Biochimica et Biophysica Acta, 731 (1983) 491–494
Elsevier

BBA Report

BBA 70093

DESTABILIZATION OF A LIPID NON-BILAYER PHASE BY HIGH PRESSURE

PAUL YAGER and EDDIE L. CHANG

Optical Probes Branch, Code 6510, Naval Research Laboratory, Washington, DC 20375 (U.S.A.)

(Received December 13th, 1982)
(Revised manuscript received March 29th, 1983)

Key words: Lipid non-bilayer; Phase destabilization; High pressure

Pressure is found to destabilize the non-bilayer phase with respect to the bilayer in a model lipid system. The lamellar to inverted hexagonal (H_{II}) phase transition of aqueous egg phosphatidylethanolamine is shifted to higher temperatures by hydrostatic pressure. The slope of the increase in transition temperature is constant to beyond 300 bar, and is greater than that seen for other lipid phase transitions. This behavior is consistent with the hypothesis that increasing chain disorder drives the conversion from the bilayer into the hexagonal phase. If this non-bilayer lipid phase is an intermediate in membrane fusion, then pressure should inhibit the process. This may explain the inhibition of chemical transmission at neural synapses by pressure.

There is currently much interest in the possible role of non-bilayer lipid phases in controlling fusion-related events in biological membranes [1–4]. Such events include the vesicle-membrane fusion which occurs during endo- and exocytosis. An important example of this process is vesicular neurosecretion at chemical synapses. The function of such synapses is known to be strongly depressed by relatively moderate pressure [5,6], and considering the relative insensitivity of proteins, this suggests that the pressure-sensitive phase transitions of lipids [7] may be responsible for the effect. Changes in bilayer fluidity have for some time been accepted as a means of controlling the function of some intrinsic membrane proteins, and hence the functions of the biological cell. However, little is known of the effects of the environment on lipids which are not confined to the plane of a bilayer.

It is clear that any event in which two bilayers fuse to become one, or vice versa, must include a step in which there is a breakdown of the pure bimolecular leaflet, a topological 'catastrophe'. The particular non-bilayer phase which has come to the fore as a probable candidate is the inverted hexagonal (H_{II}) phase long known to exist in low-water states in lipids such as extracted brain lipids [8]. A wealth of data on the non-bilayer structures formed by extracted lipids in a variety of aqueous environments [2–4] have combined to build a promising case for the role of the H_{II} phase in important biological processes [1]. However, compared to the lamellar (L) gel and liquid-crystal phases of these lipids, little is known of the H_{II} phase or the conversion between it and the lamellar phase. Since pressure favors the phase of lower volume, the relative volumes of the two phases must be determined when considering the effect of hydrostatic pressure on a biological process which may have transient non-bilayer states.

Unsaturated phosphatidylethanolamines in excess water have at least two phase transitions: a gel-to-liquid-crystal chain melting transition at low temperature (T_m), and another at higher tempera-

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

ture (T_h) at which the bilayers convert into the invert cylinders of the H_{II} state [1,9]. The temperatures of both transitions depend on the fatty acyl chain composition of the lipid, and for egg phosphatidylethanolamine made by transphosphatidylation of egg phosphatidylcholine, the L-H_{II} transition has been reported to occur at about 56°C. To elucidate the nature of a reported hysteresis in the transitions we have measured the dependence of these two transition temperatures in this simple model system on the rates of heating and cooling at atmospheric pressure. The egg phosphatidylethanolamine was prepared by Avanti Polar Lipids (Birmingham, AL) and was used without further purification. Samples of lipid were rotovaporized to dryness, with subsequent high vacuum evaporation for at least 12 h. These were then hydrated with buffer (20 mM NaCl, 2 mM Hepes, 0.1 mM EDTA at pH 7.4) by brief sonication with a standard laboratory ultrasonic cleaner (Cole-Parmer) at room temperature, resulting in a white flocculent precipitate. An aliquot of this dispersion was transferred to a stainless steel pan and calorimetry was performed using a Perkin-Elmer DSC-2 equipped with a Bascom-Turner digital plotter. The calorimetric peaks were seen to shift as a function of scanning rate in the range of 1 to 10 K/min. Extrapolation of the transition temperatures to zero rate of change gives the following: t_m(heating) = 16°C, t_m(cooling) = 15°C, t_h(heating) = 56°C and t_h(cooing) = 47°C. The small difference in the t_m for heating and cooling is probably not significant, whereas the 9 K gap between the heating and cooling values of t_h is clearly due to an instrument-independent hysteresis. The hysteresis is presumably due to a large energy barrier to conversion from one form to the other, as might be expected for so large a topological change as must occur. Also, the differing water contents of the two phases may necessitate bulk water flow through the liposomes during the transition.

Our method for examining the influence of hydrostatic pressure on the L-H_{II} transition, although simple, is sufficiently novel to merit description. The lipid was prepared as described previously, and then pelleted in melting point capillary tubes. The final lipid concentration of the pellet was approx. 50% by weight. The capillaries were then sealed with Seal-Ease hematocrit sealer (Clay Adams, Inc.) and placed into a custom-designed capillary holder inside a high-pressure cell (NOVA SWISS) with sapphire windows. The temperature of the cell was controlled by a circulating water bath (Neslab) and measured with a thermocouple inside the cell (Autoclave Engineers). Temperature readings were converted by a digital thermometer (Omega, Inc.) to binary coded decimal form. The photon count rate and temperature were recorded with an Apple II microcomputer at 0.1 K intervals. Pressure, applied with a hand pump (Enerpac) and measured with a calibrated gauge (Aminco), was held constant during through the temperature scans. Heating scans were at a rate of about 1 K/min, which we have shown to be slow enough to introduce only negligible kinetic effects. The temperatures were determined by picking the first large change in light transmission on either heating or cooling. The cooling curves were made at greater speed (3 K/nm) particularly at higher pressures, which may have induced some curvature into the cooling data. The upper limits of the pressures accessible to us were determined not by the cell construction but by the high temperatures to which the transition shifts at such pressures. We wished to avoid degradation of the sample from exposure of the the sample to such high temperatures for extended periods, but found that atmospheric pressure transition temperatures determined before and after the high pressure scans were identical.

The capillary was located between two sapphire windows to allow entry of a focussed, polarized, low power He-Ne laser beam on one side and observation through a perpendicular polarizer with a photomultiplier tube on the opposite side (zero-angle geometry). The light level measured therefore was the depolarized component of almost randomly polarized 'transmitted' light. We found that both phase transitions were accompanied by large changes in the light level, giving a 10-fold increase in intensity. In contrast to the high sensitivity of this experimental method, the L-H_{II} transition was hardly detectable by standard light absorbance measurements on suspensions of egg phosphatidylethanolamine liposomes. The magnitude and the abruptness of the change in our method allowed us, while scanning the tempera-

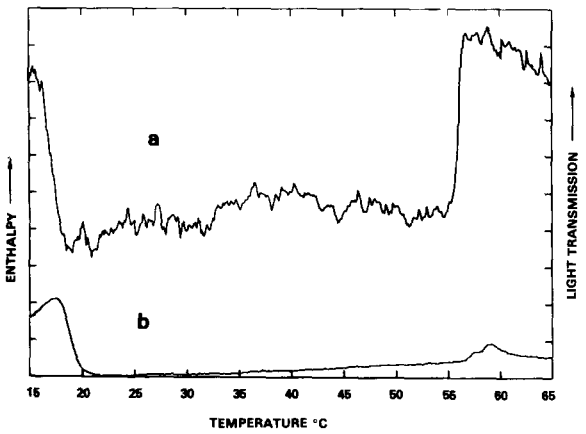

Fig. 1. Comparison of the optical transmission technique with differential scanning calorimetry for egg phosphatidylethanolamine at atmospheric pressure showing both the gel-to-liquid crystal transition and the liquid-crystal-to-hexagnal transition. Curve (a) is the depolarized transmitted signal from a 1 K/min heating scan (with the baseline shifted upwards for display clarity), and curve (b) is a calorimetric heating scan at 5 K/min. The slight displacement of the two H_{II} transitions is due to differences in the heating rates. Note the sharpness of the optical signal change as compared to the calorimetric scan. Scaling of the two curves is arbitrary.

ture of the cell, to optically ascertain the initiation of the phase transition to within one tenth of a degree (see Fig. 1).

The application of pressure to the sample raised both phase transition temperatures, but as we were concerned primarily with the properties of the H_{II}

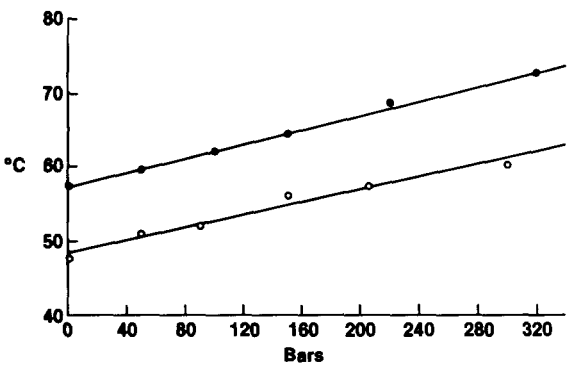

Fig. 2. The optically-observed L-H_{II} transition temperatures for heating (●) and cooling (○) of egg phosphatidylethanolamine are plotted against the applied pressure. The lines in the figure represent least-squares fits to all of the points, and the coefficient of determination is 0.998 for the heating line.

state, and the gel-to-liquid-crystal transition is quite broad in this compound, we have focussed only on the effects of pressure on the L-H_{II} transition (see Fig. 2). The increase in the transitin temperature is linear with pressure up to at least 300 bar, with constant hysteresis. The slope for the forward L-H_{II} transition, which we believe to involve less instrument error because the heating was performed more slowly than the cooling, is +0.0471 K/bar, which, for comparison, is approximately twice as steep as the slope for the chain melting transition of dipalmitoylphosphatidylcholine [9,10]. However, since the molar volume change is also proportional to the ΔH of the L-H_{II} transition, the actual increase in volume is small. If we use 0.7 kcal/mol for the value of ΔH [8], we calculate from the Clausius-Clapeyron equation that the total molar increase in volume is only 4.1 cm^3/mol, which is one seventh the value for the dipalmitoylphosphatidylcholine chain melting transition [10,11].

We have shown that the stability of a non-bilayer phase is sensitive to hydrostatic pressure. Furthermore, this is the most pressure-sensitive lipid phase transition found to date. As the bilayer form is stabilized at any temperature as a consequence of the smaller volume of this phase, processes involving the H_{II} phase as an intermediate [4] should be slowed or stopped by the application of hydrostatic pressure. Of course only one lipid has been tested so far, but many lipids undergo similar L-H_{II} transitions with increasing temperature or changing ionic concentrations [1,11]. It is well known that the number of *gauche* bonds in lipid hydrocarbon chains grows with increasing temperature and that the *gauche* conformation occupies more volume than the *trans* conformation. Our data support the conclusions of others [1,7,11] that, in the presence of excess water, the driving force for these L-H_{II} transitions is an increase in *gauche* conformations of the chains past the point where the heat groups can 'cover' a bilayer. Most L-H_{II} transitions should show a similar pressure dependence.

The pressure sensitivity of the H_{II} phase provides a basis for testing for the role of non-bilayer phases in biological phenomena such as membrane fusion. Sensitivity of membrane fusion to pressure may well prove to underlie the effects of hydro-

static pressure on the nervous system, as well as on other organs which perform secretory functions. This initial work provides a physical basis for such investigation.

This study was supported by the Office of Naval Research. One of us (P.Y.) was the recipient of a National Research Council/Naval Research Laboratory Resident Research Associateship during the period of this research.

References

1 Cullis, P.R., De Kruijff, B., Hope, M.J., Nayar, R., Rietveld, A. and Verkleij, A.J. (1979) Biochim. Biphys. Acta 559, 399–420
2 Cullis, P.R., De Kruijff, B., Hope, M.J., Nayar, R., Rietveld, A. and Verkleij, A.J. (1980) Biochim. Biophys. Acta 600, 625–635
3 Hope, M.J. and Cullis, P.R. (1979) FEBS Lett. 107, 323–326
4 Hui, S.W., Stewart, T.P., Boni, L.T. and Yeagle, P.L. (1981) Science 212, 921–923
5 Kendig, J.J. and Cohen, E.N. (1976) Am. J. Physiol. 230, 1244–1249
6 Kendig, J.J., Trudell, J.R. and Cohen, E.N. (1975) J. Pharmacol. Exp. Ther. 195, 216–224
7 Heremans, K. (1978) in High Pressure Chemistry (Kelm, H., ed.), pp. 467–487, D. Reidel Publishing Co., Dordrecht
8 Luzzati, V. (1968) in Biological Membranes (Chapman, D., ed.), pp. 71–124, Academic Press, New York
9 Boggs, J.M., Stamp, D., Hughes, D.W. and Deber, C.M. (1981) Biochemistry 20, 5728–5735
10 Liu, N.I. and Kay, R.L. (1977) Biochemistry 16, 3484–3486
11 Nagle, J.F. and Wilkinson, D.A. (1978) Biophys. J. 23, 159–175
12 Mantsch, H.H., Martin, A. and Cameron, D.G. (1981) Biochemistry 20, 3138–3145