Sucrose effects on the fluidity of natural and synthetic phospholipid bilayers

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Fluorescence anisotropy of DPH embedded in bilayers prepared from horse bean- (*Vicia faba* L. var. *minor*) root phospholipids and synthetic phospholipids, respectively, is increased by sucrose. Sucrose has both non-specific and specific effects. The non-specific effect appears at sucrose concentrations above about 1 M and is correlated with the lowering of vesicle size. The specific effect depends on the phospholipid polar heads and probably implies modifications of the hydration shell. These results show that characterization of membranes in the presence of sugars must be done with caution.

Sucrose *Phospholipid bilayer* *Fluidity* *Vesicle size* *Polar head specificity*

1. INTRODUCTION

Plants are exposed to several forms of water stress (salinity, drought, freezing). Under such conditions the cells concentrate low M_r metabolic products like glycerol [1], sucrose [2], betaine [3], proline [4]. In contrast to the effect of the accumulation of inorganic neutral salts, accumulation of these organic osmolytes allows the retention of water by the cells, without altering the activity of soluble enzymes [1,5]. However, the effects of these 'compatible' osmolytes on membrane functions are largely unknown.

Here we describe some effects of sucrose on the fluidity of horse bean-root phospholipid bilayers. As horse bean-root phospholipid extracts are rather complex [6], experiments were also performed on synthetic phospholipid bilayers. DOPC and DOPG were chosen because:

(i) their unsaturated fatty acids (oleic acid) allow bilayer fluidities of the same magnitude as those of horse bean-root phospholipid bilayers;
(ii) phosphatidylcholine, the major polar head in natural lipid extracts, is Zwitterionic at neutral pH whereas phosphatidylglycerol carries a negative charge at the same pH [7].

2. MATERIALS AND METHODS

Phospholipids were extracted from horse bean- (*Vicia faba* L. var. *minor*) roots of 6-day-old seedlings, purified, and characterized as in [6]. Purity of DOPC and DOPG (Sigma) was checked by thin-layer chromatography. Fatty acid oxidation, as judged by the change in absorbance at 231 nm, was always less than 0.4% in all extracts used [8].

Unsonicated vesicles were prepared in the following way: chloroformic solutions of phospholipids (0.5 μmol) and DPH (0.25 nmol) were dried under argon. After addition of 3 ml of 15 mM Hepes buffer (pH 7.4) containing 100 mM KCl, 1 mM EDTA and various sucrose concentrations, phospholipids were dispersed by 10-min agitation in a vortex mixer. Small, unilamellar vesicles were obtained by sonicating the resulting

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Abbreviations: DOPC, 1,2-dioleoyl-*sn*-3-phosphocholine; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phospho-rac-(1-glycerol); HB-PL, horse bean-root phospholipids; DPH, 1,6-diphenyl-1,3,5-hexatriene; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

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dispersion for 45 min in a refrigerated bath. DPH was incorporated into the vesicles after sonication, as in [9].

DPH fluorescence anisotropy and 90° light scattering experiments were performed in a thermostatted Jobin-Yvon spectrofluorometer [6]. DPH fluorescence anisotropy $\langle r \rangle$ is related to membrane fluidity [10,11] and 90° light scattering to vesicle size [12].

3. RESULTS AND DISCUSSION

3.1. *Sucrose concentration effects*

Increasing sucrose concentration up to about 0.9 M linearly increased DPH fluorescence anisotropy in horse bean-root phospholipid bilayers (fig.1). At higher sucrose concentrations more pronounced increase in $\langle r \rangle$ was observed. Analogous results were obtained for DOPG bilayers. In contrast, the fluidity of DOPC bilayers was practically insensitive to sucrose up to about 0.9 M, and decreased, as in other systems, at higher concentrations.

The effect of sucrose on vesicle size was also investigated by 90° light scattering (fig.2). Sucrose lowered the vesicle size, in the same way, in all 3 kinds of bilayers studied. The smallest size, obtained with 1.5 M sucrose, is analogous to that of the sonicated vesicles (fig.2, open symbols). The reason for this sucrose effect was not investigated. We suppose that increase in the viscosity of the medium hinders assembly of numerous phospholipid molecules into large structures. It is well known that small vesicles experience more pronounced packing strains than large ones, and this leads to lower fluidity [9]. This non-specific sucrose effect on vesicle geometry should account for the non-specific rigidification observed at sucrose concentrations above 1 M (fig.1). At lower concentrations, the occurrence of this non-specific effect cannot be ruled out but behavioural differences between DOPC and other phospholipid bilayers clearly indicate the existence of a specific sucrose effect.

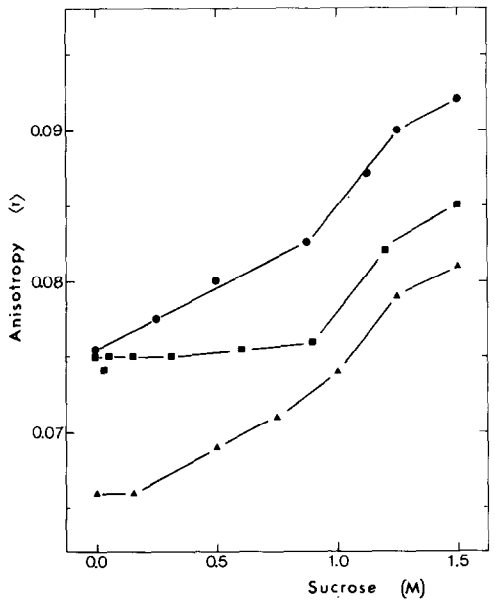

Fig.1. Fluorescence anisotropy $\langle r \rangle$ of DPH at various sucrose concentrations. (●) Horse bean-root phospholipids at $T = 25°C$, (■) DOPC at $T = 30°C$, (▲) DOPG at $T = 30°C$. All samples were prepared without sonication.

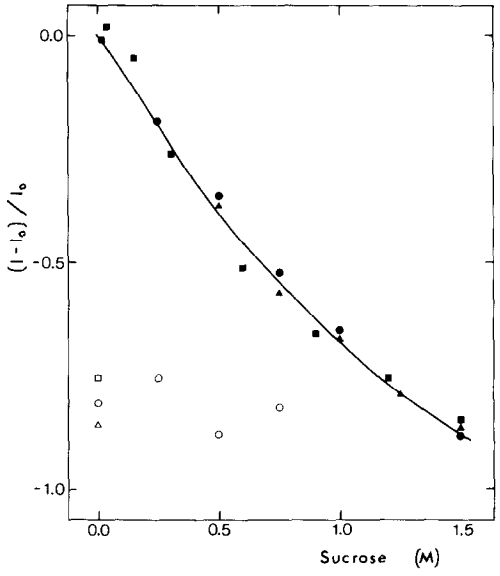

Fig.2. 90° Light scattering at various sucrose concentrations. (○, ●) HB-PL at $T = 25°C$, (□, ■) DOPC at $T = 30°C$, (△, ▲) DOPG at $T = 30°C$. Full symbols, unsonicated samples; open symbols, sonicated samples. I, light intensity at $\lambda = 330$ nm when vesicles are dispersed in a solution containing sucrose; I_o, corresponding light intensity in the absence of sucrose.

3.2. *Temperature dependence of the sucrose effect*

Temperature has a powerful effect on membranes. Arrhenius plots of fluorescence anisotropy give the following information:

(i) The gel to fluid state transition can be detected by slope breaks [9,13];

(ii) In the fluid state, the slope value depends on the nature of phospholipids and on vesicle size [9].

We studied the effect of temperature on $\langle r \rangle$ between 10 and 40°C (table 1). The absence of slope breaks indicates that the phospholipid bilayers remain in a fluid state in all studied conditions due to the presence of unsaturated fatty acids [6,9,14]. Sonicated samples give Arrhenius slope values higher than those of the corresponding unsonicated ones, as reported for phosphatidylcholine vesicles [9]. In the case of DOPC bilayers, high sucrose concentration and sonication increase the Arrhenius slope values to the same extent. This confirms that high sucrose concentration mainly acts by lowering the vesicle size. In the case of DOPG bilayers, high sucrose concentration and sonication lead to opposite slope modifications: sonication increases whereas sucrose decreases the slope value. This emphasizes again the existence of a specific effect of sucrose on phospholipids other than DOPC. However, for horse bean-root phospholipid mixture the slope value does not appear to be a useful indicator of sucrose effects, since we have no information about the Arrhenius slopes of polar heads other than phosphatidylcholine and phosphatidylglycerol: the observed increase in the presence of sucrose does not allow us to discriminate between the two kinds of effects. These observations give no direct information about the nature of the specific sucrose effect. Similar investigations on the interaction of poly-ethyleneglycol [15] and ethanol [16] with phospholipid bilayers have led to the conclusion that the observed effects reflect modifications of the network of hydrogen bonds at the membrane surface. In this case, the specific effect of sucrose should be related to:

(i) The ability of each kind of polar head to associate with the solvent network of hydrogen bonds;

(ii) The sensitivity of interactions between polar heads and the network to foreign solutes such as sucrose.

3.3. *Methodological and physiological implications*

The results presented here show that, most likely, sucrose lowers bilayer fluidity by altering polar head solvation in a way depending on their nature. These observations question several current methodological procedures. First, 15% (w/v) sucrose is often used in phospholipid dispersion media in order to limit vesicle settling. This sucrose concentration has no effect on the fluidity of phosphatidylcholine vesicles ([17], fig.1) but can lead to erroneous conclusions when other phospholipid vesicles or proteoliposomes are studied. Secondly, purification of natural mem-

Table 1

Effect of temperature on fluorescence anisotropy $\langle r \rangle$ of DPH

	Arrhenius slope values		
	HB-PL	DOPC	DOPG
0.0 M Sucrose (unsonicated)	1.66 (0.998)	1.85 (0.999)	2.34 (0.999)
0.0 M Sucrose (sonicated)	–	1.95 (0.997)	2.51 (0.998)
1.5 M Sucrose (unsonicated)	1.87 (0.998)	1.97 (0.999)	2.27 (0.999)

Arrhenius slope values $d \ln\langle r \rangle / d\ (10^3\ T^{-1})$ were calculated by linear regression from 7 points between 10 and 40°C. Values in parentheses correspond to correlation coefficients. HB-PL, horse bean-root phospholipid vesicles

brane fractions involves the use of gradients, usually made up of sucrose. Complete elimination by washing of the gradient molecules is necessary to avoid osmotic and other, specific effects on membrane-bound enzymes and, therefore, to allow membrane fraction identification [18].

Finally, effects of osmolytes on the activity of membrane-bound enzymes cannot be understood simply in terms of direct osmolyte–enzyme interactions and/or in terms of osmolyte-induced enzyme solvation changes, as proposed for the activity of soluble enzymes [19,20]. In fact, it is likely that alterations in the phospholipid vesicles by the osmolyte have to be taken into account.

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