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Thermal structural evolutions of DMPC-water biomimetic systems investigated by Raman Spectroscopy

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ABSTRACT

Many cell membranes of living organisms can be represented as phospholipid bilayers immersed into a water environment. The physical-chemical interactions at the membranes/water interface are responsible for the stabilization of the membranes. In addition, the drug efficiency, the pharmaceutical mechanism and the improvement of the drug design can be addressed to the interactions between the membranes-water interface with the drug and to the membrane-drug interface. In this framework, it is important to find membranes models able to simulate and simultaneously simplify the biological systems to better understand both physical and chemical interactions at the interface level. Dimyristoylphosphatidylcholine (DMPC) is a synthetic phospholipid used in order to make Multilamellar Vesicle (MLV), Large Unilamellar Vesicle (LUV) and Giant Unilamellar Vesicle (GUV). In order to understand the mechanisms of vesicle formation, we have analyzed mixtures of DMPC and water by micro-Raman spectroscopy at different temperatures in the range between 10 and 35 °C. Particularly, we analyzed the temperature dependence of the CN vibrational frequency, which appears well correlated to the order degree of the various phases. These investigations, beyond the determination of phospholipid hydrocarbon chains order, provide information about the conformation of the lipid membranes.

We have identified the mixture of DMPC/water that is best suited for Raman studies and can be used as an in-vitro model for biological systems.

A peculiar frequency shift across the transition gel-ripple-liquid crystalline phases has been proposed as a useful diagnostic marker to detect the “order degree” and subsequently the phases of biomimetic membranes made by DMPC.

1. Introduction

Phospholipids represent the major components of most cell membranes. Because of their amphiphilic nature, with a hydrophilic head group bonded by a glycerol backbone to hydrophobic long acyl chains [1], they assume a bilayer configuration when they are immersed into a water environment [2]. During the last decades great interest has been devoted to the study of phospholipid-water mixtures in order to evaluate the structural and functional properties of these systems [3]. In fact, beside their role in protein misfolding diseases, drug delivery, pathogenesis, gene therapy, etc. [4,5], biological membranes have attracted great attention as components for applications in electronics and bioprocessing [6]. This wide range of applications has supported the development of membrane mimetic models, such as lamellar vesicles [7]. Lipid vesicles are closed compartments where the interior is made by a small water volume enclosed by one (uni) or more (multi) bilayers constituted by amphiphilic molecules [8]. By selecting the preparation method it is possible to obtain vesicles of different diameter in the range between some nano-(like small and large unilamellar vesicles, SUVs and LUVs, respectively) and tens of micrometer (like giant unilamellar vesicles (GUVs) and multilamellar vesicles (MLVs)) [8,9].

Phospholipid bilayers present different phases as a function of the temperature: gel (L_β), ripple ($P_{\beta'}$) and liquid crystalline (L_α) [10,11]. In the L_β phase the acyl chains are arranged in a rigid configuration, while above the chain-melting temperature, they assume a more disordered arrangement typical of the lamellar liquid crystal phase (L_α). This behavior is responsible of a faster diffusion of molecules in the plane of the bilayer compared to the L_β phase. The intermediate phase $P_{\beta'}$ is

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characterized by a long range periodicity of the structure in the plane of the bilayer [10,11].

Most of the structural and dynamic information on phospholipid bilayer membranes comes from studies on MLVs by deuterium NMR spectroscopy [12–20], X-ray diffraction [21] and molecular dynamic simulations [22,23]. Nevertheless, further efforts need to be done in order to obtain information about the hydrocarbon chain order and mobility in the bio-mimetic membrane in order to fully understand the "order degree" of the different phases and the mechanisms of the phase transition. Raman spectroscopy is a very powerful technique to be used in this field. In fact, it has been already used to study the molecular organization of phospholipids in relation to the bilayer structure, affecting the fluidity and the conformational order in lipid mesophases and to determine the average orientation of the mesophases molecules inside gratings [24–26].

In this framework, we have used Raman spectroscopy to follow the thermal evolution, in the range between 10 and 35 °C, of biomimetic membranes made by the synthetic phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and water, in order to investigate the mechanisms of the phase transition. Such study allows us to define the shift of a Raman band as a marker of the phospholipid bilayer phase and to propose a qualitative model of the interaction between the water molecules and the lipid chains.

2. Materials and methods

Multilamellar vesicles were prepared as described previously [17]. Briefly, the dry 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) (Echelon Biosciences Inc., Salt Lake City, UT, USA) powder, was hydrated with double distilled water in the weight ratio 90 to 10, respectively. The mixture was then mixed and freeze-thawed with liquid nitrogen in order to obtain a homogeneous dispersion.

Raman spectra were recorded with a software controlled micro-Raman LABRAM apparatus equipped by a He—Ne laser (λ = 632.8 nm emission wavelength) (Horiba Jobin-Yvon, Milan, Italy); a 50x Mplan Olympus objective (Numerical Aperture 0.90) was used, focusing the laser spot to 2–3 μm of diameter. The spectral resolution using a grating with 1800 grooves/mm combined with the CCD detector was of the order of 1 cm^{-1}. The temperature was controlled by placing the samples in a LINKAM THMS cell (LINKAM Scientific Instruments, Tadworth, Surrey, United Kingdom). The measurements were performed from 10 to 35 °C using a heating rate of 1 °C/min. Before to perform the measurements, the samples were held at the selected temperature for 20 minutes for thermal stabilization.

3. Results and discussion

The Raman investigation on thermal evolution has been preceded by the attribution of Raman modes of the DMPC. In Fig. 1 is shown the representative Raman spectrum collected on the DMPC as powder in the range between 300 and 3100 cm^{-1}; such range has been divided in three parts to better identify the DMPC Raman bands.

The main Raman features, indicated in Fig. 1, have been assigned taking into account the data reported elsewhere [26–28]. The bands at 2856 and 2892 cm^{-1} are assigned to the symmetric ($\nu_s(CH_2)$) and antisymmetric ($\nu_a(CH_2)$) stretching modes of methylene groups. The band at 2740 cm^{-1} is due to a combination of the scissoring and wagging ($\gamma(CH_2)$) methylene groups. The two modes at 2969 and 3046 cm^{-1} correspond to the antisymmetric stretching modes ($\nu_a(CH_3)$) of the methyl terminal groups of the chains of the phosphocholine head. The band at 2946 cm^{-1} is assigned to the overtone of the methylene scissoring mode ($\delta(CH_2)$) enhanced by Fermi resonance with the $\nu_s(CH_2)$ mode. The bands at 1302 and 1465, cm^{-1} are assigned to the methylene scissoring mode ($\delta(CH_2)$) and the band at 1448 cm^{-1} is assigned to the methylene twisting mode ($t(CH_2)$). The modes between 1069 and 1134 cm^{-1} (enclosed the modes at 1092 and 1111 cm^{-1}) are assigned

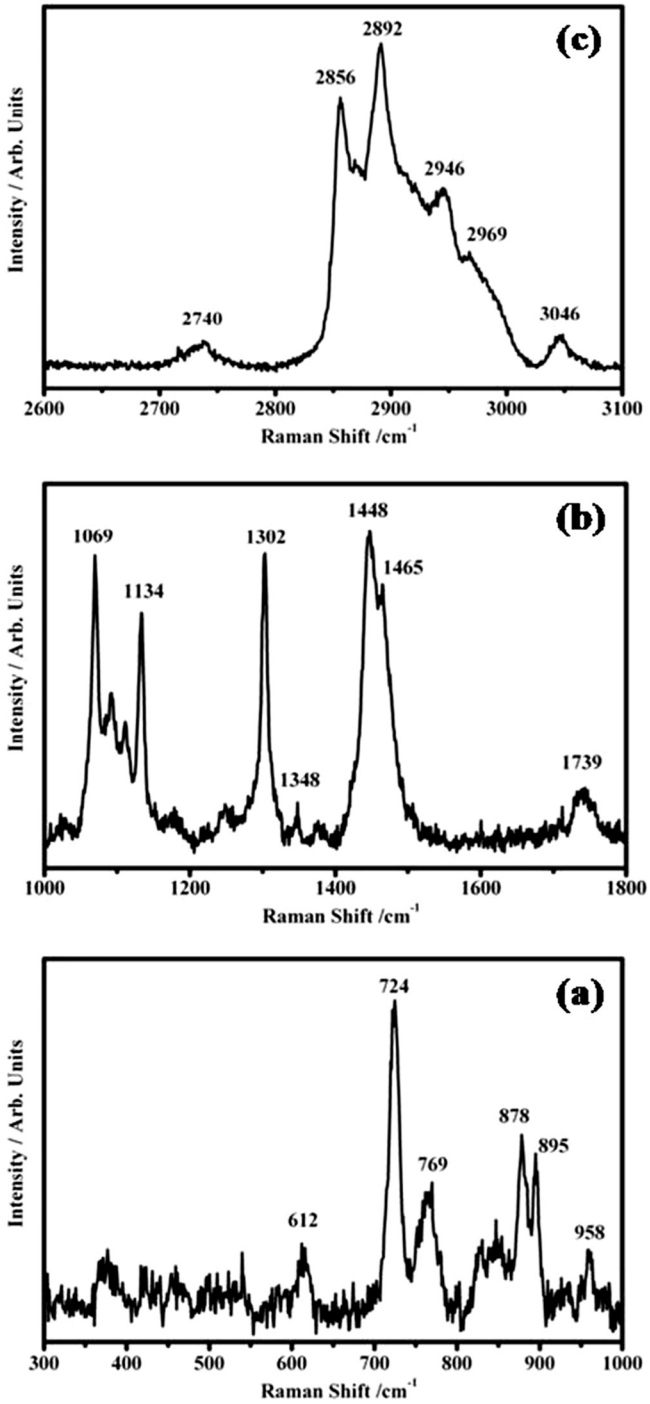

Fig. 1. Representative Raman spectra collected on the 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) powder in the range between 300 and 1000 cm^{-1} (a), 1000 and 1800 cm^{-1} (b) and 2600 and 3100 cm^{-1} (c).

to the skeletal vibrations of the C—C bonds. In particular, the modes at 1111 and 1134 cm^{-1} are assigned to the trans C—C bonds, whereas, the mode at 1092 cm^{-1} has been assigned to the stretching of the C—C skeleton of different kinds of gauche structures. The stretching of the C═O ester groups is responsible of the mode at 1739 cm^{-1}. The modes at 769 and 895 cm^{-1} are assigned to the C—N stretching of the O—⊖C—C—N^+ in the *trans* conformation while the choline gauche conformation is associated to the modes at 724 and 878 cm^{-1}. The mode at 958 cm^{-1} is assigned to the CH_2 rocking mode.

Fig. 2 reports the representative Raman spectra collected on the DMPC/water mixture as a function of the temperature.