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Organization of water molecules by adhering to oriented layers of dipalmitoylphosphatidyl serine in the presence of varying concentrations of cholesterol

I.R. Miller *, D. Bach

Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel

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Abstract

About seven water molecules adhere to one molecule of dipalmitoylphosphatidyl serine (DPPS) in an oriented surface layer as inferred from the increase of the dichroic ratio R of their OH stretching vibration band (3400 cm^{-1}) from 2 in the random bulk state to about 2.8 when adhering to DPPS. In DPPS–cholesterol mixtures the number of water molecules adhering to the phospholipid molecules and oriented by them increases as cholesterol content increases. This increase is very steep between molar fractions of cholesterol $X(\text{chol}) = 0.2$–0.4 and at $X(\text{chol}) = 0.6$ about 13 water molecules adhere and are oriented by one DPPS molecule.

Keywords: Dipalmitoylphosphatidyl serine; Attenuated total reflection

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1. Introduction

In a previous publication [1], it has been shown that the number of water molecules rendered unfreezable by phospholipids increases steeply around cholesterol concentrations at which phase separation starts between a nearly pure cholesterol phase and a phospholipid–cholesterol phase. [2,3]. It has also been shown [4] that the number of unfreezable water molecules per phospholipid molecule corresponds qualitatively to the number of water molecules adhering tightly enough to the different polar residues of the oriented phospholipids to prevent their random rotation. As a result, the oriented phospholipids induce a change in the dichroic ratio of the OH stretching band of water at 3400 cm^{-1} from 2 (corresponding to random orientation in relatively thick layers (> 1 μm)) to 2.8 when all the water molecules adhere to the aligned phospholipid layers and are oriented by them. The dichroic ratios of the water stretching vibration band result from an average orientation by the different polar residues, and serve just as a measure for the fractions of the water molecules which are either free and rotating randomly, or are on average, at fixed orientation, as determined by their attachment to the different polar residues of the phospholipids.

The aim of the present report is to show how the presence of cholesterol in the oriented layers of dipalmitoylphosphatidyl serine (DPPS)–cholesterol mixtures influences the adherence and thereby the orientation of the water molecules to DPPS in the surface layer.

The variation of the number of oriented water

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* Corresponding author. Fax: +972-8-934-4112;
E-mail: israel.miller@weizmann.ac.il

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molecules by PS with cholesterol content, as inferred from the dichroic ratio measurements, may run in parallel, but is not necessarily identical with the number of unfreezable water molecules [1].

2. Materials and methods

DPPS sodium salt was purchased from Sigma (St. Louis, MO), cholesterol was from Nu-Chek-Prep (Elysian, MN). Mixtures of DPPS with cholesterol were obtained by mixing their solutions in chloroform/methanol (2:1) at the desired ratios then evaporating the solvent, by a stream of nitrogen, and keeping the samples under high vacuum for 3 h. The aligned layers on the surface of the germanium prism (5 cm, 0.2 cm, 45°) for attenuated total reflection (ATR) and for polarized ATR were obtained by spreading a measured quantity of aqueous suspensions of the above lipid mixtures on the germanium surface and aligning the lipid layers by sheering the spread layer with a plastic tip in the course of water evaporation. Evaporation was carried out by heating in an oven. The average thickness of the spread layer was obtained from the surface area of the spread layer and from the amount spread. An excess of up to 100 water molecules per DPPS was added to the lipid layer. The wet lipid layers were sealed with rubber gaskets to eliminate any evaporation in the course of measuring the spectra. Then the gaskets were successively opened for controlled water evaporation. At every stage, the cells were resealed and consecutive spectra were taken in the presence of reduced water quantity. The number of water molecules per DPPS was estimated from the measured water to phosphate absorbance ratios ($A_{3400\mathrm{cm}^{-1}}/A_{1230\mathrm{cm}^{-1}}$). In the presence of PS or cholesterol, the 3400 cm^{-1} band also contains a contribution from the OH of cholesterol and from the NH_3 of PS. These contributions are small compared to the contribution of water and were eliminated by substraction of the dry lipid spectra. Water calibration was performed by measuring spectra of determined DPPS/water mixtures in sealed ATR cells or in sealed transmission cells with CaF_2 windows. The two sets of calibrations gave identical results within experimental error.

The ATR measurements were carried out on a Perkin Elmer Model 1600 FTIR spectrophotometer. A Graseby/IR grid polarizer (CBS, Suffolk, UK) was used.

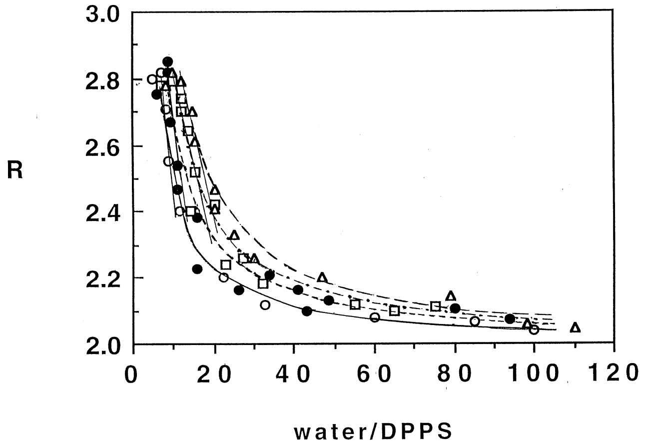

Fig. 1. Dependence of the dichroic ratio R on the number of water molecules added per molecule of DPPS as determined from the measured ratio $A_{3400\mathrm{cm}^{-1}}/A_{1230\mathrm{cm}^{-1}}$ after appropriate calibration [4] for different concentrations of cholesterol. Molar fractions of cholesterol (X): ○, $X=0$; ●, $X=0.2$; □, $X=0.3$; △, $X=0.6$. Points, experimental curves drawn according to Eq. 1.

3. Results and discussion

In Fig. 1 the dichroic ratio at 3400 cm^{-1} is plotted as a function of the number of water molecules per molecule of DPPS for different molar fractions of cholesterol in the lipid mixtures. In each case, the dichroic ratio reaches a limiting value of ~2.8 at a critical number of water molecules per DPPS ~7 for pure DPPS and ~13 with a cholesterol mole fraction −0.6. These numbers of water molecules were obtained by extrapolation of the experimental dichroic ratios to the limiting dichroic ratio of 2.8 (straight lines in Fig. 1). These critical numbers of water molecules per DPPS may be considered to be the number of water molecules adhering to DPPS and being oriented by it. Previously by differential scanning calorimetry we determined the number of water molecules rendered unfreezable by interaction with phosphatidyl serines (natural phosphatidyl serine (PS) and dimyristoylphosphatidyl serine (DMPS) and by dimyristoylphosphatidyl choline in the presence and absence of cholesterol [1]. It was found that the number of unfreezable water molecules is smaller in the case of the charged phospholipid and increases strongly when phase separation of cholesterol takes place. The number of unfreezable water molecules as obtained from ATR measurements in the present work for DPPS in the presence and absence of cholesterol is bigger than the number of unfreezable water molecules per phosphatidyl serines investigated in [1] (PS and DMPS) at the corresponding cholesterol concentrations [1]. Only part of the difference can be attributed to the determination of the water concentrations. While in the present experiments the water content of the phospholipid surface layer was obtained from the OH/CH_2 stretching vibration amplitude ratio ($A_{3400\mathrm{cm}^{-1}}/A_{2920\mathrm{cm}^{-1}}$) or OH/$PO_2$ vibration amplitude ratio ($A_{3400\mathrm{cm}^{-1}}/A_{1230\mathrm{cm}^{-1}}$) after appropriate calibration [4] in the DSC measurements, the dried phospholipid may contain, in addition, two water molecules per molecule of lipid. However, it should be recognized that a change of the hydrocarbon chain length may also influence the number of unfreezable water molecules as determined by DSC.

If we assume a two-state model in which the dichroic ratio in the presence of excess water is due to contributions of the two components: (1) the water molecules adhering to the polar groups of DPPS and polarized by them with the dichroic ratio ($R_1 = 2.8$); and (2) from randomly oriented bulk water molecules ($R_2 = 2$) then according to Marsh [5]:

$$R = \frac{R + G}{1 - f_1\,(R_\mathrm{p} - R^*_\mathrm{o})/(R_\mathrm{b} + G)}$$

$$R = \frac{2 + G}{1 - f_1\,0.8/(2.8 + G)} \qquad (1)$$

where $G = 2(E_Z^2 - E_X^2)/E_Y^2$ equals for a thick lipid layer on a Ge prism $G \approx 1.41$ and

$$f_1 = \frac{n_\mathrm{p}}{n_\mathrm{o} + n_\mathrm{p}}$$

where n_p is the number of water molecules adhering to the phospholipids and polarized by them and thus acquiring the mean dichroic ratio $R_\mathrm{p} = 2.8$, n_o is the number of randomly oriented bulk water molecules with the dichroic ratio $R^* = 2$. The curves in Fig. 1 were reconstructed using Eq. (1) and taking for n_p the extrapolated values at the different cholesterol concentrations. Some of the experimental points deviate significantly from the curve, reconstructed with the aid of Eq. 1. Moreover, at low water contents, when all the water molecules adhere to the polar residues, their dichroic ratio R at 2400 cm^{-1} fluctuates between 2.70 and 2.85. This may be due to the low accuracy of water content determination. However, it is more likely that the fluctuations stem from the variation in the orientation of the water molecules adhering to the different polar residues. Careful exploration of this phenomenon might elucidate the sequence and the relative strength of hydration of the different polar residues, which is beyond the scope of this investigation. In any event, experimental error does not affect the extrapolation of the points to the limiting value $R = 2.8$, to obtain the number of water molecules n_p oriented by one molecule of DPPS. The values of n_p in the presence of different concentrations of cholesterol are plotted as a function of the molar fraction of cholesterol (Fig. 2). This curve is similar to the curves obtained for the 'unfreezable' water molecules as a function of the molar fraction of cholesterol in dimyristoylphosphatidyl serine and in natural phosphatidyl serine [1], only, as mentioned before, differing in the number of oriented water molecules as compared to the number of unfreezable water molecules calculated in reference [1]. The results indicate that a larger number of water molecules is oriented by the phospholipid than the number of very tightly bound unfreezable ones.

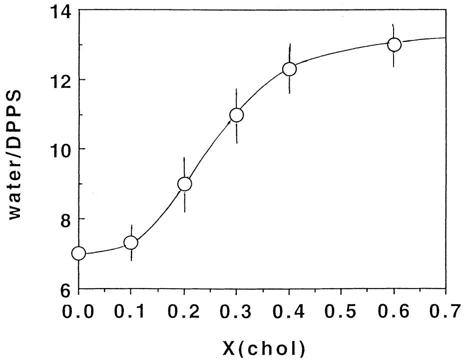

Fig. 2. Number of water molecules adhering and oriented by a molecule of DPPS as a function of the molar fraction of cholesterol in the mixture.

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