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THE THERMOTROPIC TRANSITION OF LARGE UNILAMELLAR (LUV) VESICLES OF DIMYRISTOYL PHOSPHATIDYLCHOLINE BY RAMAN SPECTROSCOPY

H. Van Dael, P. Ceuterickx, J.P. Lafaut and F.H. Van Cauwelaert

Interdisciplinair Research Centrum

Katholieke Universiteit Leuven Campus Kortrijk, B-8500 Kortrijk

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By observing Raman spectra in the 2800-3000 cm^{-1} region, we determined the gel to liquid transition characteristics of large unilamellar vesicles of dimyristoyl phosphatidylcholine. This transition occurs between 22 °C and 28.5 °C. The large unilamellar vesicles can be well distinguished from the small unilamellar vesicles, which gel to liquid transition is spread out from 10 °C to 27 °C. The multilamellar vesicles on the other hand melt in a highly cooperative phenomenon at 23.7 °C. The intermediate character of the transition of large unilamellar vesicles is also illustrated by data of the lateral order parameter.

INTRODUCTION

Phospholipids are with proteins the main components of the cell membranes. Fundamental studies on lipid-protein interactions and on the physico-chemical characteristics of lipids are carried out by model systems (1,2). Three forms of model systems are used : small unilamellar vesicles (SUV), large multilamellar vesicles (MLV) or liposomes and large unilamellar vesicles (LUV). There is much interest in the latter type of vesicles since they have a large internal volume which allows a higher entrapment of different molecules in the vesicle. These vesicles are then used as carriers in various practical applications (2). Different methods have been used to prepare such vesicles : LUV are prepared by the ether vaporization method of Deamer and Bangham (3), by the dialysis method by Milsmann and al. (4) and by the reverse-phase evaporation method by Szoka and Papahadjopoulos (5). Differences between the SUV and MLV due to the change in bilayer curvature and to multilamellarity have been described with different techniques (6-9) and also with Raman spectroscopy (10-12). Much less information is available however on the large uni-

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lamellar vesicles. They differ from the SUV only in curvature but compared with the MLV, they change the number of bilayers as well. In the course of a study that compares the behavior of SUV, MLV and LUV towards the protein α-lactalbumin (13), we studied the thermotropic transition characteristics of these three forms of vesicles of dimyristoyl phosphatidylcholine by Raman spectroscopy.

It is the aim of this paper to compare the gel to liquid transition of the LUV with the known data on the SUV and MLV forms of dimyristoyl phosphatidylcholine.

MATERIALS AND METHODS

Dimyristoyl phosphatidylcholine (DMPC) was purchased from Sigma. Purity and composition were confirmed by thin-layer and gaschromatography. Since our study (13) on the interaction of these different types of vesicles with α-lactalbumin is carried out at pH = 4 in 0.1 M NaCl and 0.01 M acetate buffer, the same conditions were maintained here. Small unilamellar vesicles (SUV) were prepared as described before (14). Multilamellar vesicles (MLV) or liposomes were prepared by vortexing the buffer solution of the phospholipid for 10 min. at room temperature. Large unilamellar vesicles (LUV) were prepared by the reverse-phase evaporation method (5). Diethylether is used as original solvent. 50 µl of these samples were transferred into Kimax melting point capillaries and they were sealed after a centrifugation during 10 minutes. Raman spectra were recorded with a Coderg double monochromator using the 514.5 nm line of a Coherent Radiation argon ion laser at a typical power of 300 mW at the sample. The spectral band width was 5 cm^{-1}. All spectra were taken in order of descending temperature and were controlled by measurements as a function of ascending temperature. The temperature of the sample was held constant to ± 0.1 °C by fitting it in a copper block, that is thermostated by internal circulation of water at constant temperature. Temperature was measured by a chromel-alumel thermocouple mounted very near to the sample. All temperature readings were corrected by 1.7 °C to account for laser heating. This correction was determined by observing the melting point of methylmyristate, methylpalmitate and methylstearate at respectively 19 °C, 30 °C and 39.1 °C by Raman spectroscopy.

RESULTS AND DISCUSSION

The different molecular structures of DMPC-vesicles can be studied by observing the ratio of the 2890 cm^{-1} band to the 2850 cm^{-1} band in the Raman spectrum. The former band is assigned to the methylene asymmetric CH stretch and the latter one to the symmetric CH stretch. A disruption of a regular chain packing like in a melting proces results in a decrease of the 2890 cm^{-1} intensity by the breakdown of the Fermi resonance between neighbouring chains.

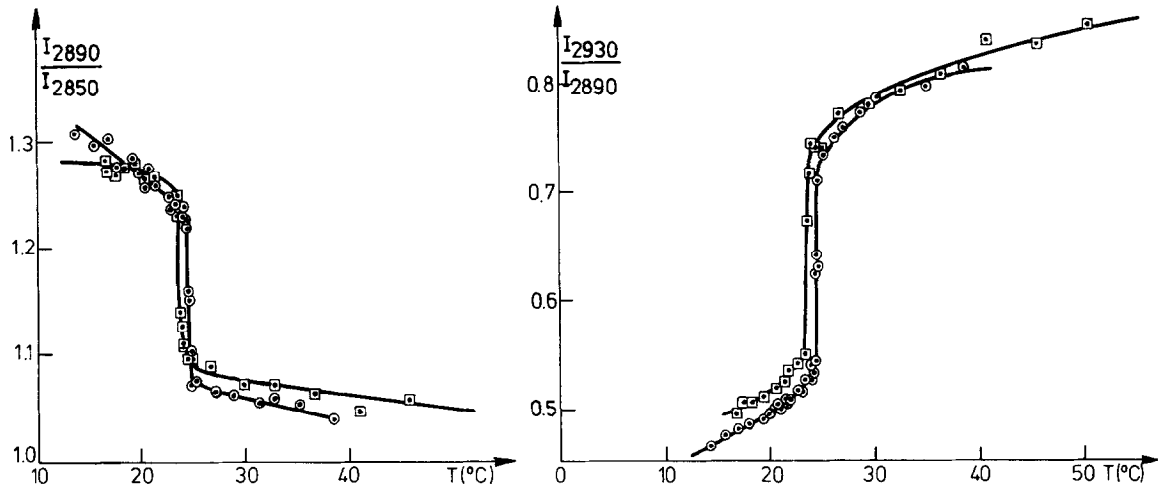

Figure 1. Temperature dependent plots of the I_{2890} cm^{-1}/I_{2850} cm^{-1} and the I_{2930} cm^{-1}/I_{2890} cm^{-1} ratio's for multilammelar vesicles of dimyristoyl phosphatidylcholine at pH = 7 (⊡) and at pH = 4 (⊙).

The intensity ratio, noted as I_{CH_2}, can be used (12) to define the lateral order parameter $S_L = \frac{I_{CH_2} - 0.7}{1.5}$. This parameter, having the meaning of a probability, is zero for the hexadecane liquid and one for the hexadecane solid. Without attributing an absolute quantitative value to S_L, it seems to be a good measure for the lateral chain order.

The melting of DMPC can also be studied by taking the ratio of the methylene asymmetric stretching modes at 2930 cm^{-1} and 2890 cm^{-1} or by comparing the 2930 cm^{-1} to the 2850 cm^{-1} feature. Transition temperatures determined in this way, completely coincide with these determined by the I_{CH_2} ratio (15). In fig.1, we used the I_{CH_2} and $\frac{I_{2930}}{I_{2890}}$ ratio to construct the temperature profile for MLV of DMPC in H_2O at pH = 7 and pH = 4. At pH = 7 the transition from the gel to the liquid crystal state is very sharp at 23.7 °C, completely in agreement with the 23.7 °C (16) and 23.9 °C (7) found by differential scanning calorimetry measurements. This transition temperature is obviously higher than the transition temperatures from other Raman experiments on DMPC vesicles. Lavialle found respectively 22.5 °C (17) and 23 °C (18) and from the plots of Susi (19) a transition temperature around 22 °C can be derived. The skeletal

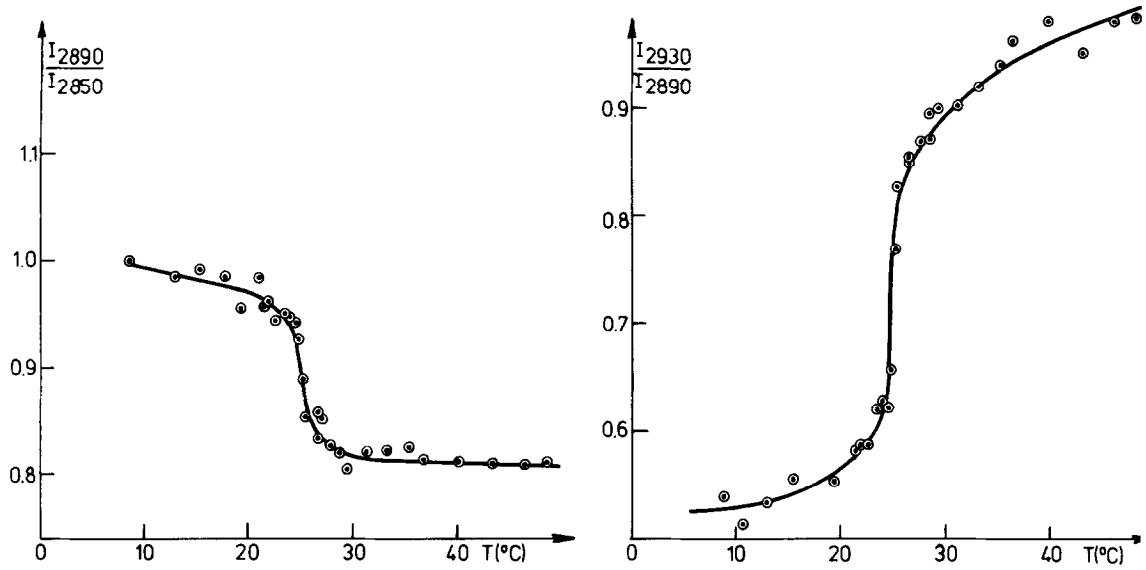

Figure 2. Temperature dependent plots of the I_{2890} cm^{-1}/I_{2850} cm^{-1} and the I_{2930} cm^{-1}/I_{2890} cm^{-1} ratio's for large unilamellar vesicles of dimyristoyl phosphatidylcholine at pH = 4.

optical modes in the 1100 cm^{-1} region, that are also appropriate to study the gel to liquid transition, confirm the same sharp transition temperature. The pretransition, that is found by differential scanning calorimetry at 14 °C (7,19), is not observed in these measurements nor in the Raman experiments of Susi (20) either. Decreasing the pH to 4, the transition remains abrupt but the transition temperature increases a little bit to 24.5 °C, in accordance with fluorescence polarization measurements at the same pH (21). At high temperatures S_L = 0.24 while the low temperature value approximates 0.40.

A small broadening of the transition region from 22 °C to 28.5 °C is found in the LUV melting curve (fig.2). In comparison to the abrupt transition for MLV, the gel to liquid transition progresses more gradually. At high temperature, I_{CH_2} remains practically constant corresponding to S_L = 0.07. At low temperature, the shape of the melting curve seems to differ for various samples possibly due to some aggregation that occurs as is apparent from electron microscopy graphs taken as a function of time (13). At 10 °C, S_L is included between 0.15 and 0.19.

The transition behavior of the SUV is characterized by a very smooth transition ranging from 10 °C to 27 °C (fig.3). The medium of this transition

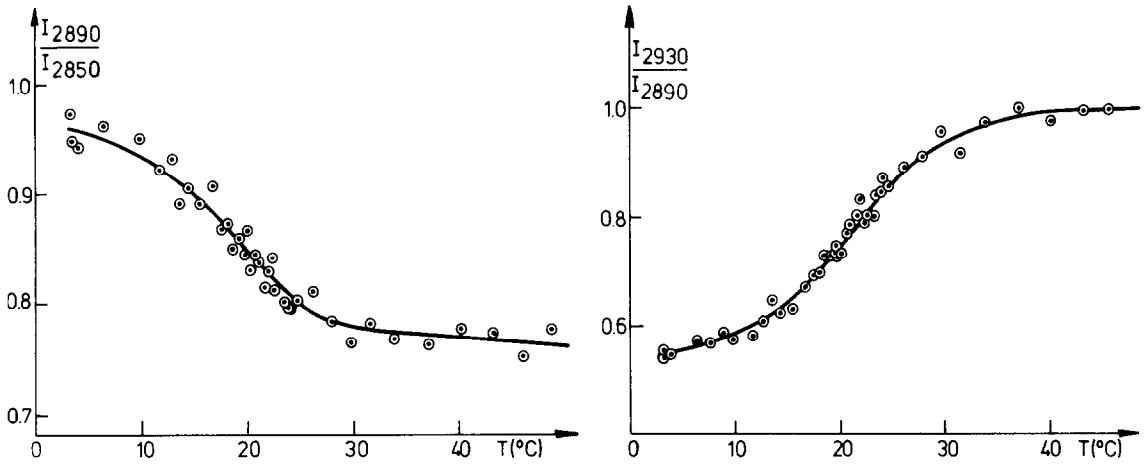

Figure 3. Temperature dependent plots of the I_{2890} cm^{-1}/I_{2850} cm^{-1} and the I_{2930} cm^{-1}/I_{2890} cm^{-1} ratio's for small unilamellar vesicles of dimyristoyl phosphatidylcholine at pH = 4.

range is 18 °C, an obvious lower value than that of the LUV's and the MLV's. This is in accordance with similar measurements by Gaber and Peticolas (12), who found a sharp transition temperature for a dipalmitoyl phosphatidylcholine dispersion at 41.5 °C, while the vesicles melt with a broad transition centered at 37 °C. The I_{CH_2} of our samples becomes nearly constant above 30 °C so that the S_L goes to a constant value of 0.04 at 50 °C. That low value indicates a nearly complete absence of lateral interaction and suggests a pure liquid character. Different and independent measurements shows that not only the broad transition is reproducible but also the I_{CH_2} and S_L values in the transition region and at high temperatures. The behavior of these parameters below the transition temperature seems to be more specific because at these temperatures, a slow transformation from single lamellar into bigger aggregates occurs in a time dependent process (8). The S_L parameter as a function of the temperature is given in table 1. The LUV value falls down more rapidly than the SUV value but remains higher in the whole temperature range. Small unilamellar vesicles (radius 300Å) indeed have a less ordered hydrocarbon chain packing by the small radius of curvature of these vesicles. Electron microscopy photographs of our LUV reveal a size between 1000 Å and 2000 Å (13). The greater radius of curvature gives rise to a more ordered packing between the

TABLE 1

S_L PARAMETER FOR THE THREE VESICLE FORMS AS A FUNCTION OF TEMPERATURE

T(°C)	MLV	LUV	SUV
12	0.388	0.193	0.146
18	0.383	0.184	0.111
24	0.269	0.157	0.070
30	0.252	0.076	0.052
36	0.245	0.073	0.048
42	0.241	0.072	0.045

hydrocarbon chains but the structure remains far from the highly ordered one of the MLV. At this moment it is still not clear in what proportion the change in curvature on the one side and the conversion to multilamellarity on the other hand, influences the great difference in lateral order between LUV and MLV.

The structural difference between the three forms is also clearly seen on fig.4 where the Raman spectra in the CH stretch region are given at 23.3

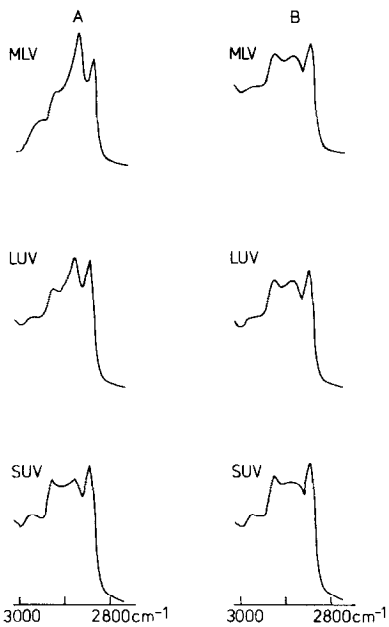

Figure 4. Raman spectra of MLV, LUV and SUV in the methylene CH stretch region : A at 23.3 °C and B at 28.5 °C.

°C and 28.5 °C. In fig. 4A (T = 23.3 °C) the 2890 cm^{-1} feature which is very strong for the MLV, diminishes for the LUV and becomes less strong than the 2850 cm^{-1} feature for the SUV. A comparison of the spectra at 23.3 °C and 28.5 °C reveals a drastic change for the MLV according to a sharp transition, a less drastic one for the LUV and a small change for the SUV because of the smooth transition.

As conclusion we can state the large unilamellar vesicles prepared by the method of Papahadjopoulos have a structure that is well different from multilamellar and small unilamellar vesicles. This structure difference can be seen clearly by monitoring the methylene CH stretch intensities by Raman spectroscopy. Our results show a substantial decrease in lateral chain packing order from MLV → LUV → SUV. From this point of view the LUV resemble the SUV. It is clear however that the small broadening of the transition region and the scarcely affected transition temperature point to a high degree of cooperativity like in the case of the MLV.

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