Influence of sucrose on the phase behaviour of phospholipid model systems

Z Slavkova, N Drinova, H Chamati, J Genova

Georgi Nadjakov Institute of Solid State Physics, Bulgarian Academy of Sciences, 72 Tzarigradsko Chaussee, 1784 Sofia, Bulgaria

E-mail: julia.genova@issp.bas.bg

Abstract. This article focuses on the influence of sucrose on the properties of SOPC (1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine) lipid systems. Differential scanning calorimetry is used to investigate the phase behaviour of the lipid system in the presence of sucrose. This method is appropriate and widely used to study the phase transitions parameters for organic compounds, in particular, lipids and the influence of various admixtures on these properties. Research results reveal that sucrose affects both, mechanical and physico-chemical characteristics of lipid systems. There are tangible differences in the bending elasticity moduli and enthalpies of the phase transitions due the addition of sucrose.

1. Introduction

It is well known that sugars, besides fats and proteins, are a primal source of energy in the complex metabolic processes [1]. Besides, some sugars protect cell’s membranes against drought [2,3], while others such as trehalose and sucrose could be cryoprotectors through their bond with the membrane, which is based on substitution of water by the sugar molecules [4,5]. Nowadays, sucrose steps into the preparation of all sorts of food not only as an ingredient, but also as a preservative. In figure 1, we present the chemical structure of sucrose. This disaccharide contains two monomers—glucose and fructose connected by a glycosidic linkage. Different types of carbohydrates and especially sucrose are widely used in food and pharmaceutical industry. They might be found in all sorts of conventional foods and beverages. According to the latest data from the World Health Organization, the number of cases suffering from all types of diabetes (type I, type II, including insulin resistance) as well as obesity has substantially increased in the past decades. There is a debate on the extent to which the reason for the observed increase lies in the excessive consumption of sugars. Thus, fundamental research aiming at gaining insights into the influence of sugars on the physicochemical properties of the lipid membrane is extremely important.

Lipid bilayers are an important constituent of the membrane and synthetic lipids are often used in scientific research to gain better knowledge about their physical (mechanical) properties. Moreover, liposomes are considered to be the simplest model of the biological cell. Phosphatidylycholines are the main building units of lecithin and cell membranes. Numerous works on the bending elasticity modulus Kc of (1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine) SOPC quasi-spherical giant vesicles have been published throughout the years, including investigations involving sugars and sucrose in particular [6—12]. A very important characteristic of phospholipids is the transition temperature from gel (Lβ)→(Lα) liquid crystal phase. This temperature varies from 3.45 °C to 4.63 °C for SOPC depending on the hydration and heating velocity [13]. According to previous studies [14—16] this transformation
takes place at about 6.7 °C. A crucial requirement of the method of thermally induced shape fluctuation analysis, used for the calculation of $K_c$, is that the studied lipid system is in the liquid crystalline state at room temperature. In the present work, we aim to investigate the influence of 300 mM water solution of sucrose on the phase behavior of SOPC (figure 2) lipid system via Differential scanning calorimetry (DSC).

![Figure 1. Schematic presentation of sucrose.](image1.png)

![Figure 2. Structure of l-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine, SOPC.](image2.png)

2. Material and methods

l-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine ((C18:0/C18:1) used in the experiment were purchased from Avanti Polar Lipids Inc., USA. The used sucrose with purity of 99.5% was obtained from SigmaUltra®. SOPC was dissolved in chloroform (Sigma-Aldrich, purity ≥99%) with concentration of 1 mg/ml. One ml of this solution was placed under vacuum for 4 hours. Afterwards, 250 µl of 300 mM sucrose solution in double distilled (dd) water solution were added so that the water would be 20 w. % to the lipid. The specimen was left in an ultrasonic bath at approximately 35 °C to hydrate for 12 hours.

DSC is a very precise technique for the investigation of the phase transition behaviour in organic compounds. The apparatus contains two crucibles – one being for the sample under study and the other one serves as a reference. The equipment maintains both samples at the same temperature along the experiment. Therefore, when a phase transition occurs, certain amount of heat flows to the sample. Comparing the difference between the heat flows that are required to maintain a certain temperature for both pans, heat is either released (exothermic process) or absorbed (endothermic process). DSC equipment used in the study is Discovery 250 (TA Instruments, USA). In all the performed experiments, the heating rate has been 5 °C/min.

3. Results and discussion

For many years, it was a common belief among scientists that carbohydrates (and particularly mono- and disaccharides) did not affect $K_c$ of model lipid membranes. They were extensively used in micropipette technique in order to maintain constant volume of the manipulated giant vesicles [17,18].
With the development of experimental techniques, it was shown that sugars sufficiently influence the elastic properties of model lipid systems [9,10]. According to those very same studies there is a substantial change in the bending elasticity moduli of SOPC membranes with the addition of sucrose up to 300 mM in the aqueous solution, and thereafter the process attains saturation. To this end, we chose this quantity of that very same disaccharide. Phase transitions in organic compounds are harder to trace compared to inorganic compounds, since such transformations are associated with lower enthalpy values. The investigation is even more difficult, when the studied transition is close to another phase transition; like in this particular case, the \((L_\beta) \leftrightarrow (L_\alpha)\) transition of SOPC is close to the phase transition of pure water \((0^\circ C)\). Thus, the results interpretation could be very complicated. It was already established that the best DSC signal is collected when the model lipid system’s hydration is around 10 w. % [13]. Moreover, since carbohydrates are soluble in water and have been added within the water solution, we chose to work in this particular system with hydration rate of 20 w. % of dd water. In such a way, we get reasonably good phase transition detection and at the same time, we can ensure sufficient contact of sucrose with lipid molecules.

In table 1, we give the obtained results by DSC measurement in presence of 300 mM sucrose in the water environment (the phase transition temperature and the corresponding enthalpy) and the bending elasticity modulus along with previously published data for pure SOPC system for comparison [13]. The obtained thermograms of hydrated at 20 w.% SOPC and SOPC in the presence of 300 mM sucrose are given on figure 3. Both samples were heated with the same velocity. As it is seen from the obtained results, the enthalpy for \((L_\beta) \leftrightarrow (L_\alpha)\) is of one order of magnitude lower for the model lipid system containing 300 mM sucrose compared to that of the pure lipid system. Yet, there is no substantial difference in the phase transition temperatures. As it is visible from the dependence of the normalized heat flow on the temperature of the studied sample the first order phase transition from gel to liquid state is very well pronounced for pure SOPC system in dd water. On the other hand, when it comes to the system with sucrose one can observe a process over large interval of temperature starting from approximately 0 °C and finishing at 10 °C. It seems that the disaccharide smears out the phase transition and the integrated area under the peak results in substantially lower enthalpy. This effect is most likely driven by the binding of OH-groups from the carbohydrate to the phosphate from the lipid’s head group via hydrogen bonding as it has been suggested for another disaccharide-trehalose [19]. One plausible explanation is given by Cacela and Hincha [20]. They consider molecular dynamic simulations and concludes that disaccharides bind more stably to the lipid membrane compared to water and are even able to bridge over several lipid molecules in certain conditions.

To deepen our knowledge further, other means of examination, such as infrared spectroscopy studies are needed. In order to reveal the details on the influence of carbohydrate on the phase behavior of the model lipid system it would be interesting to examine a wider concentration range of the studied sugar.

| Table 1. Values obtained by DSC for SOPC and 20 w. % dd water and SOPC in the presence of 300 mM sucrose and 20 w. % dd water. |
|--------------------------|-----------------|-----------------|
|                          | \(T_m\) (°C) | \(\Delta H\) (J/g) | \(K_c\) \((10^{19}J)\) |
| SOPC + 20 w. % dd water  | 4.34           | 0.145           | 1.88 ±0.17                   |
| SOPC + 20 w. % 300 mM sucrose dd water solution | 4.46           | 0.040           | 0.77±0.11                    |
4. Conclusion
In the present study, the phase behaviour of SOPC lipid system in the presence of 300 mM sucrose solution is compared with the same lipid system in pure dd water. The obtained results show that the addition of 300 mM sucrose to a SOPC model lipid system influences not only the bending elasticity modulus but also significantly affects the (L_β)→(L_α) transition process. At addition of 300 mM sucrose, the phase transition from gel to liquid crystal phase starts at lower temperature but is less noticeable and pronounced resulting in one order of magnitude lower enthalpy. When added to previous findings about the reduction of the bending elasticity moduli values with the introduction of sucrose to the phospholipid [8–10] the information coincides with the cryo protective properties of sugars. We believe that this study should be continued via other methods, such as Fourier transform infrared spectroscopy, that would give information about the bonds within the structure. This result is encouraging to, furthermore, include other concentrations of sucrose, compare results, and come to some new knowledge about the influence of sucrose on important from biophysical point of view properties of SOPC model lipid systems.

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