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The effect of hydration stress solutes on the phase behavior of hydrated dipalmitoylphosphatidylcholine

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We have investigated the interaction of solutes found to accumulate in biological systems during chilling, dehydration, and salt stress with fully hydrated multilamellar and unilamellar vesicles of dipalmitoylphosphatidylcholine (DPPC). We have focused on a series of mono-, di-, and tri-substituted amines (glycine, 4-hydroxyproline, proline, and betaine) and contrasted the action of these solutes to trehalose, a protective disaccharide. Differential scanning calorimetry studies show that when DPPC is scanned in the presence of increasing concentrations of these solutes (up to 3 M), there is a moderate increase in the pre-transition temperature (1–6°C) with a smaller increase (1–2°C) in the main transition temperature of hydrated multilamellar vesicles of DPPC. Other calorimetric parameters (ΔH, $\Delta T_{1/2}$, Cp_{max}) determined for the pre-transition and main transition were similar independent of the solute. In each case, the main phase transition was broadened with increasing solute while the transition enthalpy was not significantly affected.

Introduction

One approach toward understanding how organisms and plants can survive environmental extremes such as high salt conditions, freezing and desiccation has focused on the interaction of solutes that accumulate during the induction of these stress states with biological membranes. This work is based on the view that the maintenance of membrane integrity during stress is essential for biological structural and functional viability when conditions become more favorable. While the action of protective solutes may vary depending on the conditions under which an organism is exposed (and the resulting organismal response, e.g. dormancy, reduced metabolic states), the effect of particular solutes (amino acids, carbohydrates) on biological membranes in freeze-thaw and freeze-dried systems has been extensively studied [1–4]. In addition, liposomes have provided good model membranes for which to study the effects of particular solutes on membrane integrity and phospholipid phase behavior [5–9].

One of the suggested mechanisms for some protective solutes, such as the disaccharides, is the replacement of water at membrane hydration sites during drying or freezing events [10]. Although a large amount of work has been generated on the effect of these solutes in the frozen or dry state, less is known about the interaction of these solutes with membranes in bulk solution. The interaction of the protective solute and water may play an important role in the effect of these solutes on membranes (and other macromolecular assemblies) in bulk solution.

One such solute which has significant effects on the bulk properties of water is the imino acid proline which have been shown to accumulate in diatoms, several higher plants, and intertidal invertebrates that experience hydration or salt stress [11–13]. Calorimetric, spectroscopic, and solution viscosity studies of proline at high concentration (up to 6 M) suggest that proline self-assembles into alternate stacks, forming a hydrophilic colloid [14,15]. The interaction of these stacks with protein hydrophobic groups has been speculated as a mode of protective action for proline [16].

Although there is a greater understanding of the role of the protective substituted amines in the maintenance of protein native conformation, the mode of

Abbreviation: DPPC, dipalmitoylphosphatidylcholine.

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action of these solutes and their interactions with membrane phospholipids is not well understood. In the present work we examine the calorimetric effect of increasing glycine, proline, 4-hydroxyproline, betaine, and trehalose concentrations on the pre- and main phase transition characteristics of multilamellar and large unilamellar vesicles of DPPC.

Materials and Methods

L-Proline and 4-hydroxyproline were purchased from Calbiochem (San Diego, CA). Glycine and betaine were obtained from Sigma (St. Louis, MO). Trehalose was purchased from Pfanstiehl Laboratories (Waukegan, IL). The solutions used for this study were prepared by dissolving an appropriate amount of the solute in distilled water and filtering the solution through a 0.22 μm filter unit. Dipalmitoylphosphatidylcholine (DPPC) was purchased from Avanti Polar Lipids (Pelham, AL) and used without further purification. DPPC aliquots in chloroform were dried over a stream of N_2 to a lipid film and placed under vacuum. Multilamellar vesicles were formed by rehydrating the dried lipid film with water, an amino acid or trehalose solution to give a final phospholipid concentration of 5 mg/ml. To assure complete mixing, the lipid dispersion was warmed to 55°C and vortexed vigorously. Large unilamellar vesicles used in this study were formed by passing multilamellar vesicles through a Microfluidizer (Model 110-S, Microfluidics Corp., Newton, MA) at 30 psi for 120 strokes.

Large unilamellar vesicles were examined for vesicle size using photon correlation spectroscopy. The experimental setup incorporates a Jodon HN50 helium-neon laser (Ann Arbor, MI) with a Brookhaven Instruments BI200 photon correlation spectrometer (Holtsville, NY). A Langley-Ford correlator (Amherst, MA) was interfaced to an Apple IIe. Single exponential and cumulance exponential analyses were performed to give the best fit hydrodynamic radius and polydispersity values for the sample.

Calorimetric experiments were performed using a Microcal MC-2 differential scanning calorimeter connected to a Haake Buchler programmable water bath. The calorimetric unit was interfaced to a Compaq microcomputer for automatic data collection and analysis using an AID board (Data Translation, DT-2801) and software provided by Microcal. In each case, aliquots of freshly prepared samples were diluted to 1.5 μmol/ml in the solution of choice and loaded into the sample cell. The reference cell was loaded with the same solution used to prepare the lipid vesicles. Each cycle consisted of equilibration at 15°C for 30 min, heating at 30 C°/h to 55°C, equilibration at 55°C for 30 min and cooling at 30 C°/h back to 15°C. This cycle was repeated a second time for each sample to check for any changes in the samples during the experiment. Following the calorimetric run, the sample was removed and phospholipid concentrations were determined using the method of Stewart [17]. All calorimetric measurements were repeated twice using independently prepared samples.

Results and Discussion

The effect of increasing proline concentration on the gel to liquid-crystalline phase transition characteristics of hydrated multilamellar vesicles of DPPC as measured by differential scanning calorimetry (DSC) can be seen in Fig. 1. As the proline concentration increases, the transition temperature (T_m) for the main phase transition increases and the transition broadens. In 3 M proline the T_m of this transition is 43.4°C, approximately 1.8 C° higher than in the control DPPC sample. At this concentration of proline, the half-height width ($\Delta T_{1/2}$) is 0.33 compared to 0.14 for the control, indicating that the temperature range over which the transition is observed is increased and the cooperativity is reduced. In addition, the cooperative unit size of approximately 477 lipid molecules for the control is reduced to approximately 292 lipid molecules in 3 M proline. The enthalpy of the transition is slightly decreased in the highest proline concentrations (1 M and 3 M) by 1–1.5 kcal/mol.

A greater effect of proline is seen on the pre-transition of DPPC multilamellar vesicles (Fig. 2). As the proline concentration increases, the temperature of the pre-transition increases, with the highest increase ob-

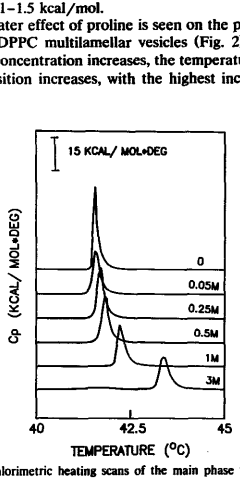

Fig. 1. Calorimetric heating scans of the main phase transition of DPPC multilamellar vesicles (1.5 mM) in increasing concentrations of proline. See Methods for thermal history of the sample. Scans have been vertically offset for presentation purposes.

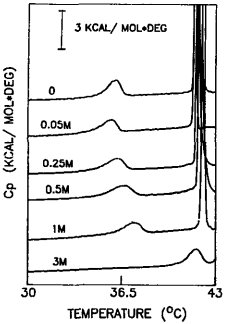

Fig. 2. Calorimetric heating scans of the pre-transition of DPPC multilamellar vesicles in increasing concentrations of proline. Scans have been offset for presentation purposes.

served from 35.9°C to 41.7°C in 3 M proline. The result of this action is to move this transition closer to the chain melting gel to liquid-crystalline phase transition. The enthalpy of this transition is unchanged as the concentration of proline is raised.

To determine if the thermotropic behavior exhibited by hydrated DPPC multilamellar vesicles in the presence of proline in bulk water is an unique property of proline or shared by the other protective solutes, a characterization of DPPC hydrated with 4-hydroxyproline, betaine, glycine or trehalose was performed using DSC. As depicted in Fig. 3, there is a linear increase in the phospholipid main (Fig. 3A) and pre-transition (Fig. 3B) phase transition temperatures with the addition of increasing amounts of each solute. Of the solutes studied, betaine shows the greatest increase in the temperature of the pre-transition at 2 M, with the disappearance of the pre-transition in 3 M betaine. As measured for proline, the enthalpy and $\Delta T_{1/2}$ properties of the pre-transition are not affected by the presence of increasing amounts of the substitute amino acids or trehalose. Similarly, the thermotropic effect of each of these additional solutes on the main transition properties of DPPC shows a decrease in the amplitude of the main transition peak (Cp_{max}) with little change in the transition enthalpy.

The effect of proline on large unilamellar vesicles made by the high pressure sheer apparatus is seen in Fig. 4. A significant broadening of the transition is observed as the concentration of proline increases. The half-height width ($\Delta T_{1/2}$) increases from 0.925 in control DPPC vesicles to 4.0 in 500 mM proline with little change in the T_m of this transition. At 3 M proline, a sharp peak appears with a transition temperature of 43.2°C. Deconvolution of this scan reveals that two peaks are present: a broad lower temperature peak centered at 41.1°C, (70% of total area) with a cooperative unit size of 30, and a higher temperature peak centered at 42.3°C with a cooperative unit size of 357. The size of the vesicles produced by this process were all between 0.06 and 0.2 μm, except for the 3 M proline sample which was 0.4 μm. Similar results were obtained for liposomes microfluidized in the presence of trehalose.

One postulated role for the accumulation of particular solutes during hydration stress is to act by increasing the intracellular solute concentration, osmotically balancing the cell as water is removed [14]. In addition, it has been suggested that intracellular solutes reduce the mole fraction of intracellular ions in reduced water

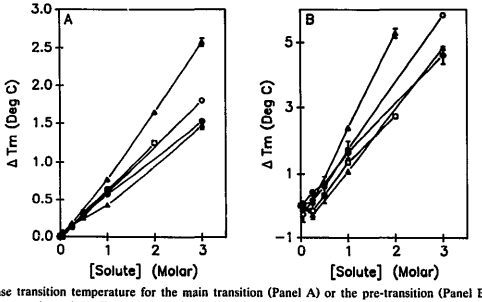

Fig. 3. Change in the phase transition temperature for the main transition (Panel A) or the pre-transition (Panel B) of DPPC multilamellar vesicles as a function of protective solute. Each point represents the mean ± S.E. based on two independent experiments. ○, proline; ●, 4-hydroxyproline; △, glycine; ▲, betaine; □, trehalose.

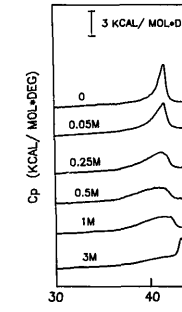

Fig. 4. Calorimetric heating scans of DPPC large unilamellar vesicles (1.5 mM) in increasing proline concentrations. Thermal history of the samples can be found in Methods. Scans have been offset for presentation purposes.

states, increasing the mole ratio of ions that may result in toxicity to cellular function [14,18]. Both of these possible roles involve the colligative action of these solutes in solution. Other possible modes of action may rely on more direct interaction with biomacromolecules experiencing hydration stress. These could involve direct binding to hydration sites on biomacromolecules during sever hydration stress such as lyophilization. This has been postulated for the role of disaccharides in preserving the structure and function of dry lipids and proteins [2]. In the case of hydrogen bonding of disaccharides, there is some indication that the amount of water remaining is negligible [19]. Thus, the penetration of the hydration shell by the protective solute for this mode of interaction is obligatory.

However, in scenarios where a considerable amount of bulk water remains, conditions may not be favorable for solute access to biomacromolecule hydration sites. In these cases, the interaction of the solute with bulk water may play an important role in the effect of the solute on biomacromolecular structure and function. This affect could be mediated through alterations in the hydration shell. In the present work we have examined a series of mono- di- and tri-substituted amines with increasing hydrophobicity. The order these solutes have on increasing the pre-transition temperature of DPPC multilamellar vesicles follows the order of increasing hydrophobicity with betaine [with greater shielding of the amine and increased hydrophobicity) showing the greatest increase in T_m of the pre-transition and glycine showing the least increase. It is interesting to note the similarity between the increase in the transition temperature of hydrated multilamellar vesicles of DPPC in the presence of proline and the increase observed in other stabilizing solutes such as the disaccharides, trehalose and sucrose [9,19–23]. It has been proposed that the increase observed in the phase transition temperature of hydrated multilamellar vesicles of DPPC in the presence of high concentrations of protective disaccharides is attributed to hydrogen bonding of the sugar to the lipid head groups [19]. Whether these solutes have similar mechanisms of interaction is outside the scope of this work, but can be approached by examining functional groups which may participate in interfacial interactions with phospholipid bilayers.

Conclusions

The stabilizing solutes glycine, 4-hydroxyproline, proline, and betaine increase the pre- and main phase transition temperature of hydrated multi- and unilamellar vesicles of DPPC. This increase is monotonic with increasing concentration of solute. Examination of enthalpic values for these transitions shows only slight decreases in the highest concentration of solute examined. The effectiveness of the solutes examined follows the order of amine substitution with the tri-substituted amine (betaine) showing the largest increase in both the pre- and main chain melting phase transition. In addition, there is evidence in the highest concentration of betaine examined (3 M), that the interfacial region is perturbed as no pre-transition is observed. In some cases, where significant interactions between the solute and bulk water take place (as in high concentrations of proline), changes in the nature of the hydration shell must be considered. Further experiments will be aimed at direct observations of the effect of these solutes at the lipid-water interface and hydration shell of lipid assemblies.

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References

1 Heber, U., Tyankova, L. and Santarius, K.A. (1971) Biochim. Biophys. Acta 241, 578–592.

2 Crowe, J.H., Crowe L.M., Carpenter, J.F., Rudolph, A.S., Wistrom, C.A., Spargo, B.J. and Anchordoguy T.J. (1988) Biochim. Biophys. Acta 947, 367–384.
3 Crowe, J.H. and Crowe, L.M. (1982) Cryobiology 19, 317–328.
4 Rudolph, A.S. and Crowe, J.H (1985) Cryobiology 22, 367–377.
5 Anchordoguy, T.J., Rudolph, A.S., Carpenter, J.F. and Crowe, J.H. (1987) Cryobiology 24. 324–331.
6 Crowe L.M., Womersley. C. Crowe, J.H., Reid, D, Appel, L. and Rudolph, A.S. (1986) Biochim. Biophys. Acta 861, 131–140.
7 Crowe, L.M., Crowe, J.H., Rudolph, A.S., Womersley, C.W. and Appel, L. (1985) Arch. Biochem. Biophys. 242, 240–247.
8 Womersley, C., Uster, P.S., Rudolph, A.S. and Crowe, J.H. (1986) Cryobiology 23, 245–255.
9 Rudolph, A.S., Crowe, J.H. and Crowe, L.M (1986) Arch. Biochem. Biophys. 245, 134–143.
10 Clegg, J.S. (1986) in Membranes, Metabolism, and Dry Organisms (Leopold, C.A., ed.), pp. 169–187, Comstock Publishing Associates, Ithaca, NY.
11 Chu, T.M., Jusaitis, M., Aspinall, D. and Paleg, L.G. (1978) Physiol. Plant. 43, 254–260.
12 Levy, D. (1983) Physiol. Plant. 57, 169–173.
13 Gilles, R. (1979) in Mechanisms of Osmoregulation in Animals, (Gilles, R., ed.), pp. 93–99, John Wiley and Sons. New York.
14 Schobert, B. (1977) J. Theor. Biol. 68, 17–26.
15 Schobert, B. (1978) Biochim. Biophys. Acta 541, 270–277.
16 Rudolph, A.S. and Crowe J.H. (1986) Biophys. J. 50, 423–430.
17 Stewart, C.M.J. (1980) Anal. Biochem. 104, 10–14.
18 Shearwin, K.E. and Winzor, D.J. (1988) Biophys. Chem. 31, 287–294.
19 Crowe, L.M. and Crowe, J.H. (1988) in Physiological Regulation of Membrane Fluidity, Vol. 3 (Aloia, R.C., Curtain, C.C. and Gordon, L.M., eds.), pp. 75–101, Alan R. Liss, New York.
20 Strauss, G., Schurtenberger, P. and Hauser, H., (1986) Biochim. Biophys. Acta 858, 169–180.
21 Chowdhry, B.Z., Lipka, G. and Sturtevant, J.M. (1984) Biophys. J. 46, 419–422.
22 Tsvetkov, T.D., Tsonev, L.I., Tsvetkova, N.M., Koynova, R.D. and Tenchov, B.G. (1989) Cryobiology 26, 162–170.
23 Koynova, R.D., Tenchov, B.G. and Quinn, P.J. (1989) Biochim. Biophys. Acta 980, 377–380.