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Acoustical investigation of the liposome-saccharide interaction

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High precision measurements of ultrasonic velocity in aqueous suspension of phosphatidylcholine liposomes in the presence of trehalose, sucrose and glucose have demonstrated its dependence on the concentration of the two disaccharides. This finding is interpreted in terms of replacement by the latter of water molecules bound to phosphate head groups on the liposome surface.

Trehalose and sucrose are found at high concentrations (as much as 20% of dry weight) in many organisms capable of surviving complete dehydration [1]. Survival of dehydration is correlated with synthesis of trehalose and sucrose during dehydration; following rehydration these disaccharides undergo degradation. In the absence of disaccharides biomembranes lose their functional and structural integrity during dehydration [2]. In recent years, evidence has accumulated that certain saccharides may replace the water bound to polar residues in membrane phospholipids, maintaining their integrity in the absence of water. Infrared spectroscopy of dry phospholipids in the presence of trehalose showed that vibrational bands assigned to the phosphate head group were strongly affected, e.g. the P=O asymmetric stretch was displaced to lower frequencies. Addition of water to the dry lipid had the same effect [3]. Furthermore, molecular modeling of the phosphatidylcholine-trehalose interaction suggests that hydrogen bonding of trehalose to the phosphate group may take place, too [4]. Evidence for the possible direct interaction between disaccharides and phospholipid polar head group comes also from studies on the effect of heavy metals, e.g. Eu^{3+} on the ability of saccharides to stabilize lipid membranes [5]. Eu^{3+} ions were found to abolish the ability of saccharides to protect phospholipid vesicles [6, 7], indicating that phospholipids and Eu^{3+} interact with, and compete for, the same site on the membrane.

Recently a new method called ultrasonic velocimetry has been developed [8, 9]; it is based on the high-precision measurement of ultrasonic velocity in solution, and has been successfully applied to the study of nucleic acid hydration [10, 11], hydration of proteins [12] and polyethylene glycol induced dehydration of liposomes [13]. The aim of this work was to study, by the ultrasonic velocimetry method, direct interaction between phosphatidylcholine unilamellar liposomes and disaccharides.

MATERIALS AND METHODS

Liposome preparation. Lipid – egg yolk phosphatidylcholine (Sigma, U.S.A.) – was dissolved in organic solvent (mixture of chloroform and ethanol, 2:1, v/v) and the lipid solution was evaporated under vacuum in a rotary evaporator. After evaporation of the solvent, redistilled water was added into the flask containing the lipid film, and the mixture was shaken mechanically. The suspension thus obtained was then sonicated with a Brown Lab-

sonic 2000 sonicator at 80 W for 15 min under nitrogen in an ice-bath, to obtain small unilamellar liposomes.

Ultrasonic velocity measurement. The changes in ultrasonic velocity were measured by a device RADA-2 working at 7.0 - 7.2 MHz frequency (Institute of Biological Physics, Pushchino, Russia), based on the so-called resonance method. The apparatus is equipped with a measuring and a reference cell (acoustic resonators) each of 0.8 ml volume. Changes in the ultrasonic velocity were determined by measuring the changes in the resonance frequency at the maximum of the resonance peak of the present harmonic component of the cell using the expression $(u-u_o)/u_o = (f-f_o)/f_o$, where f and f_o are frequency values at the maximum of the resonance peak of the resonance harmonic component of the cells filled by a solution and a solvent, respectively, and u and u_o are the corresponding velocities of ultrasound.

The acoustic titrations of liposome solutions were performed as follows. The measuring and reference cells were filled with the solution and water with a high-precision micrometer. The titration with saccharides was performed using a microsyringe with a precision micrometer. The concentration of saccharides was varied by adding a concentrated solution of trehalose, sucrose or glucose (all obtained from Sigma) both to the measuring and reference cells containing equal volumes of liposome solution and solvent, respectively. Initial concentration of phospholipid was 2.4 mM and changed in the course of titration by less than 3%.

RESULTS AND DISCUSSION

When dry phospholipids are hydrated they swell and absorb various amounts of water, which constitutes the main hydration shell. Dehydration of phospholipid bilayers can lead to several sorts of damage, e.g. fusion, leakage and lateral phase separation. It seems that resistence to dehydration in the presence of disaccharides is associated with changes in the hydration shell. Therefore we have studied the disaccharide concentration-dependent changes in ultrasonic velocity, which permits to assess the adiabatic compressibility of the hydration shell; this, in turn, might be helpful in gaining more understanding of the phospholipid-disaccharide binding.

Apparent molar adiabatic compressibility ϕ_{ks} is defined by the following relation

$$\phi_{ks} = (K - n_1 K_1^o)/n_2 \quad (1)$$

where K is the adiabatic compressibility of a solution which contains n_1 moles of the solvent and n_2 moles of the solute (in this work, n_2 is number of moles of the phosphatidylcholine in solution) and K_1^o is molar compressibility of the pure solvent. For dilute solutions we have the equation [14]

$$\phi_{ks} = 2\beta_o(\phi_v - A - M/2\rho_o) \quad (2)$$

where ϕ_v is the apparent molar volume, M - molecular weight of solute and ρ_o and β_o are the density and adiabatic compressibility coefficients of the pure solvent, respectively. The concentration increment of ultrasonic velocity A is defined by

$$A = (u - u_o)/u_o c \quad (3)$$

where u and u_o are the ultrasonic velocities in the solution and pure solvent, respectively, and c is molar concentration of the solvent (phosphatidylcholine).

For a dilute solution ϕ_v is given by

$$\phi_v = V_M + \Delta V_h \quad (4)$$

where V_M is the intrinsic molar volume of a solute molecule inaccessible to surrounding molecules of the solvent, and ΔV_h represents the hydration contribution, from the change in volume of the solvent surrounding the solute molecule, resulting from the solute-solvent interactions and the void volume between the solute molecule and the surrounding solvent.

Similarly ϕ_{ks} is given by

$$\phi_{ks} = K_M + \Delta K_h \quad (5)$$

where K_M is the intrinsic molar compressibility of the solute, ΔK_h represents the hydration contribution and, like ΔV_h, depends on the solute-solvent interaction.

In this work we were interested in the relative changes of A induced by replacement of water molecules surrounding liposomes by saccharide molecules. From eq. (2) ΔA is given by

$$\Delta A = \Delta\phi_V - \Delta\phi_{ks}/(2\beta_o) \quad (6)$$

The relation of ΔA to molecular characteristics is

$$\Delta A = \Delta(\Delta V_h) - \Delta(\Delta K_h)/(2\beta_o) \quad (7)$$

The ΔK_h and ΔV_h values are determined by the water-liposome interaction, i.e. by liposome hydration. They reflect the hydration-de-

pendent change in the compressibility and density of water surrounding the liposome.

When saccharides are added both to the measured and reference cell, the change in A is caused by replacement of water molecules by saccharide molecules. In Fig. 1 the dependence of relative increment ΔA on the concentration of saccharides is shown (acoustic titration curves of liposomes with saccharides). As we can see, trehalose and sucrose strongly interact with liposome surface whereas the interaction of glucose is negligible. These findings are in agreement with those obtained previously [2 - 7]. The structure of liposome-bound water is similar to that of ice [15]. As shown in Figs. 2 and 3, all structural parameters of galactose on nonreduced end of the sucrose are very similar to those of ice-I [16]. Disaccharides which form

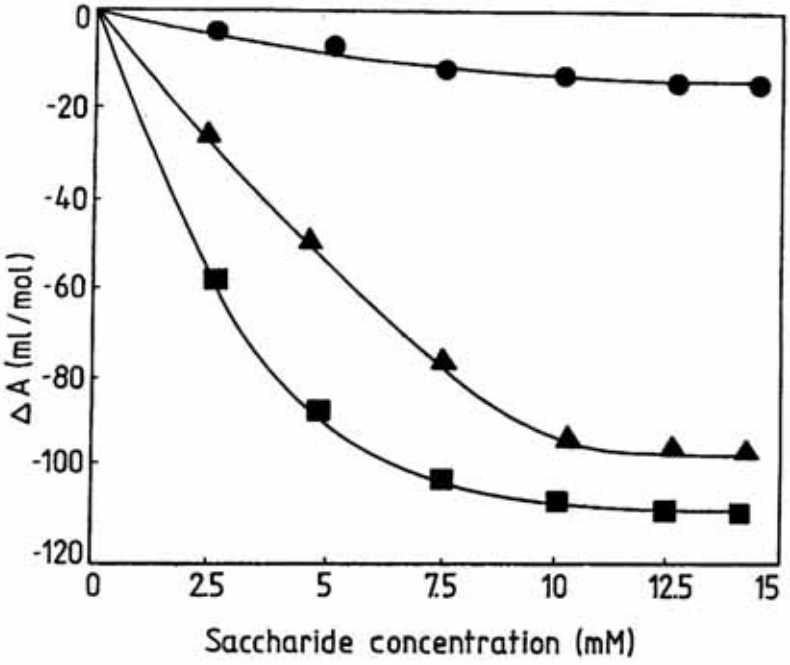

Fig. 1. Dependence of the relative concentration increment of ultrasonic velocity, ΔA, in liposome solution on saccharide concentration: (■) trehalose, (▲) sucrose and (●) glucose.

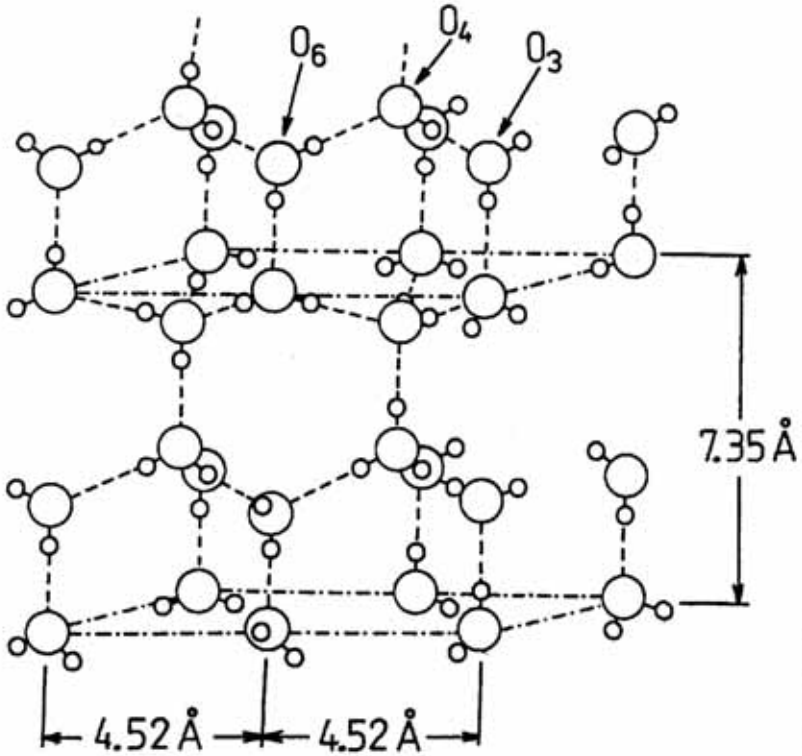

Fig. 2. Hexagonal structure of ice-I. The sites, which are complementary to galactose oxygens (cf. Fig. 3), are shown by arrows [16].

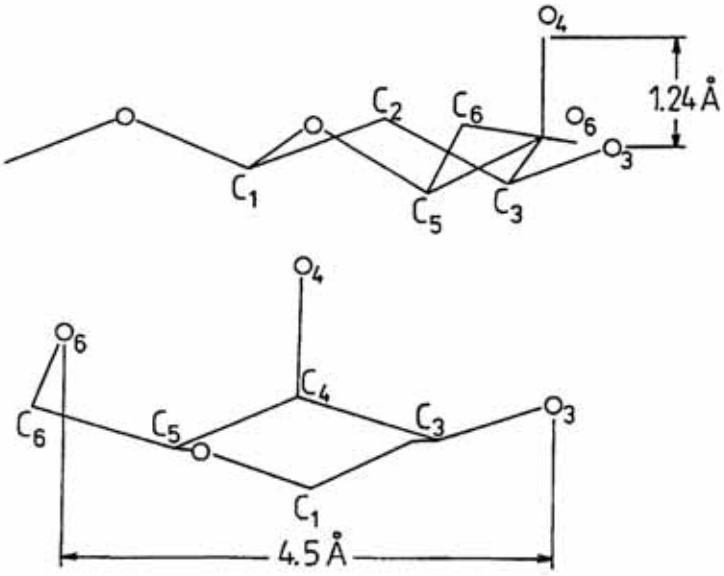

Fig. 3. Structure of galactose on the non-reduced end of sucrose [17].

a hydrogen-bonded complex with the polar head groups of membrane phospholipids, due to their structural similarity with bound ice-like water, probably form a cover around membranes, replacing water in maintaining the structural and functional integrity of membranes on their dehydration.

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