CHOLESTEROL-INDUCED MODULATION OF MEMBRANE HYDRATION STUDIES BY THERMAL ANALYSIS

Lisbeth TER-MINASSIAN-SARAGA and Georgette MADELMONT

Physico-Chimie des Surfaces et des Membranes, Equipe de Recherche du CNRS associée à l'Université Paris V, UER Biomédicale, 45 rue des Saints-Pères, 75270 Paris cedex 06, France

Received 18 November 1981

1. Introduction

Incorporation of cholesterol Ch into dipalmitoylphosphatidyl choline DPPC multibilayers induces disorder in the low temperature gel state and order in the liquid crystalline state as shown by IR spectroscopy [1]. Differential scanning calorimetry DSC [2–4] and X-ray diffraction studies [1,5] of fully-hydrated, mixed DPPC + Ch bilayers show that their behaviour is complex at low Ch content. At low Ch content and temperature the system is biphasic [5]. One of the phases is DPPC in the gel state. The second displays a 3.5 nm thick aqueous spacing broader than the 1.9 nm thick aqueous spacing of the DPPC-hydrated gel phase.

We have used thermal analysis [6,7] (DSC differential scanning calorimetry) to measure directly the amount of water forming the interbilayer aqueous spacing in a multibilayer system. We apply this technique to the systems studied by other techniques such as the X-ray diffraction technique [5] and various dynamic techniques [8]. The agreement between our results and those in [5,8] are better than qualitative.

Cholesterol modulates membrane antigenity expression by affecting the membrane microviscosity [9] and may be involved in modulating the ionic channels in reconstituted acetyl choline receptors vesicles [10]. We have shown [7] that bilayer fluidity and hydration modulation are correlated properties. Furthermore, membrane–membrane interaction, adhesion and fusion may be modulated by its state of hydration. We wonder whether membrane antigenity is not related to both membrane fluidity and to state of hydration, which are correlated membrane properties.

2. Experimental

Ch and DPPC were purchased from Fluka and Merck, respectively and were used as received. Samples were prepared from stock solutions in chloroform/methanol, 9/1 (v/v). The solvents were of spectroscopic grade purity.

Ch and DPPC solutions were deposited inside the DSC cups and the solvent evaporated in vacuum and dark. The dry sample composition is expressed as mol fraction of DPPC, x_{DPPC}. It weighed ~0.5 mg ± 0.005 mg. An equal amount of water was added. The cup was sealed and incubated at 60°C for 32 h. Leakage of water out of the cups was controlled by weighing. The samples were stocked at –18°C.

The thermograms (fig.1) were obtained with a 990-910 Du Pont de Nemours Thermal Analyzer equipped with a mechanical cooling accessory. Heating–cooling cycles were performed at 2°C min^{-1} between –65 and 60°C. In general the sensitivities used were 48 $\mu cal \cdot s^{-1} \cdot cm^{-1}$ or 96 $\mu cal \cdot s^{-1} \cdot cm^{-1}$. The areas of the peaks were measured with a planimeter and converted into heat amounts. The values for the molar enthalpy for the gel–liquid crystal transition of DPCC were obtained by dividing the heat of the high temperature endothermic transition (fig.1b) by the amount of DPPC only. The temperatures at the peaks maxima are shown in fig.2 for the various systems. The endothermic low temperature (<0°C) peaks and the 'exothermic' peaks at 0°C (fig.1a) correspond to frozen water melting inside the sample and inside the reference cups, respectively. A reference thermogram [6,7] (not shown) established with pure water in the reference cup and an empty cup on the sample holder calibrates the apparatus. The interpretation of these peaks has been given elsewhere [6,7]. It is based

Published by Elsevier/North-Holland Biomedical Press
00145793/82/0000–0000/$02.75

on the hypothesis that the state of the water molecules between bilayers is perturbed by the phospholipid molecules polar head. This water will freeze and melt at a temperature different from that of pure bulk water. If the fully hydrated sample contains also excess external water, the external ice will melt at 0°C and the corresponding heat will be deduced from the heat involved in the reference ice melting. The net heat of ice melting inside the reference cup is proportional to and is converted into the number of the water molecules inside the sample perturbed by the bilayers.

3. Results

The thermograms shown in fig.1b represent the high temperature end of the thermal cycle. Shown are the results for x_{DPPC} = 1, 0.95, 0.9, 0.85, 0.5 and 0. Not shown are those for x_{DPPC} = 0.75, 0.60, 0.40. The peaks corresponding to DPPC chains transition may be considered to be located at the same sample temperature, the small (≃0.5°C) temperature shifts between the cooling and the heating modes being due presumably to the small (≃±0.25°C) thermal lag between the sample and its holder. Only the x_{DPPC} = 1 sample might have shown some supercooling. The temperature T_t-values at the peak maxima are compared in fig.2 with the values reported by [4]. Our values for T_t agree with those in [3]. They are 0.5°C lower than the values reported in [4] for the compositions $1 > x_{DPPC} > 0.85$. Our results of T_t and those reported by [3,4] are higher than the ideal chain freezing temperature T_{id} calculated according to [11] on the assumption of an ideal solution of Ch in DPPC above T_t with pure DPPC separation at T_t (see fig.2 legend). The value of T_t for x_{DPPC} = 0.75 corresponds to a second order transition and has been disconnected. As reported in [3,4] we confirm that for $x_{DPPC} < 0.75$ the thermograms did not display high temperature sharp peaks. Also we note as in [3,4] that as x_{DPPC} decreases the sharp peaks area decreases and a broad peak with a maximum which might be located around 41°C emerges. The two peaks areas are converted into ΔH_{DPPC} the molar enthalpy of DPPC chain melting and plotted *vs* DPPC content in fig.3. Results from [3,4] are also shown. The agreement with [3] is satisfactory. The results of [4] are lower because the authors used only the sharp peak areas.

The number of water molecules per bilayer mole-

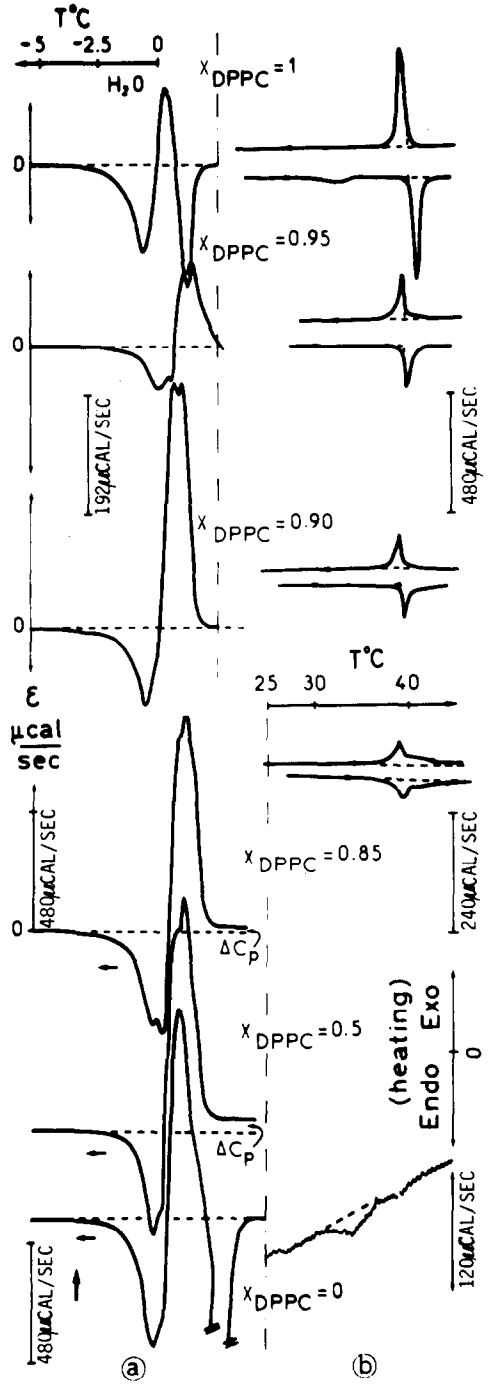

Fig.1. Thermograms for heating–cooling cycles with DPPC + Ch fully hydrated bilayers: overall hydration, 50% (w/w); x_{DPPC}, DPPC% (mol/mol); (a) low temperature cycles obtained between −65 and ~0°C allowing only partial melting of ice inside the reference cup; ΔC_p, shift in the heat capacity of the sample at 0°C; (b) heating (→) and freezing (←) scans. Temperature of peaks maximum (see fig.2).

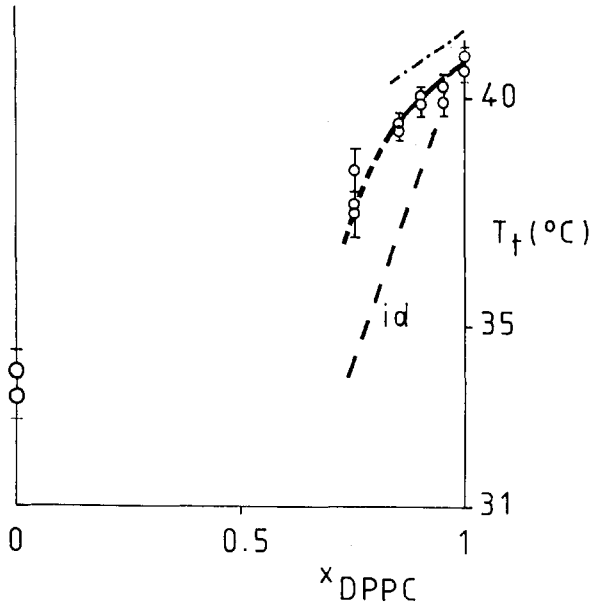

Fig.2. DPPC chain melting and freezing temperatures in fully hydrated DPPC + Ch bilayers: x_{DPPC}, % DPPC (mol/mol). The ideal phase diagram T_{id} (x_{DPPC}) is calculated as in [11] using the equation $\ln x_{DPPC} = -(\Delta H_{DPPC}/R)(T^{-1} - T_{DPPC}^{-1})$ with ΔH_{DPPC} = 8.5 kcal . mol^{-1}, R = 1.98 cal . mol^{-1} . deg^{-1}, T_{DPPC} = 314 K. The result for x_{DPPC} corresponds to a second order transition (no peak). Dashed line: results of [4].

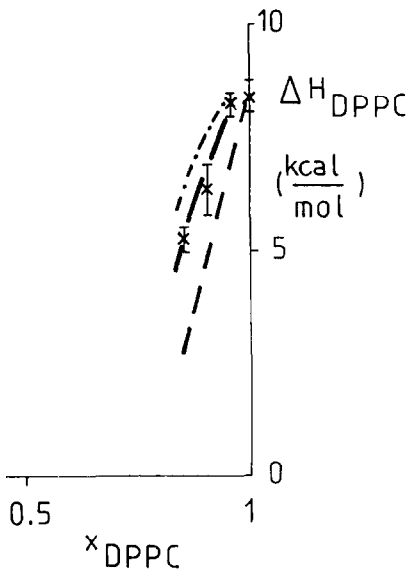

Fig.3. Molar enthalpy of DPPC chain melting ΔH_{DPPC} in fully hydrated DPPC + Ch bilayers: x_{DPPC}, DPPC% (mol/mol); (×) this report; (○) [4]; (+) [3].

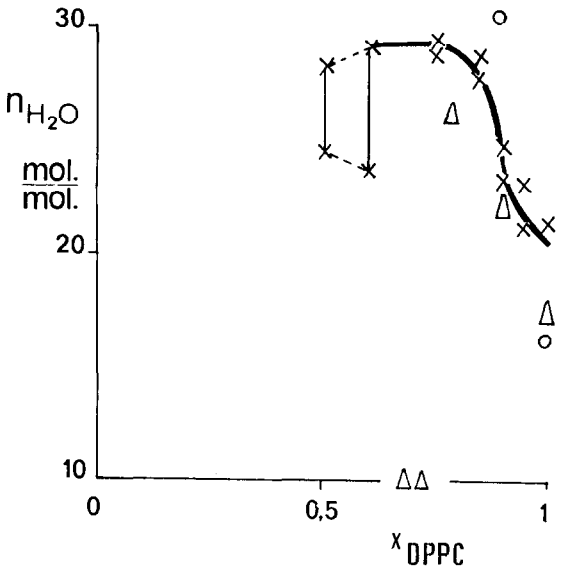

Fig.4. Hydration of fully hydrated mixed DPPC + Ch bilayers: x_{DPPC}, DPPC% (mol/mol); (○) [5]; (△) [8]; (×) this report. Note the drop in hydration around the 20–30% (mol/mol) Ch content for both monolamellar vesicles [8] and for swollen 'planar' multibilayers (this report).

cule is determined by thermal analysis from the exothermic peak area at 0°C. The corresponding heat of ice melting inside the reference cup is divided by L_0 = 1.4 kcal . mol^{-1} the molar enthalpy of bulk ice melting and by the number of bilayer molecules (DPPC + Ch). The results are shown in fig.4 as a function of bilayers composition. They are compared to the results reported in [5,8] for mixed or pure phospholipid multibilayers or monolamellar vesicles. The 3 sets of results confirm that low contents of cholesterol increase the bilayer hydration.

4. Discussion and conclusion

Our results (fig.2, fig.3) and the results in [3,4] for the low content Ch bilayers point clearly to either a very small activity of Ch inside the fluid DPPC bilayers or to a small difference between the compositions of, respectively, the fluid or the frozen DPPC bilayers; the bidimensional 'solutions' as argued in [10].

Spectroscopic studies [1,11] point to the second possibility. Yet, there is no clear picture of Ch–DPPC molecular interactions in the hydrated bilayers. Both the polar and the non-polar parts of DPPC molecules

seem to be involved as shown by spectroscopic studies [1,12].

These results and those in [5,8] show that Ch incorporation into DPPC bilayers up to 15–25% mol/mol enhances the bilayer hydration by 30–50% (fig.4). This enhancement of hydration is observed below 0°C (our results), over 5–40°C [5], and at 20°C ([8] for egg lecithin). It is displayed for very concentrated multibilayers (our paper and [5]) as well as by sonicated dilute monolamellar suspensions [8]. Therefore under all these different conditions the hydration effects are comparable. However, some bilayers are formed by egg lecithin [8] and others by synthetic DPPC (here, [5]); in [8] hydration is deduced (indirect determination) from elaborate studies of dynamic properties (vesicles diffusion constant and sedimentation velocity) while in [5] and in this study hydration is measured at equilibrium by static methods (X-ray diffraction and thermal analysis). Hence bilayer hydration is a well-defined, quatifiable property of these systems.

The discontinuity in the bilayer hydration evolution with Ch content at about x_{Ch} = (0.2–0.3)% (mol/mol) might be relevant to the modulation of membrane antigenicity expression through its effect on cell adhesion. Membrane cholesterol depletion below the critical x_{Ch} = (0.2–0.3)% (mol/mol) might induce an increase in cell membrane hydration which might modulate cell adhesion to other cells or to a foreign substrate. This result is complementary to that in [9] on cholesterol modulation of membrane microviscosity. The modulation of membrane hydration by cholesterol might be relevant also to the modulation of membrane-induced ionic permeability of acetyl choline receptor-reconstituted vesicles [10].

It is most interesting that 25% cholesterol (mol/mol) is a critical bilayer composition for both bilayer hydration (this report) and for vesicle membrane permeability [10].

Acknowledgements

V. Malet-Ferroni has typed the manuscript and J. M. Lepecq has drawn the graphs.

References

[1] Cortijo, M. and Chapman, D. (1981) FEBS Lett. 131, 245–248.
[2] Ladbrooke, B. D., Williams, R. M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340.
[3] Estep, T. M., Mountcastle, D. B., Biltonen, R. L. and Thomson, T. E. (1978) Biochemistry 17, 1984–1989.
[4] Mabrey, S., Mateo, P. L. and Sturtevant, J. M. (1978) Biochemistry 17, 2464–2468.
[5] Rand, R. P., Parsegian, V. A., Henry, J. A. C., Lis, L. J. and McAlister, M. (1980) Can. J. Biochem. 58, 959–968.
[6] Ter-Minassian-Saraga, L. and Madelmont, G. (1981) J. Colloid Interface Sci. 81, 369–384.
[7] Ter-Minassian-Saraga, L. (1981) Pure and Appl. Chem. 53, 2149–2166.
[8] Newman, G. C. and Ching-Hsien Huang (1975) Biochemistry 14, 3363–3370.
[9] Shinitzky, M. and Souroujon, M. (1979) Proc. Natl. Acad. Sci. USA 76, 4438–4440.
[10] Dalziel, A. W., Rollins, E. S. and McNamel, M. G. (1980) FEBS Lett. 122, 193–196.
[11] Lee, A. G. (1977) Biochim. Biophys. Acta 472, 285–344.
[12] Seelig, J. and Seeling, A. (1980) Quart. Rev. Biophys. 13, 19–61.