ELSEVIER

Bioelectrochemistry 58 (2002) 193–196

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Interrelation between hydration and interheadgroup interaction in phospholipids ☆

Israel R. Miller [a,*], Diana Bach [a], Ellen J. Wachtel [b], Miriam Eisenstein [b]

[a] *Department of Biological Chemistry, The Weizmann Institute of Science, P.O.B. 26, 76100 Rehovot, Israel*
[b] *Department of Chemical Services, The Weizmann Institute of Science, Rehovot, Israel*

Received 18 July 2001; received in revised form 23 November 2001; accepted 20 December 2001

Abstract

The stability and the ionic conductivity of biological membranes and of lipid bilayers depend on their hydration. A small number of water molecules adhere strongly to the different residues of the lipid headgroups and are oriented by them. An additional number of water molecules adhere more weakly, preserving their freedom of rotation, but are essential for bestowing the thermodynamic properties of hydrated bilayers and of biological membranes. Around six water molecules are attached so strongly to the headgroups of different phospholipids (PL) that they are rendered unfreezable, or their freezing is extended over such a wide range of temperatures that it cannot be detected by differential scanning calorimetry (DSC). If cholesterol is added to the PL above the concentration at which phase separation of the cholesterol phase occurs, the number of unfreezable water molecules per PL increases, indicating that the PL molecules on the border line between the two phases attach nearly twice as many water molecules as those in the middle of the phase. The orientation of about seven or eight water molecules attached to PL headgroups (seven to phosphatidyl serine (PS)) can be detected by polarized FTIR. The dichroic ratio of the successively adhering water molecules to the headgroup of PS fluctuates between 2.6 and 2.9, with the cumulative value of about 2.8 for the seven water molecules adhering to the headgroup of PS. In addition, in this case, the number of water molecules oriented by PL molecule residues on the border line of the two phases is much larger (~ 13 for PS). Interaction between two opposite negatively charged layers containing PS approaching each other may lead, after correlated electrostatic attraction, to change in the conformation of the headgroups with concomitant dehydration. This process is enhanced by Ca^{+} and by Li^{+}, but it may also occur with Na^{+} and K^{+} as counter-ions if the layers are mutually aligned. This process may be important in the fusion mechanism of biological membranes, and its molecular modeling has been carried out.

Keywords: Phospholipids; Differential scanning calorimetry (DSC); FTIR modeling

1. Introduction

Bioelectrolytes, including phospholipids (PL), act in the aqueous solution in the hydrated form. Hydrating water molecules adhere to polar dipoles. The same polar residues tend to interact when they are juxtaposed. Thus, competition between hydration and interpolar group interaction is expected. In bilayer layers, as in multilamellar liposomes, the headgroups are hydrated, but their degree of hydration may be restricted by the competition with the lateral interaction between the headgroups. To demonstrate this, one has to compare the degree of hydration of the phospholipids in continuous uninterrupted bilayers, and in bilayers where the continuity is interrupted and boundary lipids lacking nearest neighbors to interact with are exposed. Two hydration strengths of phospholipids have been observed. The first few (up to six or seven in different phospholipids and more than 20 in gangliosides) [1,2] hydrating water molecules adhere to the phospholipids so strongly that they are rendered unfreezable. This means that either the free energy of the adhering water molecules is lower than that of ice in the whole temperature region, or that every consecutive water molecule attached to the phospholipid binding sites freezes at different temperatures and the transition region extends over a wide temperature range and cannot be discerned from the background in differential scanning

☆ This paper was presented at the XVIth International Symposium on Bioelectrochemistry and Bioenergetics, Bratislava from June 1 to June 6, 2001. A collection of these papers has also been published in previous issues of this Journal 55/1–2 and 56/1–2.

* Corresponding author. Tel.: +972-8-9344059/2413; fax: +972-8-9344112.

E-mail address: bmiller@weizmann.weizmann.ac.il (I.R. Miller).

1567-5394/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S1567-5394(02)00035-X

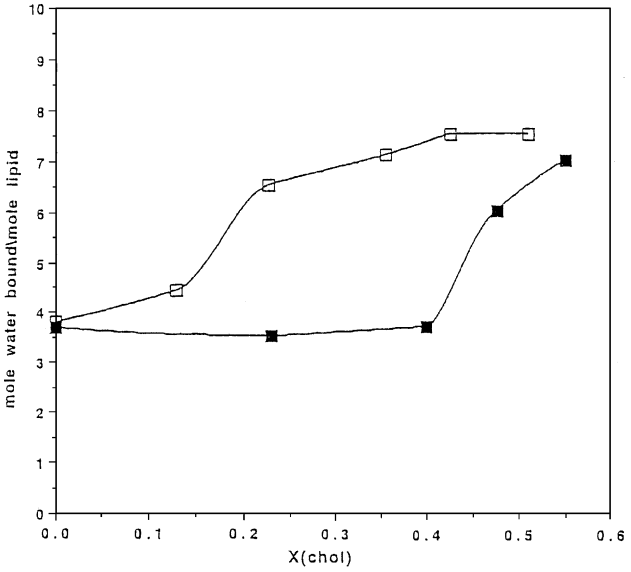

Fig. 1. Hydration of phospholipid–cholesterol mixtures, moles of water bound per mole phospholipid as a function of molar fraction of cholesterol X (chol), □□, DMPS–cholesterol mixtures; ■■, DMPC–cholesterol mixtures.

calorimetry (DSC) measurements. A larger number of water molecules is needed to bestow on the phospholipids all the properties required for functioning in the aqueous environment [3,5]. When phase separation of cholesterol dissolved in the phospholipid starts, an increasing number of phospholipid molecules become situated on boundary lines, with restricted lateral interaction possibilities with other phospholipids. This may affect and probably increase their hydration. On the other hand, interbilayer interaction between headgroups induced by correlated electrostatic attraction may cause their dehydration. These phenomena were shown experimentally and their feasibility was demonstrated by molecular modeling.

2. Experimental

Dimiristoyl phosphatidyl serine sodium salt (DMPS) was purchased from Avanti Polar Lipids. Dimiristoyl phosphatidyl choline (DMPC) and dipalmitoyl phosphatidyl serine (DPPS) were purchased from Sigma and phosphatidyl serine from bovine spinal cord (SCPS) was purchased from Lipid Products (South Nutfield, UK). Cholesterol from Merck was extra pure and recrystallised from ethanol before use. The lipid mixtures were prepared in chloroform/methanol (2:1, v/v). After removing the solvent by a stream of nitrogen and in vacuum, weighed samples were either dispersed in water (for preparation of oriented multilayers on the Ge crystals for the ATR-FTIR measurements), or weighted amount of water was added to them in the pans for differential scanning calorimetry (DSC). The FTIR measurements were performed on a Perkin Elmer Model 1600 FTIR spectrophotometer, for ATR using a 2-mm-thick Ge prism cut to 45*. The calorimetric measurements were carried out on a Du Pont 990 Thermal Analyzer, equipped with cell base 2. The number of water molecules per phospholipid in the oriented phospholipid layer was inferred from the vibration band intensity ratios of the water OH stretching band at 3250 cm^{-1}, and the CH_2 stretching at 2920 cm^{-1} or CO stretching band at 1730 cm^{-1} (in the presence of cholesterol) [1,4,5]. Low angle X-ray diffraction was carried out on thin layers deposited on the outer surface of a Li glass capillary. An Elliot GX-6 generator, at 1.2 kW, with a Cu anode 0.2-mm focus was the X-ray source [7].

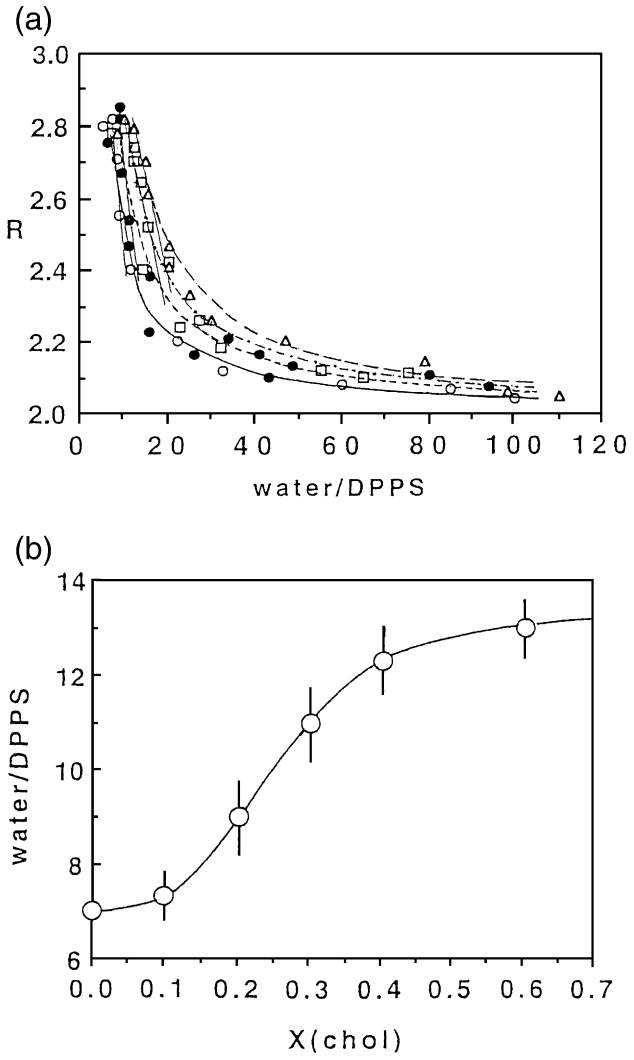

Fig. 2. (a) Dependence of the dichroic ratio R on the number of water molecules added per molecule of DPPS as determined from the measured ratio A (3400 cm^{-1})/A (1230 cm^{-1}) after apropriate calibration [4] for different concentrations of cholesterol. Molar fractions of cholesterol (X): ○, $X=0$; ●, $X=0.2$; □, $X=0.3$; △, $X=0.6$. Points—experimental curves drawn according to Eq. (1). (b) Number of water molecules adhering and oriented by a molecule of DPPS as a function of the molar fraction of cholesterol in the mixture.

3. Results and discussion

Phospholipid hydration by tightly attached (unfreezable) water molecules is expected to be enhanced, when interphospholipid interactions are interrupted by cholesterol clusters and the phospholipid molecules at the phase boundary line lack part of their phospholipid neighbors and are exposed instead only to the competing water molecules.

This is shown in Fig. 1 and in Ref. [1] for dipalmitoyl phosphatidyl choline (DPPC) and for dipalmitoyl phosphatidyl serine (DPPS). The firmly attached water molecules are also expected to be oriented in aligned phospholipid multibilayers [4] and this is indeed the case as shown in Fig. 2a, where the dichroic ratios of water OH stretching frequency band, are given as a function of the number of water molecules added per phospholipid molecule. The dichroic ratios vary from 2, characteristic for random orientation at large excess of water, to about 2.8 when all the water molecules are oriented. In addition, the number of oriented water molecules per phospholipid increases, as shown for DPPS in Fig. 2b, when phase separation of cholesterol starts. Phase separation, as indicated by the increase of water attachment to phospholipids, occurs at lower cholesterol concentration than by any other method. This shows that water attachment to phospholipids starts increasing already in the presence of small cholesterol clusters, undetectable either by PSC or X ray, which allows for a large number of phospholipids to be at the lipid cholesterol boundary line. A number of water molecules, which do not adhere tightly to the phospholipids but affect their thermotropic properties, also lower the vibration frequencies of different polar residues. A convenient way to determine the frequency shift is from the difference spectra, between the spectra in the presence and in the absence of water. The frequency shift yields positive and negative peak in the difference spectrum [5]. In Fig. 3, the positive and negative peak difference of different bands is plotted against the number of water molecules per phospholipid added, calculated from the measured ratios of the water OH peak to the CO peak. The spectral results correspond qualitatively, but not quantitatively, with the DSC results as only similar but identical properties are measured by the two methods.

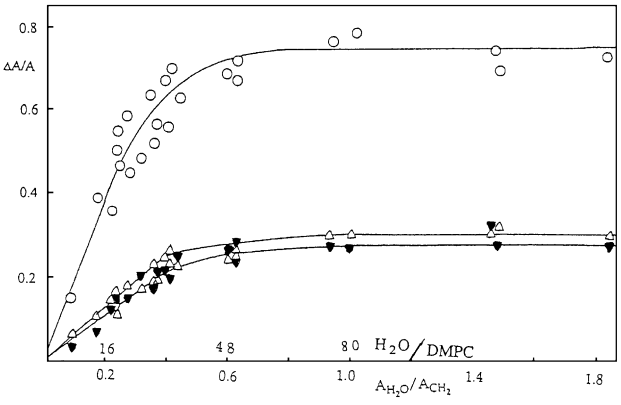

Fig. 3. Difference absorbance amplitude (difference between positive and respective negative peaks) of DMPC due to the frequency shift by hydration of different residues, normalised with respect to their vibration band absorbance against the number of water molecules per DMPC, expressed as A_{H_2O}/A_{CH_2}: ○○, asymmetric stretching of PO_{2-}; △△, symmetric stretching of PO_{2-}; ▼▼, stretching and of CO.

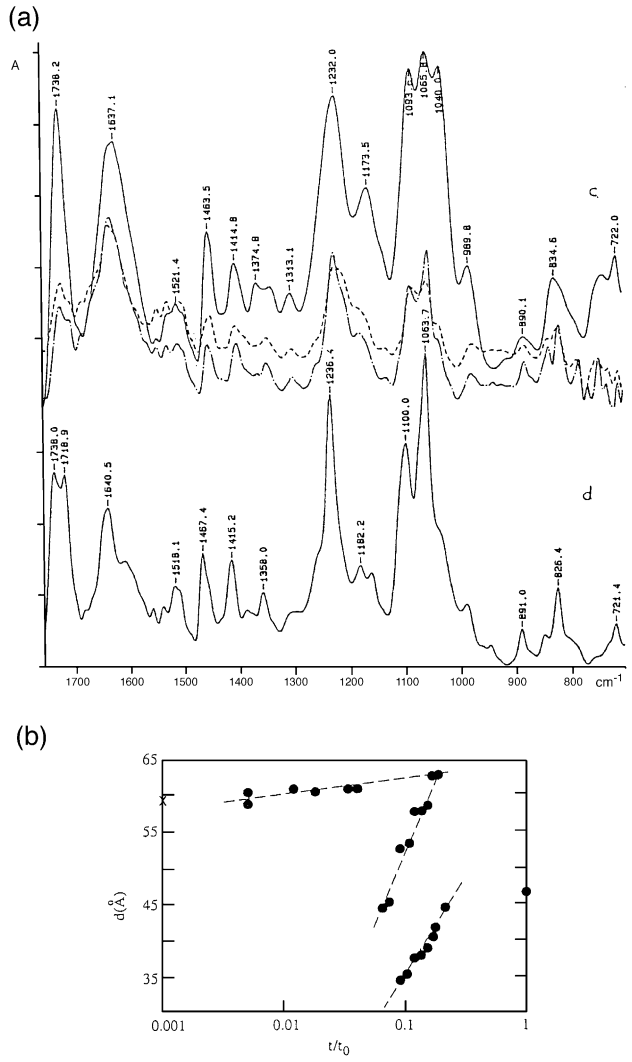

Fig. 4. (a) Time dependence of the ATR-FTIR spectra of PS multibilayers wetted by H_2O. Phosphatidylserine from spinal cord (SCPS) (__) spectrum of film freshly prepared from aqueous suspension. (- - -) Spectrum obtained during 5-min scan immediately after wetting by H_2O. (-.-) Spectrum obtained at room temperature 15–20 min after wetting. In the last two cases, water spectra were subtracted. (d) Spectrum obtained after 2-h drying at 37 °C. (b) Time dependence of the measured d-spacings of the most intense X-ray reflections of a DMPS film, during controlled exposure to water (●). $t_0 = 1050$ min. (X) after withdrawal from the hydrating environment.

In the same way as decrease in lateral interaction between headgroups can enhance hydration, enhanced interaction between headgroups, be it lateral or between opposite polar layers, can cause dehydration. Such a dehydrating interaction between facing each other surfaces of phospha-

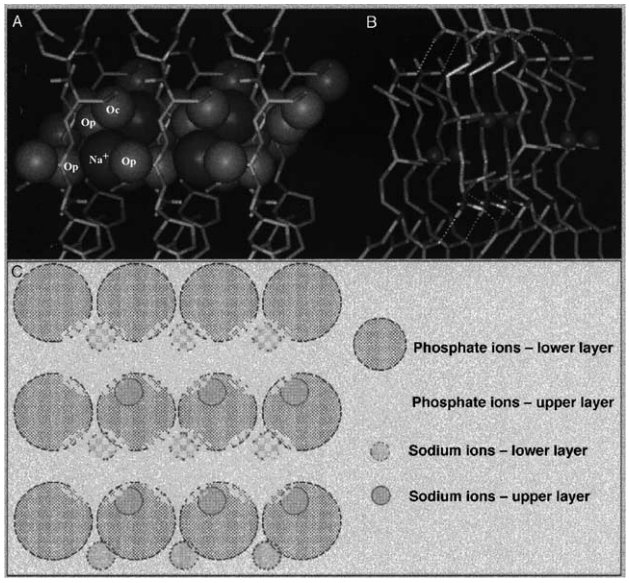

Fig. 5. Conformation of opposite polar layers of PS interacting through salt bridge and hydrogen bond formation. (A) Salt bridges with coordination of the counter-ion (violet) with oxygen (red) stemming from phosphates and from carboxylate — as indicated — are highlighted. Phosphates indicated only by yellow bonds. (B) Hydrogen bonds between ammonia from one plane with sn-carbonyl in the other plane highlighted. (C) Schematic presentation of the salt bridging between phosphates of opposite planes by the counter-ions.

tidyl serine can be induced by multivalent cations, e.g. Ca^{2+}, which results in dehydrated cochlate formation and characteristic change in IR spectrum [6]. Such a change in spectrum with consecutive dehydration, demonstrated by a decrease in the proton by deuteron exchange rate in the ammonium residue by many orders of magnitude, can also be obtained in the presence of univalent counter-ions, if the multibilayers are aligned in planar configuration, eliminating the possibility of spontaneous liposome formation. The change in the IR spectrum and in the repeat distances with time [7] is shown in Fig. 4a and b. These conformational changes proceed only in the presence of water even though the final configuration is dehydrated. Molecular modeling, taking into account molecular radii and minimizing the interaction energies [8], yielded a multimolecular conformation, including the aligned headgroups and their counter ions as shown in Fig. 5.

References

[1] D. Bach, I.R. Miller, Hydration of phospholipid bilayers in the presence and absence of cholesterol, Biochim. Biophys. Acta 1368 (1998) 216–224.
[2] D. Bach, B. Sela, I.R. Miller, Compositional aspects of lipid hydration, Chem. Phys. Lipids 31 (1982) 381–394.
[3] C. Faure, L. Benakdar, E.J. Dofourc, Determination of DMPC. Hydration in the L_α and and L_β phases by ^2H solid state NMR of D_2O, FEBS Lett. 405 (1997) 263–266.
[4] I.R. Miller, D. Bach, Organization of water molecules by adhering to oriented layers of dipalmitoyl phosphatidylserine in the presence of varying concentrations of cholesterol, Biochim. Biophys. Acta 1468 (2000) 199–202.
[5] I.R. Miller, D. Bach, Hydration of phosphatidyl serine multilayers and its modulation by conformational change induced by correlated electrostatic interaction, Bioelectrochem. Bioenerg. 48 (1999) 361–367.
[6] H.L. Casal, H.H. Mantsch, Studies of fully hydrated phosphatidyl serine bilayers. Effect of Li^+ and Ca^{2+}, Biochemistry 26 (1987) 4408–4416.
[7] I.R. Miller, E. Wachtel, Interbilayer interaction and deuterium–proton exchange in thin films of phosphatidyl serine, Bioelectrochem. Bioenerg. 45 (1998) 203–214.
[8] I.R. Miller, M. Eisenstein, Structure of phosphatidyl serine (PS) involved in interbilayer salt bridging and hydrogen bonding, Bioelectrochemistry 52 (2000) 77–81.