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Fourier-transform infrared and Raman characterization of bilayer membranes of the phospholipid SOPC and its mixtures with cholesterol

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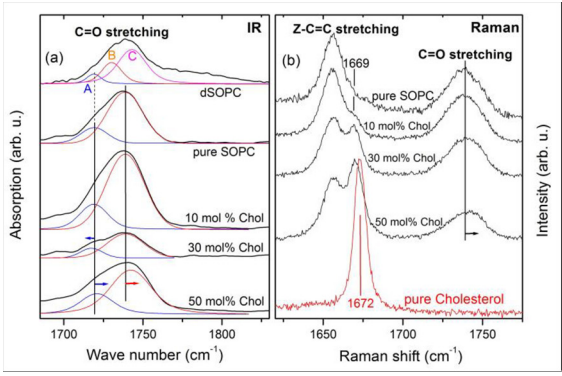
GRAPHICAL ABSTRACT



IR absorption (a) and Raman (b) spectra of pure SOPC and SOPC/cholesterol mixtures in the region of vibrations of the SOPC carbonyl group. The spectrum of the dSOPC (dry) sample obtained from pure SOPC by subsequent dehydration (see text) is also shown. With each IR spectrum the components resulting from the deconvolution of its C=O stretching band are shown.

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ABSTRACT

One of the primary roles of cholesterol (Chol) in the biological cell is to modulate the physical properties of the bilayer phospholipid membrane. Moreover, the effect of cholesterol on lipid bilayers is strongly dependent on the concentration, hence it can easily adapt to the changes in the cell temperature. Incorporation of cholesterol in membranes induces diverse changes in the bilayer properties, including variation of the bilayer thicknesses and changing the lipid order. Taking into consideration these physical and structural characteristics of the lipid membranes with cholesterol, as well as their optical birefringence, we apply the typical structural methods for studying these complex biological systems. We have used Fourier transform infrared (FTIR) and micro-Raman spectroscopy, aiming on study of the specific physical characteristics of the lipid membrane of the type 1-stearoyl-2-oleoyl-sn-glycerol-3-phosphocoline (SOPC). The analysis of the FTIR and Raman fingerprint spectral range, including band deconvolution, indicated that hydrogen bonds (HBs) exist between the hydroxyl groups of cholesterol and the carbonyl ester groups of the polar–apolar interface of the bilayer membrane. Upon insertion into the bilayer Chol actively participates in H-bonding at the C=O sites, facilitates H-bonding of water to the PO_2-site and relaxes the "improper H-bonding" of H_2O molecules to the choline moiety. We also establish an overall ordering effect of Chol on the lipid bilayer. The interplay of cholesterol and water in realization of HB with the phospholipid moieties, in dependence on the Chol concentration, was analyzed.

Abbreviations: Chol, cholesterol (5-Cholesten-3-ol); FTIR, Fourier transform infrared; SOPC, 1-stearoyl-2-oleoyl-sn-glycerol-3-phosphocoline; DPPC, 1,2-diphytanoyl-sn-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; H_2O, water; PL, phospholipid; PC, phosphatidylcholines; OH, hydroxyl; HB, hydrogen bonding; LC, liquid crystal; MDS, molecular dynamics simulation

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1. Introduction

Biological membranes are heterogeneous assemblies composed of various lipids differing in chemical structure [1–10]. Because of the amphipathic nature of phospholipids, and the presence of a hydrophilic 'head' and hydrophobic 'tail', they spontaneously arrange in a lipid bilayer, which separates the cell from the water environment [11,12]. The structural and thermodynamic properties of the model lipid membranes were object of very intensive research with the purpose to create an adequate model for natural biological membranes. Potential model systems are binary lipid mixtures of phospholipids and cholesterol [13,14]. Cholesterol is a representative member of the steroid group. The most important function of cholesterol in the cells is related to the fact that it is a component of the cell membrane, providing its sufficient stabilization and fluidity, as well as control the membrane permeability [15–17]. Cholesterol possesses a characteristic arrangement of four interconnected cycloalkane rings, one of which is linked with a hydroxyl (OH) group. The hydroxyl group of cholesterol acts as a proton donor and engages in hydrogen bonding (HB) both to phosphate group in the polar head and to the ester carbonyl group in the interfacial region of PCs [13–17]. The H-bond of cholesterol to the ester carbonyl groups is very important for the control over the order-disorder effect of the hydrophobic hydrocarbon domain, which is decisive for the elastic properties of the bilayer membrane. Currently, the role of HB in the mixtures of phospholipids and Chol are still insufficiently understood. It turns out that the presence of hydrogen bond between the hydroxyl group of Chol and the carbonyl group of phospholipids is difficult for detection due to the complex influence of different factors including conformations in the hydrophobic interface [18–24].

The fundamental role of the HB in the biological functions of the bilayer lipid membranes prompts contemporary structural methods to be applied for detection and control of such important lipid interactions. The most effective method for detection of the hydrogen bonds is the Fourier transform infrared spectroscopy (FTIS) [25–29], in coordination with Raman, particularly micro-Raman, spectroscopy [30,31]. These techniques are expected to comprise an efficient combination for the HB study in the phospholipid field.

The goal of the present work is applying these two efficient structural methods to detect the influence of cholesterol on the bilayer phosphate lipid structure. We expect the impact of Chol to modulate the physicochemical properties of the bilayer lipid membranes and hence its biological functions [13,15,17]. Most of the cholesterol interactions with the carbonyl, phosphate and choline groups one expected to be based on HB interactions [32,33]. Intercalated cholesterol also causes modification of the order and the distance between hydrocarbon chains inside the bilayer lipid membrane, enabling a control on the transmembrane permeability [13].

For that reason, we detect and describe the specific spectral signature of the phospholipids-cholesterol hydrogen bonded interactions applying an adequate analysis for the FTIR and Raman spectra. We expect, also by discovering the interactions of cholesterol and phospholipid in dependence of the Chol concentration, to specify the study of the mechanical and electrical properties of this complex biological system. The results could be a base for a molecular dynamic simulation, impending in the near future, and allowing the exploration of the new physical and structural characteristics of the lipid membranes.

The physical characteristics and the optimization of the bio-functions of the bilayer lipid membranes strongly depend on the degree of hydration and the nature of hydrogen bonding of water to moieties located at or near lipid polar/apolar interfaces in the lamellar liquid-crystalline phase. The small changes in the chemical structure of the polar/apolar interfacial region of a lipid molecule are expected to significantly affect the lipid–lipid and lipid–water interactions progressing in bilayer assemblies [34,35]. This should lead to conformational changes in the glycerol backbone of the lipid and a concomitant increase in intermolecular, hydrocarbon chains spacing, respectively. As was indicated [34], the increase in both the lipid hydrocarbon chain length and disorder is associated as well with an increase in the mobility of the hydrogen-bonded ester carbonyl groups. Such a higher mobility, however, may influence the strength of the carbonyl group interactions with the corresponding hydrogen-bond donors.

2. Materials and experimental procedures

2.1. Materials and methods

2.1.1. Materials

The lipid films were prepared from l-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) (C18:0/C18:1)-(Avanti Polar Lipids Inc. (USA)), containing appropriate quantities cholesterol (5-Cholesten-3β-ol, Sigma–Aldrich). The lipid was dissolved in chloroform with concentration 1 mg/ml. The cholesterol was dissolved in methanol with concentration 2 mg/ml. The solvents chloroform (purity ≥99%) and methanol (purity ≥99.8%) were purchased by Sigma–Aldrich. All the chemicals were used without any further purification. The final lipid–cholesterol solution was prepared by mixing SOPC and cholesterol solutions in the desired proportion for each cholesterol content.

2.1.2. Sample preparation

We prepared the sample for FTIR and Raman study as follows:

(i) for FTIR, we constructed a transmission cell with silicon windows for IR measurements in the transmission mode by spreading the SOPC-cholesterol mixtures and pure SOPC over Si plate surfaces by spin coating method. To avoid any evaporation the cell was sealed. The average thickness of the spread layer was determined from it's surface area and from the amount spread. The obtained thickness was 10 μm and the illuminated (working) area was approximately 1 cm^2. Under these preparation conditions the hydration degree of the samples results from the air humidity, leading to a thermodynamic equilibrium at ambient conditions. Such a spontaneous hydration yields intermediate water concentration between anhydrous and fully hydrated state, and regulates the water content in the interface polar–apolar bilayer region and in the interfacial bilayer region. For subsequent dehydration of the pure SOPC sample after the first measurement series, we introduced it in a desiccator under vacuum for 24h in the dark.

(ii) For the Raman experiment, the same material was spread over a conventional glass plate, thus again obtaining a phospholipid multilayer, with thickness ≈ 10 μm and area approximately 1 cm^2. The degree of hydration was estimated from the ratio of peak IR intensities of the water OH-stretching band at about 3400 cm^{-1} and the asymmetric stretching vibration of CH_2 groups at app. 2920 cm^{-1} after suitable calibration [36,37]. The sharp line at 2920 cm^{-1} is not present in Cholesterol IR spectra and can be fitted separately in the framework of deconvolution of the high-frequency C—H stretching domain while the contribution from the OH group of cholesterol to the 3400 cm^{-1} band is small compared to the contribution of water. In this way the water content of the SOPC-Chol samples with 0 mol%, 10 mol%, and 30 mol% Chol concentration was estimated to be 15–20 water molecules per lipid. For 50 mol% Chol concentration a higher hydration of 25–30 H_2O molecules per lipid was established. The reference SOPC sample without Chol will be referred to as "pure SOPC" in what follows.

2.1.3. Fourier transform infrared (FTIR) and Raman spectroscopy

FTIR spectra were recorded on a Bruker Fourier transform infrared (FTIR) spectrometer in the transmission mode. With a temperature control unit we maintained 26 °C temperature during acquisition of the IR spectra. For each Chol concentration two scans were performed with resolution 2 cm^{-1}. The Raman spectra were measured in backscattering geometry on a HORIBA Jobin Yvon Labram HR visible spectrometer (1800 grooves/mm) equipped with a Peltier-cooled CCD detector. The 514.5-nm line of an Ar+ laser was used for excitation, the absolute accuracy being better 0.5 cm^{-1}. The laser beam was focused on a spot