temperature, the result is lateral phase separation of the PC, other lipid classes, and intramembrane

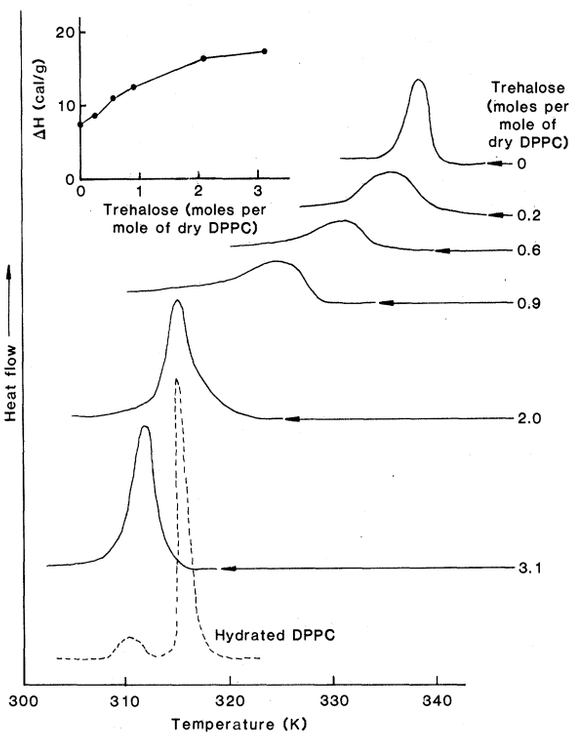

Fig. 1. Differential scanning calorimetry traces of dry and hydrated dipalmitoyl phosphatidylcholine (DPPC) and dry DPPC in the presence of various amounts of trehalose. The inset illustrates the rise in enthalpy of the main phase transition of dry DPPC in the presence of increasing amounts of trehalose (*26, 27*).

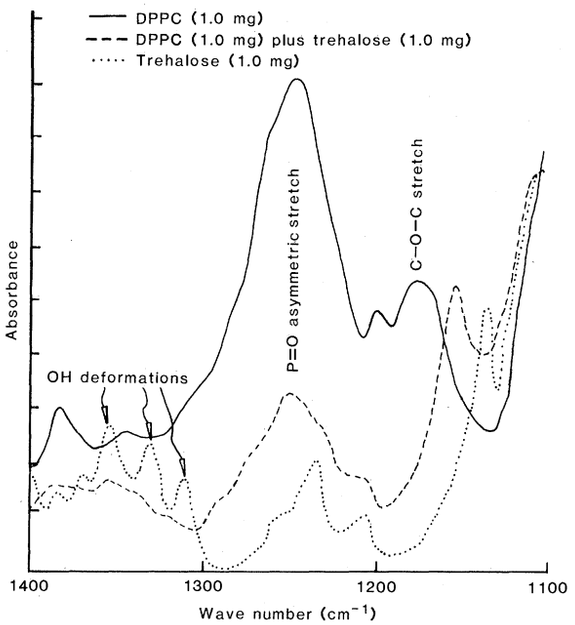

Fig. 2. Infrared spectra of DPPC, DPPC plus trehalose, and trehalose in the phosphate region (*28*).

proteins (*16*). It follows that damage to the membrane might be alleviated if the initial event in this sequence, transition of PC to its gel phase, is prevented. The most likely mechanism for an inhibition of the phase transition by trehalose is an interaction between trehalose and phosphate head groups that increases the spacing between head groups. This "water replacement" hypothesis was proposed more than a decade ago (*6*). One prediction from this hypothesis is that dry lipids in the presence of trehalose have physical properties similar to those of hydrated lipids.

We have measured T_c for mixtures of DPPC and trehalose (Fig. 1). The dry and hydrated DPPC show T_c's of 341 and 314 K, respectively (*15*). When even small amounts of trehalose were added to the DPPC before it was dried, the endotherm was broadened and displaced to a lower temperature. With increasing concentrations of trehalose, T_c decreased steadily until, at the highest concentration, it was actually below that of fully hydrated DPPC. Coincident with this decrease in T_c, as the mole ratio of trehalose to DPPC was increased, there was an increase in the enthalpy of the phase transition (Fig. 1). Hydration of dry phospholipids similarly results in depression of T_c and elevation of the enthalpy of that transition. In other words, addition of trehalose to dry phospholipid in some respects mimics addition of water.

To clarify the mechanism by which trehalose exerts these effects on PC, we have recorded infrared spectra (*17*) of dry DPPC in the presence and absence of trehalose (Fig. 2). The most striking changes were in the bands that have been assigned to vibrations of the phosphate head group (*18*); the band at 1246 cm^{-1} was broadened, depressed, and shifted to 1240 cm^{-1} in the presence of trehalose. Similarly, a band at 1084 cm^{-1} was depressed, although the details of this change were obscured by strongly absorbing bands in the regions that are assigned to trehalose (data not shown). These effects can be titrated by adding varying amounts of trehalose. Concurrent with the changes in the phosphate vibrations, alterations in bands assigned to trehalose were seen. For example, the series of bands between 1400 and 1300 cm^{-1}, assigned to OH deformations, were completely missing after DPPC was added. We interpret these data to mean that there is an interaction between the OH of the trehalose and phosphate head group, probably by means of hydrogen bonding (*19*).

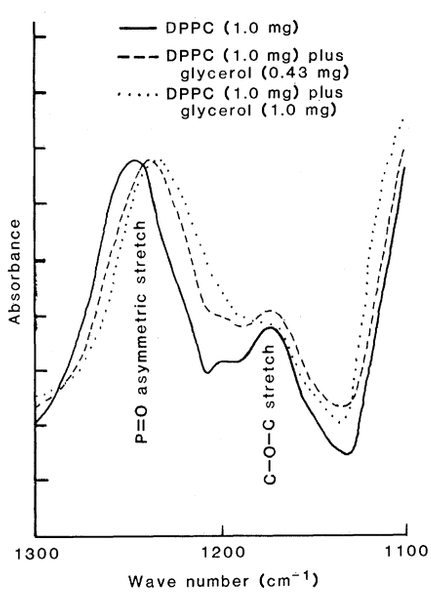

Fig. 3. Infrared spectra of DPPC and DPPC plus glycerol in the phosphate region (*28*).

We have conducted similar studies on several other carbohydrates and show (*20*) that the ability of these carbohydrates to preserve membrane structure in the dry state is correlated with their relative efficiencies in decreasing T_c and in interacting with the phosphate head group, as assessed with infrared spectroscopy. For example, calorimetry and infrared spectroscopy indicate that, in contrast to trehalose, inositol does not interact with dry phospholipids and does not preserve structure and function in dry membranes (*13*). The one apparent exception to this pattern is glycerol, some properties of which we describe further. McDaniel *et al.* (*21*) reported that glycerol reduces T_c for dry DPPC. We have confirmed that glycerol depresses T_c, although not to the same extent as trehalose; 5.2 moles of glycerol per mole of DPPC were required to reduce T_c to that of hydrated DPPC; with trehalose, only 2.0 moles per mole of DPPC were required to achieve the same T_c. Furthermore, in contrast to the results with trehalose, T_c in the presence of glycerol was not depressed below the T_c for fully hydrated DPPC, even at the highest concentrations of glycerol.

The results for glycerol appear to contradict the hypothesis presented above concerning the mechanism of preservation of membranes in the dry state in that even though glycerol depresses T_c, it does not preserve membrane structure and function in the dry state (*13*). Thus, we have sought an explanation for this apparent inconsistency. There is evidence that the mechanisms of interaction with phospholipids are different for trehalose and glycerol. For example, glycerol has well-known fusion-producing properties (*22*), which are lacking in trehalose (*12*). Also, when either trehalose or glycerol is added to the subphase of PC spread on a water surface, the monolayer is spread by the carbohydrates. However, the monolayer must be in a partially expanded state for glycerol to affect it (*23, 24*). A possible explanation for this is that glycerol interacts with the phospholipids by intercalating between the head groups. Similar nonspecific interactions between other low molecular weight alcohols and phospholipids also occur (*25*). Thus, glycerol might depress T_c in dry phospholipids by intercalating between head groups, which in turn would increase intermolecular spacing

and decrease van der Waals interactions between hydrocarbon chains. Trehalose, by contrast, is capable of expanding even fully condensed monolayers (*24*) and therefore must associate with the PC by a different mechanism. Infrared spectroscopic studies of dry PC-trehalose (Fig. 2) and PC-glycerol (Fig. 3) mixtures are consistent with this explanation. The data for glycerol (Fig. 3) show that in place of the complex interactions seen in the trehalose-PC mixtures, the only change in the PC spectrum was in the band at 1246 cm^{-1}, which was shifted to 1240 cm^{-1} with increasing concentration of glycerol. The band broadening seen in the trehalose-PC spectrum was missing, as were the changes in OH stretching bands in the carbohydrate.

These data show that while the ability of a molecule to depress the melting temperature of a dry phospholipid is probably important in preserving biomembrane structure in the dry state, it is not in itself sufficient; glycerol depresses T_c, but its mode of interaction with the phospholipid, probably intercalation between the head groups, carries with it deleterious side effects such as fusion. As a result, glycerol does not preserve dry membranes. Such side effects are not found with trehalose, probably because its interactions with phospholipids involve hydrogen bonding between OH groups in the trehalose and the phosphate head group. These hydrogen bonds may replace the same or similar hydrogen bonds between the lipid and water that occur in bulk water. The relative specificity of the bonding between trehalose and phosphate head groups may therefore be an important factor in the ability of this molecule to replace water around the head group of a phospholipid and thereby to stabilize dry membranes.

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27. DPPC (purissimum) was purchased from Fluka and used without further purification. Samples were prepared by dissolving 10 to 20 mg of DPPC (weighed to the nearest 0.01 mg) and an appropriate amount of trehalose in 2 ml of methanol to which 1 ml of benzene was added after the samples were fully dissolved. The solution was frozen in liquid nitrogen and lyophilized. Subsamples of the dry preparations were weighed and were sealed in DSC pans, and calorimetric curves were recorded with a Perkin-Elmer DSC-2 calorimeter. At least three heating endotherms were recorded for each sample to confirm stability of the sample. The data were converted to digital form with a Hewlett-Packard graphics computer-plotter, normalized to a standard weight of lipid, and replotted in the form presented in Fig. 1. T_c was measured according to (*25*). Enthalpies (reported as calories per gram of lipid) were calculated from the areas of the heating endotherms, using published values for the main transition of hydrated DPPC as a standard (*15, 26*).
28. Subsamples (approximately 1 mg) from the same preparation (*27*) were weighed to the nearest 0.01 mg, ground with 100 mg of KBr, and pressed into disks. Spectra were obtained with a Perkin-Elmer IR681 spectrometer linked to a Perkin-Elmer 3500 data station. The data, obtained in digital form, were corrected for small differences in sample weights by introduction of the appropriate factor on the data station, smoothed by the Savitsky-Golay function, and replotted as shown in Fig. 2.
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Lymphokine Production by Cultured Human T Cells Transformed by Human T-Cell Leukemia-Lymphoma Virus-I

Abstract. *Cell-free conditioned media from human T cells transformed by human T-cell leukemia-lymphoma virus (HTLV-I) were tested for the production of soluble biologically active factors, including several known lymphokines. The cell lines used were established from patients with T-cell leukemia-lymphoma and from human umbilical cord blood and bone marrow leukocytes transformed by HTLV-I in vitro. All of the cell lines liberated constitutively one or more of the 12 biological activities assayed. These included macrophage migration inhibitory factor (MIF), leukocyte migration inhibitory factor (LIF), leukocyte migration enhancing factor (MEF), macrophage activating factor (MAF), differentiation inducing factor (DIF), colony stimulating factor (CSF), eosinophil growth and maturation activity (eos. GMA), fibroblast activating factor (FAF), γ-interferon and, in rare instances, T-cell growth factor (TCGF). Some cell lines produced interleukin 3 (IL-3), platelet-derived growth factor (PDGF), or B-cell growth factors (BCGF). Such cells should prove useful for the production of lymphokines and as sources of specific messenger RNA's for their genetic cloning.*

T cells play an important role in cell-mediated immunity both as effector cells and as modulators of cell proliferation and function. Many of these T-cell functions are mediated by soluble, biologically active molecules called lymphokines (*1*). These include among others: macrophage migration inhibitory factor (MIF) (*2*), leukocyte migration inhibitory factor (LIF) (*2*), leukocyte migration enhancing factor (MEF) (*2*), neutrophil migration-inhibitory factor (NIF-T) (*3*), macrophage activating factor (MAF) (*4*), differentiation inducing factor (DIF) (*5*), colony stimulatig factor (CSF) (*6*), eosinophil growth and maturation activity (eos. GMA) (*6*), interleukin 3 (IL-3) (*7*), fibroblast activating factor (FAF) (*8*), chemotactic factors (*1*), lymphotoxins (*9*), T-cell growth factor (TCGF) (*10*), B-cell growth factor (BCGF) (*11*), interferon (*12*), and other activities involved with helper and suppressor T-cell function. Some of these activities are well characterized while most essentially remain phenomenological observations not associated with distinct molecules. Progress in biological and biochemical characterization of many of these activities has been hampered by difficulties in ob-