BBA - Biomembranes 1860 (2018) 673–682

Contents lists available at ScienceDirect

BBA - Biomembranes

journal homepage: www.elsevier.com/locate/bbamem

Examining water in model membranes by near infrared spectroscopy and multivariate analysis

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ARTICLE INFO

Keywords:
Membranes
Water
Near infrared spectroscopy
Principal Component Analysis

ABSTRACT

By exploiting the sensitivity of the NIR spectrum, particularly the first overtone of water, to the number and strength of hydrogen bonds, the hydrogen bond network and water polymerization in membranes of DMPA (1,2-dimyristoyl-*sn*-glycero-3-phosphate) and DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) was investigated as a function of the temperature and the presence of this two phospholipids having the same tail but different polar head. Principal components analysis performed on the spectra was used to disclose subtle spectral changes that mirror the alteration of the vibrational energy of the water O-H bonds, as a measure of the H-bond network. Temperature showed a dominating effect on the H-bond network. Increasing temperatures diminished the number of strongly H-bonded water molecules and increased the number of weakly H-bonded waters. This main effect of temperature was missing after the subtraction of the pure water spectra from the lipid-containing ones. An intriguing secondary effect of temperature was also revealed. Phospholipids exhibited an effect qualitatively similar to that of the temperature. DMPA, and particularly DMPC, disrupted the H-bond network in the neighboring lipid-water interface, reducing water polymerization and strengthening the water O-H bonds. The type of the polar head affects the H-bonds more than duplicate the concentration of the lipid. A connection between head group structure and the effect on the H-bonds network, and the existence of two populations of water molecules are discussed.

1. Introduction

Water in membranes has been an important subject in the study of the phase behavior of phospholipids since the early reports [1–3], and it is considered a paradigm for membrane hydration.

A variety of techniques were employed to study water in membranes. Near infrared spectroscopy (NIR) is a nondestructive, accurate analytical method useful to measure parameters of various systems through their single scans in the 750 and 2500 nm range (13000–4000 cm^{-1}). It reflects overtones or combinations of fundamental molecular vibrational modes of the O—H bond of water and thus informs about H-bonds network. By analyzing the first overtone of water in the 1300–1600 nm NIR region, derived from the main tension band of the O—H bond in the middle infrared (2700–3200 nm), it is possible to investigate the H-bonds network and water polymerization in aqueous systems [4,5]. Other regions of the NIR spectra have also been employed for studying water status, as the 1100–1300 nm [5,6] and 1800–2100 nm intervals [7]. The 1684–1800 nm region was recently exploited for studying the chain-melting phase transitions of lipid bilayers [8,9]. The NIR water spectrum changes according to the number and strength of hydrogen bonds and the degree of water aggregation [6]. Nearly a century ago [10], these factors were reported as being sensitive to temperature; later, they were also found as being sensitive to solutes that interact with water [11].

Instead of the direct measurement of the component of interest (salt, protein, lipid, etc.) in an aqueous system, the approach used in this work is focused on measuring the changes that the component cause to the water NIR spectra [12]. Even though water absorbs energy in the whole spectra, the NIR region is particularly interesting as the radiation is not totally reflected (as in the visible window) nor totally absorbed (as in the mid infrared) by the water. It is then possible to register the radiation sent it back from the perturbed water. In aqueous systems having abundant H-bonds this approach can be used as an indicator of the effect of a solute. Several components in aqueous systems have been

Abbreviations: NIR, near infrared spectroscopy; MIR, mid infrared spectroscopy; MLV, multilamellar vesicles; PCA, Principal Component Analysis; PC, principal component; n_w, number of water molecules per lipid; n_w', steric number of water molecules per lipid; RMSEP, Root Mean Square Error of Prediction; DMPA, 1,2-dimyristoyl-sn-glycero-3-phosphate; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine

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https://doi.org/10.1016/j.bbamem.2017.12.007
Received 18 August 2017; Received in revised form 23 November 2017; Accepted 7 December 2017
Available online 09 December 2017
0005-2736/

investigated with this methodology: metals [13], sugars [6,7,14], honey [15], milk [16], DNA [17], plants [18], inorganic salts [4,11], acids and hydroxides [11], cells and tissues [12,19].

A required step in this line of attack is the use of multivariate analysis to disentangle the subtle and hidden spectral perturbations. Broad and overlapped bands are usual and preprocessing procedures as smoothing, derivatives, mean centering, autoscaling, etc., are commonly used for the discrimination and interpretation of the spectra. Then, one or more multivariate techniques, as Principal Component Analysis (PCA), Partial Least Squares (PLS), Multiple Linear Regression (MLR), Soft Independent Modeling of Class Analogy (SIMCA), etc., are usually applied. Last but not least, the outcomes requires a meticulous examination for a correct interpretation [20,21]. See Supplementary material for a more detailed explanation of the multivariate method employed in this work, PCA.

As far as the author is aware, no work using NIR spectroscopy for the study of water in membranes has been reported. NIR spectroscopy and multivariate analysis of the spectra were combined here to investigate the effect of temperature and phospholipids on the hydrogen bond network and water aggregation in membranes. For that, multilamellar vesicles (MLV) of phospholipids having an identical fourteen carbon acyl chains but polar heads differing in the presence of an hydroxyl (phosphatidic acid) or a choline group (phosphocholine), were prepared at two concentrations and scanned at varying temperatures.

2. Experimental procedure

2.1. Materials

The DMPA (1,2-dimyristoyl-*sn*-glycero-3-phosphate) and DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) were purchased from Avanti Polar Lipids (Alabaster, AL), having a purity > 99%. Stock solutions were prepared in chloroform/methanol (2:1 v/v) at a final concentration of 1 mg/ml. All solutions were stored at − 20 °C before use.

2.2. Liposome preparation

Multilamellar vesicles (MLV) were prepared by mixing the appropriate amount of stock solution of each component in round-bottomed tubes and dried under nitrogen flow at room temperature while rotating the tube. The samples were then dried under nitrogen flux for 1 h to eliminate the remaining solvent. Milli-Q water was added to the thin lipid film and heated up to 55–57 °C, i.e., at a temperature higher than the highest transition temperature (T_m) of the lipids. At this stage, the total lipid concentration of the suspension was around 1 mg/ml. The samples were then vigorously mixed in a vortex in two steps of 1 min each, also at 55–57 °C, and incubated for 30 min in a low power bath sonicator in order to obtain a more uniform size distribution of the resulting MLVs. Lipid suspensions were then diluted with Milli-Q water to a final concentration of 250 and 500 μM.

2.3. Spectroscopic measurements

Near infrared spectra were obtained in a Brimrose Solid-state Luminar 5030 AOTF-NIR (Acousto-Optic Tunable Filter-Near Infrared) Analyzer (Brimrose, Sparks, MD), equipped with a probe attachment and a spectra analysis software (Snap32). For each measurement the probe with its reflectance attachment was immersed directly into the round-bottomed tubes containing 1.5 ml of the lipid suspension. Absorbance ($\log T^{-1}$) was registered every 1 nm in the interval 1100–2300 nm, with a layer of thickness of the suspension of 1 mm, corresponding to the gap of the immersion probe. Samples were scanned every 5 °C in the range 13–58 °C in a temperature-controlled circulating water bath (Cole Parmer Polystat) with a heating rate of 0.4 °C/min. Special care was taken for the temperature control. Due to the relatively high ratio between the mass of the probe (a 20 cm length × 1,2 cm diameter stainless steel cylinder) and that of the samples to be measured (1.5 ml ≈ 1.5 g), a significant thermal transference from (or to) the sample may occur, depending on the direction of the heat flux. To minimize possible temperature fluctuation, the probe was maintained submerged into the bath. Before sample measurement, the probe was rinsed with Milli-Q water (also at the working temperature) and further dried with tissue paper to prevent dilution effects. Each sample, with the probe inside the tube, was kept in the water bath during the spectra acquisition.

A total of sixty spectra involving samples/conditions were registered: two control samples (Milli-Q water), two different liposomes (DMPA and DMPC) at two concentrations (250 and 500 μM), and 10 temperatures (13 to 58, every 5 °C). Each of the sixty spectra was the average of 5 scanning of the corresponding sample/condition. They were averaged in view that the variation coefficient (SD ∗ 100 / Mean) of the absorbance at each wavelength was < 0.2%.

2.4. Multivariate analysis of NIR spectra

2.4.1. Principal Component Analysis

Depending on the objective, PCA was performed on different set of samples and on different spectral windows using The Unscrambler [22]. Prior to PCA, a mean centering was performed on the raw spectra. PCA was firstly applied to the raw spectra of the matrix of 60 samples (including type of phospholipid, concentration and temperature) and 1201 variables (wavelengths). PCA was also performed on separated set of samples composed of water, DMPA and DMPC liposomes. PCA was also performed on spectra after the subtraction of the pure water spectrum at the corresponding temperature. More details about the PCA procedure can be found in the Supplementary material ("Principal Component Analysis").

3. Results

3.1. Raw NIR spectra of water

Fig. 1 shows the sixty NIR spectra corresponding to two control samples (Milli-Q water), DMPA and DMPC liposomes at two concentrations (250 and 500 μM) measured every 5 °C from 13 to 58 °C (ten temperatures). Spectra were very similar and it was almost unfeasible to distinguish visually between samples according to the type of phospholipid (DMPA or DMPC) or concentration. The effect of temperature, illustrated by the arrows in Fig. 1, is the only spectral trend that can be ascertained by an examination at specific regions of the spectra. The main of the first overtone of water reflecting O—H stretching is centered around 1450–1455 nm, in agreement with other reports [23–25]. An isosbestic region is observed at 1425–1430 nm, not far from the 1440–1442 nm [26] and 1446 nm [24] reported for pure water. This band, as well as others of the NIR spectra, may shift depending on the interaction with the solute. In the presence of inorganic salt, for example, [4] observed the isosbestic point quite far from these values, at 1497 nm (6680 cm^{-1}). Increasing temperatures increase the absorbance on the left shoulder of the band and it decreases toward the right shoulder (Fig. 1), entailing a shift to shorter wavelengths (a "blue shift"), i.e. higher energy. This means that temperature weakens the H-bonds and this strengthens the O—H bonds; then, they vibrate and absorbs radiation at higher energy [24,27]. In other words, when temperature increases, the fraction of weakly bonded water increases, while that of strongly bonded water decreases.

3.2. Temperature and phospholipid effects on water are reflected in the NIR spectra

From the abundant PCA output, only those providing useful information for the present purposes are discussed in the next sections.