Specific Far Infrared Spectroscopic Properties of Phospholipids

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Abstract. We describe the specific spectral signature of different phospholipids and sphingolipids in the far infrared. Three specific spectral domains have been found: the head group contributions (600 and 480 cm⁻¹); the modes of the torsion motion of the hydrocarbon chains and of the skeleton vibration (460 to 180 cm⁻¹); and the hydrogen-bonding continuum (below 300 cm⁻¹). Marker bands for individual phospholipids are distinguished.

Keywords: Bound water, far infrared, hydrogen bonds, self-organization, phospholipids

1. Introduction

In natural membranes, a specific mixture of lipids is typically found for each organism [1]. The cell membranes of eukaryotes essentially consist of three classes of lipids: phospholipids, sphingolipids, and cholesterol. Inositol phospholipids are ubiquitous membrane lipids found in mammals as well as in bacteria and other organisms, including plants [2, 3]. Cardiolipin (CL) is a major mitochondrial anionic phospholipid with important functions in promoting cell growth, anaerobic metabolism, mitochondrial function, and biogenesis. The function of this unique phospholipid is based on two protonable phosphate groups [4] that have been shown to form an intermolecular hydrogen bond with its phosphate group [5]. Phosphatidylglycerol (PG) is a crucial phospholipid as it is the metabolic precursor in the biosynthetic pathway leading to the formation of cardiolipin and the sole phospholipid of thylakoid membranes of prokaryotic and eukaryotic oxygenic photosynthetic organisms. Sphingomyelin (SM) belongs to the sphingolipid group and it is at the same time the most abundant component of mammalian membranes in brain and nervous tissues. It plays an important role for apoptosis, aging, and signal transduction with cations [6]. Phosphatidylethanolamine (PE) is ubiquitous in eukaryotic cell membranes, being unevenly distributed between the inner and the outer leaflets of the bilayer. Phosphatidylcholine (PC) is usually
This rich molecule group, its composition and interaction, was extensively studied by means of infrared spectroscopic techniques \[7, 8\]. Importantly, functional groups involved in hydrogen bonding have IR absorption bands that are temperature and pressure dependent \[9–11\]. Different specific spectral regions can be distinguished, and so-called IR continua have been described involving hydrogen bonding features \[12–15\]. In the far infrared (FIR) region, this continuum is seen at about 400 to 0 cm\(^{-1}\), the exact position and broadness depending on the polarizability of the hydrogen bonding features. Hydrogen bonds from water molecules were found to contribute to this same spectral region \[14\] as recently described for the reorganization of the water solvation shell in the picosecond time range in the THz \[16, 17\].

Recently, we corroborated the temperature dependent phase transition from phospholipids in the mid-infrared \[18–20\] with the hydrogen bonding interaction seen in the far domain \[21\] and found that the lipid structure influenced the intermolecular hydrogen bonding interplay. In this work we examine in detail the specificity of the far infrared spectral range for the different types of phospholipids.

### 2. Materials and Methods

#### 2.1. Sample Preparation

L-\(\alpha\)-Phosphatidylinositol (PI) from Glycine max (soybean), containing 50\% of pure PI (amount of phosphatidylethanolamine and phosphatidyl acid), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE) were purchased from Sigma-Aldrich. Asolectin from soybean which contains equal proportions of PC, PE, and PI was purchased from Sigma-Aldrich. Phosphatidylethanolamine from egg yolk (EPC), phosphatidylethanolamine from egg yolk (EPE), phosphatidylglycerol from egg yolk (EPE), and sphingomyelin from egg yolk (ESM) were purchased from Lipoid (Ludwigshafen, Germany). The purity of all phospholipids is above 98\% and phospholipids were used without further purifications. The samples were dissolved in chloroform using a concentration of 20 mg/mL. For the lipid titrations, EPC and EPE were dissolved in a ratio of 1 : 1, 2 : 1 and 4 : 1 (w/v). Furthermore, PC, PE, and PI as well as EPC, EPE, and PI were added in a ratio of 1 : 1 : 1 (w/v).

#### 2.2. Spectroscopy

Far infrared absorbance spectra were recorded from 700 to 50 cm\(^{-1}\) with a diamond ATR unit at room temperature. Mid-infrared absorbance spectra were recorded from 4000 to 900 cm\(^{-1}\) with a silicon/ZnSe ATR unit. The lipid solutions were dried on the respective ATR unit. After drying, the film was rehydrated by adding 1 \(\mu\)L distilled water to the film.

Data was recorded from 700 to 50 cm\(^{-1}\) with a Bruker Vertex FTIR spectrometer equipped with a silicon beamsplitter as well as a deuterated triglycerine sulfate detector and a conventional infrared light source. Five spectra with 128 scans were averaged. The resolution of all spectra was 4 cm\(^{-1}\) and the data were acquired in the single-side, forward-backward mode. The resultant interferograms were apodized with the Blackman-Harris-3-term function and Fourier transformed with a zero filling factor of two.
Figure 1: Far infrared absorbance spectra of different phospholipids from 600 to 50 cm$^{-1}$ measured at room temperature: (A) phosphatidylinositol from Glycine max, (B) cardiolipin from bovine heart, (C) phosphatidylglycerol from egg yolk, (D) sphingomyelin from egg yolk, (E) phosphatidylethanolamine (DMPE), and (F) phosphatidylethanolamine from egg yolk.

3. Results and Discussion

Figure 1 summarizes the far infrared absorbance spectra of selected phospholipids and sphingolipids, all of them having a special function in membranes, as mentioned in the introduction.

Trace A shows the spectra of phosphatidylinositol (PI), trace B of cardiolipin, and trace C for phosphatidylglycerol form egg yolk (EPG). Trace D displays the spectroscopic properties of sphingomyelin from egg yolk (ESM), trace F represents phosphatidyl-ethanolamine from egg yolk (EPE), and the direct comparison to the synthesized phosphatidylethanolamine (DMPE) is shown in trace E. The main difference between the two samples in trace E and F is the degree of saturation of the hydrocarbon chains.

Figure 2 shows the spectroscopic properties of phosphatidylcholine from egg yolk (EPC in trace A), phosphatidylcholine C$^{13}$ isotopically labeled (trace B), synthesized phosphatidylcholine (DMPC) (trace C), as well as deuterated D$_{22}$-phosphatidylcholine (trace D), and deuterated D$_{35}$-phosphatidylcholine (trace E).

Three main domains can be depicted between 600 and 50 cm$^{-1}$ in the far infrared spectra of the phospholipids and sphingolipids seen in Figures 1 and 2. The signals between 600 and 480 cm$^{-1}$ originate from the head group domain. Between 540 and 503 cm$^{-1}$, the O–P–O wagging and rocking vibrations from the lipid head groups can be expected [22, 23]. In these lines the absorbance band at 577 cm$^{-1}$ in ESM (Figure 1, trace D) is assigned to the CN$^{+}$ (CH$_3$)$_3$ deformation vibration. In PC this band appears at 575 cm$^{-1}$ (Figure 2). In the deuterated D$_{35}$-PC variant, the related CN$^{+}$ (CD$_3$)$_3$ deformation vibration can be found at 542 cm$^{-1}$ [8]. The out-of-plane deformational motion of the...
glycerol group in PG and CL is found about 560 cm\(^{-1}\). Furthermore, the deformation vibration of the ethanolamine head group of PE is seen at 555 cm\(^{-1}\).

A second spectral domain is defined from 460 to 180 cm\(^{-1}\). It includes the coordinates of the torsion motions of the hydrocarbon chains as well as the skeleton vibration of the lipid molecules. The C–CN torsion vibration of the choline group is found around 382 cm\(^{-1}\). The vibration of the hydrocarbon chains has its maximum at about 185 cm\(^{-1}\) and it is not affected by deuteration. The CH\(_3\) torsion mode has its maximum at approximately 250 to 230 cm\(^{-1}\), the exact position depending on the lipid type.

The third prominent spectral region that can be depicted is the broad feature between 170 and 50 cm\(^{-1}\). This signal originates from the molecular breathing and from the intermolecular hydrogen bonding structure within the phospholipid bilayer [21, 24, 25]. Both CL and PG show a broad hydrogen bonding feature at higher frequencies between 300 and 100 cm\(^{-1}\). This observation is in agreement with the presence of hydrogen bonds with large proton polarizability [26]. For example in the PG bilayer, there is room for intermolecular hydrogen bonding between the glycerol hydroxyl groups and the phosphate moiety of the polar head group [27–30]. The hydroxyl groups in the head groups may also partially mimic the solvation properties of water. The hydrogen bonding feature of PC variants found from 165 to 50 cm\(^{-1}\) is just slightly affected by the deuteration of the chains. This is not surprising since isotope labeling does not strongly affect these vibrations. While deuteration is widely used in the mid-IR to perform assignments, in the far IR, deuteration is not very efficient, since the shift of the hydrogen bond is about 1–5 cm\(^{-1}\) [14, 31].
Table 1: Summary of the tentative assignments presented in the text.

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Table 1 summarizes the tentative assignments for the phospholipid contributions in the far infrared region.

4. Conclusion

Herein, we have presented the characterization of the far infrared spectra of different phospholipids and sphingolipids in the spectral range from 600 to 50 cm$^{-1}$. The far infrared spectra of phospholipids have been divided in three main domains and in addition the individual fingerprint of each lipid was depicted. The first domain included the significant signals between 600 and 480 cm$^{-1}$ which originates from the head group domain. The second domain described the range from 460 to 180 cm$^{-1}$. It included the vibrations of the torsion motions of the hydrocarbon chains as well as the skeleton vibration of lipids. Finally, the third domain discussed below 300 cm$^{-1}$ was assigned to the broad hydrogen bonding signature which originates from the molecular breathing and from the intermolecular hydrogen bonding structure within the lipid layer.

In conclusion, the far infrared spectral range allows the description specific signals for lipids.

Acknowledgments

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