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FTIR studies of temperature influence on the DPPG model membrane

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ABSTRACT

The thermal changes induced in dipalmitoyl phosphatidyl glycerol (DPPG) model membranes were studied by FTIR technique. When temperature increases, the DPPG model membrane passes from a high ordered gel phase to a few ordered liquid crystalline phase. The very quick passing between the two phases is characterized by the main phase transition temperature at which the bilayers contain equal percentages of ordered and disordered phospholipids. The main phase transition induces important changes in the symmetric and asymmetric stretching vibrations of the $-CH_2$ groups of the DPPG acyl tails. Mathematical methods for determining the main phase transition and the degree of order of the DPPG model membrane at a given temperature are proposed in this paper.

1. Introduction

Biological membranes [1–3] are crucial cellular components having multiple roles. They maintain electrochemical gradients by controlling the diffusion of ions and biomolecules. They act as a supporting matrix for embedded enzymes or receptors and they are engaged in stabilizing interactions with skeleton proteins.

The biological membrane fluidity is a natural condition of its functionality. In order to eliminate the multitude of factors influencing the biological membrane fluidity, some simplified model membranes, built from one of the main constituents of the biological membranes, are usually used.

The main constituents of the biological membranes are the phospholipids as dipalmitoyl phosphatidyl coline (DPPC), dipalmitoyl phosphatidyl glycerol (DPPG) or cholesterol. The amphiliophilic nature of the phospholipids determines the bilayers formation when the phospholipids concentration in water is appropriate [4–7]. The bilayers have the hydrophilic heads of the phospholipids in contact with water and the hydrophobic tails, oriented to the middle of the bilayer.

The DPPG model membranes can be received in a buffer solution [5–9] and studied by FTIR technique. The measurement of some spectral parameters like band frequency, width and intensity provided information regarding the possible structural interactions and conformational rearrangements taking place.

The phospholipids model membrane stability is affected by temperature and by the impurities. A phase transition of the type (1) takes place when the model membrane temperature increases [7,10,11].

$$\text{Gel phase} \Longleftrightarrow \text{Liquid crystalline phase} \quad (1)$$

The model membranes are built as lamellar symmetric bilayers forming a gel phase with a high degree of order at low temperatures. However, at high temperatures, they become disordered and pass into a liquid crystalline phase. The transition (1) is very quick and it is characterized by the main phase transition temperature, T_m. The value of the main phase transition temperature is dependent on the chemical nature of the model membrane constituents and also on their concentration [11,12]. The bilayers contain equal percentages of ordered and disordered phospholipids at the main phase transition temperature.

Important changes in the IR spectra such as spectral shifts or bandwidth modifications are induced by the phase transition from the gel to the liquid crystalline phase. FTIR spectra indicate the degree of order in the phospholipids bilayers by the shifts of the stretching symmetric and asymmetric vibration bands of the CH_2 groups belonging to the acyl chains [7,8].

On the other hands, the $-PO_2^-$ [5] and $-C{=}O$ [4,9] groups of the phospholipids hydrophobic heads are potential recipients of hydrogen bonding interactions with the water layer separating the phospholipids bilayers. So, the stretching vibrations of these groups offer information about the temperature influence on the hydrogen bonding mechanism.

Some observations regarding the temperature influence on the degree of order of the acyl chains in DPPG model membranes and a mathematical model permitting the calculation of the percentages of gel and liquid crystalline phases at a given temperature are developed here.

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2. Experimental setup

Dipalmitoyl phosphatidyl glycerol (DPPG) model membranes were used in this study. DPPG was purchased from Sigma Chemical Co., St. Louis, MO, and used without purification. Multilamellar vesicles were obtained from DPPG dried films and phosphate buffer solution, using the procedure proposed by Severcan et al. [10–13].

Fourier Transform Infrared (FTIR) spectra were registered in $CaCl_2$ cells, using a Bomeme 157 FTIR spectrometer. The interferograms were averaged for 100 scans.

An Unicam Digital Temperature Controller unit with a thermocouple located around the edge of the cell window was used for temperature monitoring. The samples were investigated in a large temperature range with increasing temperature from 26 to 61 °C. The temperature increasing rate was small enough to assure quasi-static processes [11]. Each spectrum was recorded after 15 min of temperature stabilization to make sure that the sample temperature is that displayed by the digital controller.

The water vaporous influence was eliminated by subtracting the FTIR spectra of buffered solution from the model membrane spectrum, at each studied temperature.

The FTIR spectra of the DPPG model membrane in the region 3000–3800 cm^{-1} were also analysed without buffer spectrum subtraction in order to evidence the important modifications in the hydrogen bonds interactions when temperature increased [14].

The structural formula of DPPG and also the arrangement of the hydrocarbon tails in the gel and liquid crystalline phases of DPPG are given in Figs. 1 and 2, respectively.

3. Calculation methods

Based on the idea that at the main phase transition temperature, the gel phase and the liquid crystalline phase coexist in equal proportions in sample, we determined the P_g and P_{lc} percents of the molecules in the gel and in the liquid crystalline phases in a model membrane, at each temperature.

Let us consider a system consisting from N amphiphilic molecules that can have only two thermodynamic phases gel and liquid crystalline ones. Let suppose that the transition between these phases is a reversible thermodynamic transformation (1). Relation (1) suggests that by the increasing temperature of the system, molecules can pass in the liquid crystalline phase and, by the system cooling, they can return in the gel phase.

Let be N_g the number of the molecules in gel phase and N_{lc} the number of the molecules in liquid crystalline phase in the studied system. These numbers are dependent on temperature and satisfy the equation:

$$N_g + N_{lc} = N \tag{2}$$

At low temperatures, when the system is in its gel phase, we can consider that N_g tends to N, while at the temperature higher than the melting point, N_g is near zero, because the system passed in its liquid crystalline phase and N_{lc} tends to N.

Such a condition is satisfied by DPPG acyl chains from the DPPG model membrane. By temperature increasing, N_{lc} increases and N_g decreases as relation (1) predicts. Contrariwise, N_g increases and N_{lc} decreases when the model membrane is cooled, in order to assure the returning of the system into its gel phase.

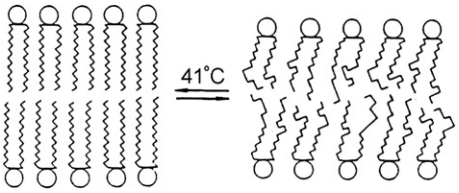

Fig. 2. Changes induced by temperature in the order degree of the long acyl chains of DPPG.

The reversibility of the transformation (1) has been experimentally demonstrated [12] by using wavenumber modification of CH_2 stretching modes measured in the DPPG model membranes. So, by temperature increasing the wavenumbers of the CH_2 stretching modes increased, showing a sharp modification at 41.5 °C (the melting point of DPPG) and then, at the sample cooling, they decreased in the same way to the values of the wavenumbers corresponding to the gel phase.

Supposing that all the phospholipids acyl chains are ordered in the gel phase of the sample one can write:

$$N(\varepsilon_g^f - \varepsilon_g^i) \cong h \cdot c(\bar{\nu}_g - \bar{\nu}_0) \tag{3}$$

where ε_g^f and ε_g^i are the interaction energies between $(N-1)$ molecules in their ground vibration state and one molecule of the sample in its final (f) and initial (i) states of the spectral transition, when all molecules are in the gel phase (g): $\bar{\nu}_g$ is the wavenumber measured in the gel phase of the system and $\bar{\nu}_0$ is the wavenumber measured in the gas phase of the same system.

A similar relation can be written for the liquid crystalline phase of the system:

$$N(\varepsilon_{lc}^f - \varepsilon_{lc}^i) \cong h \cdot c(\bar{\nu}_{lc} - \bar{\nu}_0) \tag{4}$$

by using the interaction energies ε_{lc}^f and ε_{lc}^i between $(N-1)$ molecules of the system in their ground vibration state and one molecule of this system in its vibration states (f and i) participating to the IR transition, when all molecules of the system are in liquid crystalline phase. The wavenumbers $\bar{\nu}_{lc}$ and $\bar{\nu}_0$ correspond to the system in its liquid crystalline and gas phases, respectively.

For the system at a given temperature T, different from T_m, one can define the average ratios of the molecules from the gel and liquid crystalline phases, by:

$$P_g \equiv \frac{\bar{N}_g}{N} \quad \text{and} \quad P_{lc} \equiv \frac{\bar{N}_{lc}}{N} \tag{5}$$

The ratios P_g and P_{lc} satisfy the relation:

$$P_g + P_{lc} \equiv 1 \tag{6}$$

On the other hand, for the system at a given temperature T, having N_g molecules in the gel phase and N_{lc} molecules in liquid crystalline phase, one obtains:

$$\bar{N}_g(\varepsilon_g^f - \varepsilon_g^i) + \bar{N}_{lc}(\varepsilon_{lc}^f - \varepsilon_{lc}^i) \equiv h \cdot c(\bar{\nu} - \bar{\nu}_0) \tag{7}$$

The averages values of N_g and N_{lc}, denominated by $\bar{N}_g$ and $\bar{N}_{lc}$, respectively, were introduced because they determine the shifts

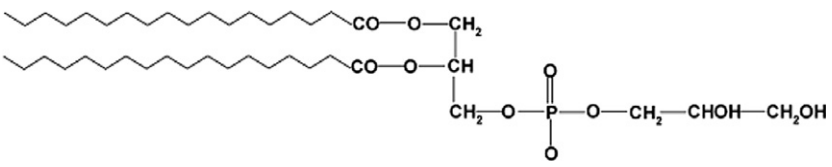

Fig. 1. Structural characteristics of DPPG.