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INCREASE IN MEMBRANE FLUIDITY IN LIPOSOMES AND PLANT PROTOPLASTS UPON OSMOTIC SWELLING

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Summary

Osmotic gradient across the membrane of nonsonicated liposomes and rose petal protoplasts are shown to induce swelling. Concomitantly, the lipid fluidity as measured by fluorescence depolarization is increased, probably due to increase in molar free volume. It is suggested that osmotic swelling can affect cell physiology via changes in membrane fluidity.

The most prominent physical effectors of membrane fluidity are temperature [1,2], pH [3], multivalent ions [1] and the effective molecular volume [4]. The latter is modulated under conditions naturally occurring in vivo upon changes in the tonicity, which determines cell and organelle volume [5—8]. In the present communication we describe the effects of osmolarity gradients on the fluidity of nonsonicated phospholipid vesicles, as well as plant protoplasts, which accompanied the volume changes.

Nonsonicated multilamellar liposomes were prepared from mixtures of egg phosphatidylcholine (Makor Chemicals), phosphatidic acid (Lipid Products) and cholesterol (BDH) at different proportions. The dry lipid mixtures were suspended by a Vortex mixer in an aqueous solution containing 2 M sucrose (Merck; for density gradients) following the technique of Bangham et al. [9]. Plant protoplasts were isolated from young rose petals (cv. Golden Wave) by enzymatic digestion, as previously described [10] and resuspended in 0.6 M mannitol.

Suspensions of phospholipid vesicles (1 mg lipid/ml) and protoplasts (10^5 cells/ml) were labelled with 10^{-6} M 1,6-diphenyl-1,3,5-hexatriene (DPH, Fluka) by a rapid dilution of $2 \cdot 10^{-3}$ M DPH in tetrahydrofuran into the aqueous mixture, followed by incubation at 37°C for 30 min. Osmotic gradients were created by diluting the above stock suspensions to yield a final

TABLE I

THE EFFECT OF SUCROSE GRADIENTS AND THE PRESENCE OF CHOLESTEROL ON THE SWELLING OF NONSONICATED LIPOSOMES OF EGG PHOSPHATIDYLCHOLINE AND 5% PHOSPHATIDIC ACID

Lipid composition (mol:mol)	Δ[Sucrose] (M)[a]	Initial swelling rate $\frac{d\ 1/A\ (\%)}{dt}$ (min^{-1})[b]	1/A (%)		
			0 h[c]	1 h[c]	24 h[c]
Egg phosphatidylcholine	0.0	0.00	100	100	99
Egg phosphatidylcholine	−1.0	1.32	100	108	108
Egg phosphatidylcholine:cholesterol (2:1)	0.0	0.00	100	100	102
Egg phosphatidylcholine:cholesterol (2:1)	−1.0	0.92	100	115	117

[a] The sucrose concentration in the medium minus its concentration inside the liposomes.
[b] A is the absorbance of the liposome suspension at 450 nm. The values of $1/A$ are presented as a percentage of $1/A$ measured immediately after dilution.
[c] Time after dilution.

concentration of 0.1—0.2 mg lipid/ml and $5 \cdot 10^4$ protoplasts/ml, respectively, in the solution of the desired tonicity. The resulting liposome suspensions were immediately used for absorbance measurements at 450 nm to determine changes in scattering which stem from volume variations [9]. When swelling was completed, about 60 min after preparation, fluorescence polarization measurements were performed, using an instrumentation and technique comprehensively described elsewhere [2]. The results were expressed as $[(r_0/r) - 1]^{-1}$, where r_0 and r are the limiting and the measured fluorescence anisotropy, respectively. Excited state lifetime was measured at 20°C under argon with a pulse sampling instrument [11] followed by a deconvolution analysis. Fluorescence polarization of single intact protoplasts was measured with a Single Cell Microviscositometer (SCM, Elscint) [12].

Unlike single walled liposomes [13], multilamellar liposomes were shown to be osmotic sensitive and to obey the Boyle-Van 't Hoff law [9]. When an osmotic gradient is created, such liposomes change their volume by swelling or shrinking.

Results on the swelling of egg phosphatidylcholine liposomes, with and without cholesterol are summarized in Table I. The swelling was found to proceed for a few minutes until reaching an equilibrium volume, which persisted for over 24 h. The initial swelling rate was slower in the presence of cholesterol, in accord with published data [14]. Swelling of rose protoplasts was achieved by transferring them from 0.6 M to 0.3 M mannitol. The swelling was followed microscopically, and at the equilibrium stage, which was reached after a few minutes, the average radius increased from about 25 to 50 μm, which is in agreement with published data [5].

Swelling was associated with decrease in the fluorescence anisotropy of the liposome suspensions, as demonstrated in Fig. 1. This was true for both

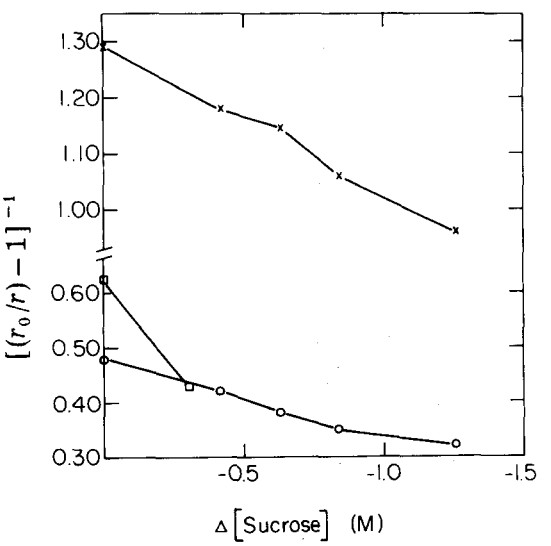

Fig. 1. The correlation between the osmotic gradient and the fluidity parameter $[(r_0/r) - 1]^{-1}$ in non-sonicated liposomes of egg phosphatidylcholine/phosphatidic acid (95 : 5) without cholesterol (○—○) and with 50% cholesterol (X—X) and in rose petal protoplasts (□—□).

types of liposomes, though those containing cholesterol exhibited higher values of fluorescence anisotropy as expected [1,2]. The lifetime of the excited state was measured and found to be 7.9 ± 0.1 ns, independent of the degree of swelling. Hence, the observed decrease in the fluorescence anisotropy, which accompanied the swelling, indicated an increase in the 'fluidity' of the hydrocarbon regions in the liposomes [2], and can be attributed to an increase in the molar free volume of the lipid constituents. Similar results were obtained upon substituting the sucrose with either mannitol or KCl.

Different instrumentation was required to perform fluorescence depolarization measurements of protoplasts upon swelling. Since exposure of plant protoplasts to hypotonic surrounding results in a high percentage of lysis, measurements had to be done on individual intact protoplasts. This difficulty was overcome by using the SCM manually. The results of a representative experiment (out of 8) are shown in Fig. 1. Similarly to the multilamellar liposomes, plant protoplasts exhibited lower fluorescence anisotropy upon swelling.

Osmotic gradients affect cell volume in both plants and animals [5—8], and generate a series of physiological events, such as increased ion transport [6,15], increased leaking of metabolites [16] and changes in membrane potential [5].

The results described in this communication raise the possibility that the overt changes in membrane function by swelling are mediated, to a major extent, by an increase in lipid fluidity.

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References

1 Chapman, D. (1975) Q. Rev. Biophys. 8, 185—235
2 Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta, in the press
3 Watts, A., Harlos, K., Maschke, W. and Marsh, D. (1978) Biochim. Biophys. Acta 510, 63—74
4 Bar, R.S., Deamer, D.W. and Cornwell, D.D. (1966) Science 153, 1010—1012
5 Racusen, R.H., Kinnesley, A.M. and Galston, A.W. (1977) Science 198, 405—407
6 Poznansky, M. and Solomon, A.K. (1972) J. Membrane Biol. 10, 259—266
7 Wang, C.T. and Nobel, P.S. (1971) Biochim. Biophys. Acta 241, 200—212
8 Kirk, I.B. and Hanson, J.B. (1973) Plant Physiol. 51, 357—362
9 Bangham, A.D., de Gier, J. and Greville, G.D. (1967) Chem. Phys. Lipids 1, 225—246
10 Borochov, A., Halevy, A.H., Borochov, H. and Shinitzky, M. (1978) Plant Physiol. 61, 812—815
11 Grinvald, A. and Steinberg, I.Z. (1974) Anal. Biochem, 59, 583—598
12 Bruck, A., Sahar, E., Agmon, E. and Shinitzky, M. (1977) Appl. Optics 16, 564—567
13 Johnson, S.M. and Buttress, N. (1973) Biochim. Biophys. Acta 307, 20—26
14 De Gier, J., Mandersloot, J.G. and van Deenen, L.L.M. (1968) Biochim. Biophys. Acta 150, 666—675
15 Venosa, R.A. (1978) Biochim. Biophys. Acta 510, 378—383
16 Burg, S.P., Burg, E.A. and Marks, R. (1964) Plant Physiol. 39, 185—195