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THE EFFECT OF PRESSURE ON THE PHASE DIAGRAM OF MIXED DIPALMITOYL—DIMYRISTOYLPHOSPHATIDYLCHOLINE BILAYERS

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Summary

The application of 136 atm of helium pressure to suspensions of mixed dipalmitoyl—dimyristoylphosphatidylcholine bilayers caused a 3—5° C elevation of points on the envelope of the binary phase diagram.

Membrane bilayers containing lateral phase separations are able to respond to external pressure by converting fluid phase phospholipids to the more compact gel phase.

There is an increasing body of evidence [1—3] which suggests that the function of membrane-bound proteins is dependent on the existence, within the membrane, of domains of lipids near their phase transition temperature. When both condensed and fluid phases coexist, a membrane bilayer is able to respond to the application of lateral forces by converting some fluid phase to the lower volume condensed phase [4]. Thus membrane-bound proteins could exist in conditions of optimum lateral compression despite changes external to the membrane. The ability of the membrane to convert fluid to condensed phase is governed by the enthalpy of phase transition (ΔH) of the bilayer lipids. The ΔH is in turn a function of the composition of the lipids in the bilayer. The Clausius—Claperyon equation quantitatively relates dP/dT to the volume increase (ΔV) created in a bilayer upon going from a condensed gel phase to a more fluid smectic liquid crystal phase. The increase in bilayer area which occurs during the gel to fluid phase transition is related to ΔV by the concomitant decrease in bilayer thickness.

We have previously shown that the application of high pressure to phosphatidylcholine vesicle bilayers decreases the internal fluidity or molecular

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Abbreviation: TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl.

motion within these bilayers [5]. More recently, we have shown that 137 atm of helium pressure raises the phase transition temperature of a dipalmitoylphosphatidylcholine bilayer by 3° C [6]. These findings suggest that high pressure would affect the distribution of solid and fluid domains within a membrane. Moreover, the application of pressure should be a good technique for studying the properties of membranes which contain mixtures of solid and fluid phases near their characteristic phase transition temperature.

Phase diagrams were constructed by the electron spin resonance (ESR) technique of McConnell [7] which consists of observing the exclusion of the spin label 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) for the condensed phase portion of the bilayer.

The application of 136 atm of helium pressure to suspensions of mixed dipalmitoylphosphatidylcholine—dimyristoylphosphatidylcholine* vesicles caused a 3—5° C elevation of points on the envelope of the binary phase diagram. Thus at an increased pressure, a higher temperature is required to reach a given state of membrane fluidity.

Ether—methanol solutions containing 100 mg of the desired mixtures of dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine** were evaporated to films in small vials. To these vials were added 800 μl of a 0.1 M sodium phosphate buffer at pH 7.4 and 100 μl of 5 mM TEMPO solution prepared in the same buffer. The vials were shaken for 20 min at 5° C above the transition temperature of the binary mixture being studied. Moderate negative pressure was maintained in order to remove any gas bubbles formed.

The vesicle suspension in a 1 mm (internal diameter) quartz ESR tube was placed vertically in a microwave cavity thermostated to within ± 0.1° C over a range of 15—55° C. The desired pressure was attained from a helium sourcell pressurized to 3500 pounds/inch^2. Before beginning a series of measurements, the ESR tube was pressurized to 80 atm with helium and vented several times to remove any residual air in the system. The presence of dissolved helium has was shown to have only a small effect on bilayer fluidity. ESR peak heights from a modified Varian EM-500 spectrometer were measured after curve fitting and smoothing on a PDP 8/e computer***. All spectra were obtained as a function of temperature, beginning 5—10° C above the transition temperature and then cooling at 5° C/h.

After each increment of temperature decrease, the sample was equilibrated for 15 min before the spectrum was measured. Each experiment was duplicated using a fresh vesicle suspension.

Fig. 1 shows the TEMPO spectral parameter f as a function of temperature as well as reciprocal temperature for the aqueous dispersion of the dimyristoylphosphatidylcholine—dipalmitoylphosphatidylcholine (50 mole %) binary system at 1 (△), 68 (□), and 136 (○) atm. The f value, which is proportional to the fractional solubility of TEMPO in the membrane, is a measurement of membrane fluidity [7]. The 1 atm curve (△) can be interpreted as

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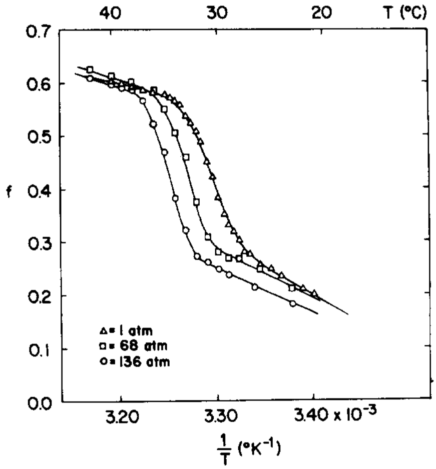

Fig.1. The TEMPO spectral parameter, f, vs T and $1/T$, for an aqueous dispersion of the binary system dimyristoylphosphatidylcholine—dipalmitoylphosphatidylcholine at 50 mole % dipalmitoylphosphatidylcholine. At helium pressures of 1 atm (△), 68 atm (□), and 136 atm (○). The parameter f is a measure of the fraction of total lipid which is in the fluid phase. Abrupt changes of slope occur at the onset and completion of phase separation.

describing a region in which there exists a two-dimensional separation,in the plane of the bilayer, between domains of smectic liquid crystalline fluid phase (F) where the fatty acid chains exhibit high mobility, and a gel phase (S) where they are stiffer and less fluid. As one lower the temperature of the dimyristoylphosphatidylcholine—dipalmitoylphosphatidylcholine binary mixture which is initially entirely in the F phase, one begins to form, at a characteristic temperature, domains of S phase in equilibrium with the F phase. The temperature which marks the transition from pure fluid phase to mixed fluid—solid configurations at 1 atm occurs at 33.5 ± 0.5°C and is determined by the abrupt change in the slope of f vs T. Below this transition temperature, regions of S phase increase until a second transition temperature, 27.5 ± 0.5°C, the binary mixture is entirely in a solid phase configuration. The curves at 68 (□) and 136 (○) atm exhibit a shift towards higher temperatures relative to the control curve (1 atm). We interpret this effect to be due to the fact that hydrostatic pressure decreases the conformational mobility and molal volume of the phospholipid components and causes the onset of solid phase formation at higher temperatures. Fig. 2 is the phase diagram for the dimyristoylphosphatidylcholine—dipalmitoylphosphatidylcholine binary system as a function of temperature and mole % dipalmitoylphosphatidylcholine system of Fig. 1, a 75 mole % dipalmitoylphosphatidylcholine system as well as 100 mole % dipalmitoylphosphatidylcholine and dimyristoylphosphatidylcholine. The application of 68 (□) and 136 (○) atm of pressure successively raise the envelope of the mixed fluid—solid region.

In a previous study [6], the Clausius—Clapyron equation was used to calculate

$$\frac{dP}{dT} = \frac{\Delta H}{T(V_F - V_S)}$$

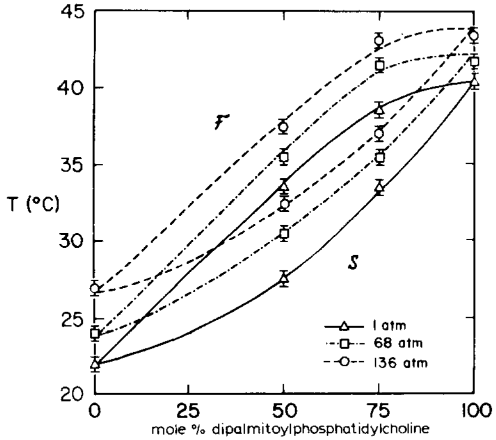

Fig.2. The fluid—solid equilibrium phase diagrams for aqueous dispersions of the dimyristoylphosphatidylcholine—dipalmitoylphosphatidylcholine binary systems: temperature vs mole % dipalmitoylphosphatidylcholine, for helium pressures of 1 atm (△), 68 atm (□), and 136 atm (○). The phase diagrams shown at the three pressures define an area above each envelope where only fluid phase (F) exists, an area within the envelopes in which two dimensional domains of fluid and solid phase exist, and an area below each envelope where only solid phase (S) exists. Pressures of 68 and 136 atm decrease the flexibility and lateral mobility of the phospholipids, causing the onset of solid phase formation at higher temperatures.

an enthalpy of phase transition (ΔH_{pt}) of 8.8 kcal/mole in the pure dipalmitoylphosphatidylcholine bilayer. This value was in good agreement with literature values (9.7 [8] and 8.7 [9] kcal/mole). The $\mathrm{d}P/\mathrm{d}T$ measured for the pure dimyristoylphosphatidylcholine bilayer together with the literature ΔV value of 2.3% [10] yields a ΔH_{pt} of 5.1 kcal/mole. The large variation in ΔV values from different investigators suggests that this value is within experimental error of the literature value for ΔH of 6.3 [8] and 6.6 [9] kcal/mole.

The results of this study provide quantitative support for the prediction that phospholipid bilayers could respond to external pressure by converting fluid phase lipids to the more tightly packed gel phase [4]. The ability to use pressure to control membrane internal fluidity and the distribution of domains of gel phase phospholipids may be useful to future investigations of membrane lipid—protein interactions.

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