PHOSPHOLIPID SURFACE BILAYERS AT THE AIR-WATER INTERFACE

II. Water Permeability of Dimyristoylphosphatidylcholine Surface Bilayers

L. GINSBERG AND N. L. GERSHFELD
National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205

ABSTRACT Dispersions of dimyristoylphosphatidylcholine (DMPC) in water have been reported to form a structure at 29°C at the equilibrium air/water surface with a molecular density equal to that of a typical bilayer. In this study, the water permeability of this structure has been evaluated by measuring the rate of water evaporation from DMPC dispersions in water in the temperature range where the surface film density exceeds that of a monolayer. Evaporation rates for the lipid dispersions did not deviate from those for lipid-free systems throughout the entire temperature range examined (20–35°C) except at 29°C, where a barrier to evaporation was detected. This strengthens the view that the structure that forms at this temperature has the properties of a typical bilayer.

INTRODUCTION

The spontaneous formation of a bilayer at the air/water surface by equilibrium aqueous dispersions of dimyristoylphosphatidylcholine (DMPC) at 29°C has been supported by direct measurement of the surface film concentration, and by thermodynamic arguments (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985). Here we provide additional evidence for the existence of the surface bilayer by measurement of the permeability of the structure to water.

Permeation of water across air/water surfaces has been studied systematically in the context of finding lipid monolayers to reduce the rate of evaporation of water from large bodies of water (LaMer, 1962). We have employed similar techniques for measuring the rate of evaporation of water from dispersions of DMPC in water at temperatures in the range where DMPC surface bilayers form. The results demonstrate the existence of a structure at the air/water surface that lowers the rate of evaporation of water at 29°C, but at temperatures 0.1°C above and below this temperature the permeability barrier virtually disappears. An analysis of this phenomenon is presented that shows it is consistent with surface density measurements and with the thermodynamic characterization of the surface bilayer (Tajima and Gershfeld, 1985).

EXPERIMENTAL METHODS

Technique of Measurement and Theory of Water Permeability from Rates of Evaporation

Rates of evaporation from aqueous surfaces were monitored continuously by a device that consisted of an inverted Teflon cup (0.5 cm deep, 2.5 cm diam) filled with anhydrous lithium chloride. The mouth of the cup was covered by 300 mesh silk-screening material that was clamped to the cup by an aluminum compression ring. The cup was suspended from the lever arm of a strain gauge (model G110b, Statham Instruments, Puerto Rico) over the water surface. As evaporation proceeded, vapor was absorbed by the LiCl crystals, and the increase in weight, as indicated by the amplified output of the strain gauge, was recorded as a function of time. By suspending known weights from the lever arm of the strain gauge, the output voltage was calibrated directly in terms of weight.

A water-jacketed cylindrical glass cell (volume 35 ml, surface area ~20 cm^2) was filled to the rim with either distilled water or a dispersion of DMPC in water (0.2 mg/ml). Temperatures were monitored by a thermistor placed in the water surface of an identical cell in series with the cell used for evaporation measurements. Constant temperature water (±0.05°) was circulated through the jackets of both cells. Since evaporation studies require an open surface, the temperature of the surface will always be cooler than the bulk solution because of evaporation. The amount of cooling will depend on the rate of evaporation, and the air temperature (Barnes and Hunter, 1982). For our studies the cooling was ~0.5°C. Rather than making extensive efforts to correct for the cooling effect, we report the recorded surface temperature. The apparatus, cells, and detector were covered by a Plexiglas box. Since room air temperatures were variable, the interior of the box was kept at a temperature near that of the water surface (±1.0°C) by electrical heating tapes placed around the cell and detector. The air temperature was monitored by a thermistor placed near the detector. The strain gauge was mounted outside the box because the heat of the air space changed the bridge resistance characteristics of the strain gauge. Temperature fluctuations of the air space of ±1.0°C did not appear to affect evaporation.

L. Ginsberg's present address is the Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, London, W.1., England.

Address all correspondence to N. L. Gershfeld.

The size of the detector and its distance above the water surface were found to influence the measurements: the measured rate of evaporation was found to vary directly with the area of the detector (1.0–20 cm^2), and inversely with distance of the detector above the water surface (0.25–1.0 cm). We used a 5-cm^2 detector at a distance of 0.25 or 0.5 cm above the surface for the experiments reported here. Modification of either detector area or its distance above the water surface had no effect on the permeability coefficient measurements. The silk-screening material did not contribute to the rate of water uptake by the detector because no weight change was noted when the detector without LiCl was placed over the water surface for a period of ~1 h.

The detector was filled with anhydrous LiCl just before suspending it from the strain gauge over the water surface. The increase in weight of the detector due to absorption of water vapor was monitored for ~15 min. The first minute of the recording was ignored since it reflected mechanical artifacts arising from positioning of the detector and establishment of the vapor pressure gradient between the detector and the water surface. (A simple calculation using the diffusion coefficient of water vapor in air indicated that it took less than a minute to establish the gradient.) A steady state rate of evaporation between 1 and 10 min after the start of the experiment was observed; a slight decrease in rate was observed over the next 30 min. This may be attributed to the fact that the lower surface of the LiCl became caked with moisture and therefore may have contained a thin layer of water that is not completely saturated with salt; this would act to raise the effective vapor pressure at the surface of the detector and concomitantly decrease the gradient and the rate of evaporation. Rates of evaporation were therefore obtained from the slope of the weight-time recording between 2 and 8 min after the start of the experiment. Note that in preliminary experiments with extremely coarse crystals of LiCl the steady state period was shortened significantly to the first 4 min rather than the 10 min found with the finer crystal preparation. In the steady state, the measured evaporation rates were independent of the crystal sizes of anhydrous LiCl. The method of analysis of the weight (m)-time data follows that of Langmuir and Schaefer (1943) and Archer and LaMer (1955).

The evaporation experiment entails a number of water transport processes linked in series: evaporation from the surface, passage through the film, diffusion through a stagnant air layer and the detector membrane, and absorption at the surface of the desiccant (LiCl).

It is convenient to analyze the contributions of each of these processes by their resistance to water transport

$$\begin{aligned} R_{\text{total}} &= R_{\substack{\text{water}\\ \text{surface}}} + R_{\text{film}} + R_{\text{air}} + R_{\text{detector}} \\ &= R_0 + R_{\text{film}}, \end{aligned} \tag{1}$$

where R_0 is the resistance in the absence of lipid film. To evaluate R_{film}, the usual procedure is to measure the rates of evaporation in the presence and absence of the film.

In the presence of a film, assuming steady state evaporation, the rate of evaporation may be written, by analogy with Ohms law,

$$(\mathrm{d}m/\mathrm{d}t)_{\text{film}} = (C_w - C_d)/R_{\text{total}}, \tag{2}$$

where m is the weight of water vapor absorbed by the detector, $(C_W - C_d)$ represents the difference in chemical potential of water between bulk water and the detector where C_w and C_d are the equilibrium concentrations of water vapor at the water and detector surfaces, respectively. The latter are calculated from the vapor pressures of water and saturated LiCl solutions (International Critical Tables, Vol. 3, p. 212, 368). For evaporation in the absence of lipid film

$$(\mathrm{d}m/\mathrm{d}t)_0 = (C_w - C_d)/R_0. \tag{3}$$

Combining Eqs. 1–3 yields

$$R_{\text{film}} = (C_w - C_d)\,[(\mathrm{d}m/\mathrm{d}t)_{\text{film}}^{-1} - (\mathrm{d}m/\mathrm{d}t)_0^{-1}]. \tag{4}$$

The specific resistance of the film, r_{film} (s/cm), is obtained from the relation

$$r_{\text{film}} = R_{\text{film}}A, \tag{5}$$

where A is the area of the detector; r_{film} is the reciprocal of the permeability coefficient.

MATERIALS

Dimyristoylphosphatidylcholine (DMPC) (Avanti Polar Lipids, Inc., Birmingham, AL) dispersions in water were prepared by vortexing the lipid at a concentration of 0.2 mg/ml in water at temperatures just above the gel–liquid-crystal transition temperature (25°–35°C). The lipid did not show any impurities by thin-layer chromatography (chloroform/methanol/water, 65:25:4, by volume); it was used without further purification.

RESULTS

The rate of evaporation from water surfaces as a function of temperature is presented in Fig. 1. The solid line is the calculated rate of evaporation from a lipid-free water surface. The relation is derived from Fick's first law with the assumption that in the steady state the rate of diffusion of water vapor in air is the rate-limiting step for evaporation, i.e., in Eq. 1, $R_{\text{air}} \gg R_{\text{water surface}}, R_{\text{detector}}$, and the vapor gradient is constant. The rate law for evaporation becomes

$$(\mathrm{d}m/\mathrm{d}t)_0 = (AD/x)\,(C_w - C_d). \tag{6}$$

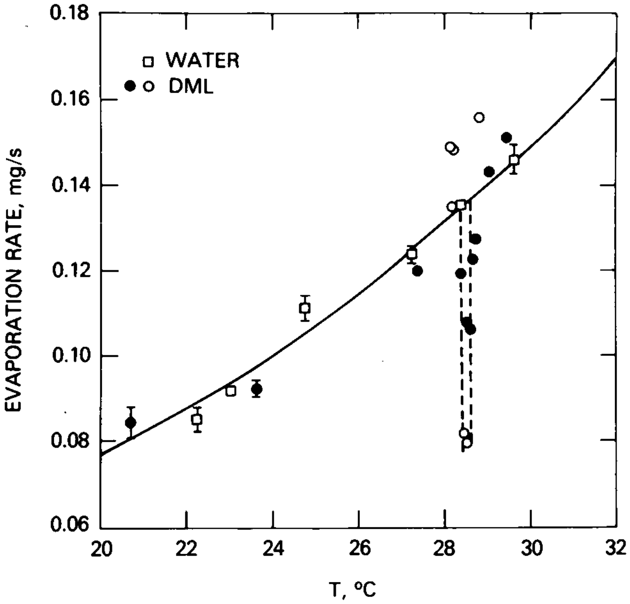

FIGURE 1 Steady state water evaporation rate is given as a function of temperature. The solid line is the theoretical relation for lipid-free system, calculated by Eq. 6 using $A = 5$ cm^2, $x = 0.25$ cm (see text). Where mean deviation error bars are given, points are the means of five independent measurements. Experimental conditions are (except as noted below): (*a*) Isothermal experiments (±0.05°C), no vibration damping provided; □, lipid-free; ●, DMPC dispersions, 0.2 mg/ml. (*b*) Temperature scanning, vibration damping; ○, DMPC dispersions, 0.2 mg/ml; data obtained with $x = 0.5$ cm, rates normalized to place on same graph with other data, normalization does not alter r_{film}.

From Eqs. 1 and 3

$$R_0 \approx R_{\mathrm{air}} = x/AD, \tag{7}$$

where A is the detector area, x is its distance above the water surface, and D is the diffusion coefficient of water vapor in air. D is a function of temperature and may be calculated from the relation $\log D = 1.75 \log T - 4.921$ (Langmuir and Schaefer, 1943). The experimental values for the rates of evaporation in the absence of film follow Eq. 6 over the entire temperature interval examined.

The rate of evaporation in the presence of DMPC dispersions is also presented in Fig. 1. The data for two different sets of conditions are presented. In the first (filled circles) isothermal (±.05°C) experiments were conducted without any serious efforts to dampen building vibrations. The data indicate that over the entire temperature interval except at 28.5°C, the rate of evaporation equals that for the lipid-free system. A 28.5 ± 0.1°C the rate of evaporation decreases by ~25%. The temperature where the rate of evaporation decreases is, within experimental uncertainty (due to surface cooling by evaporation), the temperature where pure surface bilayer occupies the whole surface, 29°C, (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985).

The second experiment for measuring the rate of evaporation for DMPC dispersions took cognizance of two aspects of the bilayer system: (*a*) single, planar bilayers are extremely sensitive to vibrations; and (*b*) the temperature range of existence of the surface bilayer is very narrow. The experiments were therefore conducted with improved vibration damping, as noted by a decrease of ~75% in the amplitude of mechanical noise, and, instead of isothermal conditions, the temperature was slowly raised through the temperature interval of surface bilayer formation. Actual recorded data are reproduced in Fig. 2 where the change in weight as a function of time is presented for three separate temperature scans: 28.1–28.33°C, 28.2–28.5°C, and 28.5–28.8°C. These temperatures were obtained by shutting off the cooling unit of the constant temperature bath, allowing the temperature to rise slowly. At 28.4°C there is a significant decrease in the rate of evaporation; the rate appears to increase again as the temperature approaches 28.5°C. Each of these runs was made with a fresh loading of anhydrous desiccant. The slight curvature that appears with the higher and lower temperature scans (28.1–28.33°C, and 28.5–28.8°C) is the result of caking of the desiccant (see Methods), and clearly is not the cause of the sudden decrease in rate at 28.4°C. The rates for two separate series of scans are plotted in Fig. 1 (open circles). The rate at 28.4°C decreases ~50% from the lower and higher temperature rates, and the rate for the lipid-free water surface. Thus, damping of surface vibrations significantly improves the evaporation resistance of the surface bilayer.

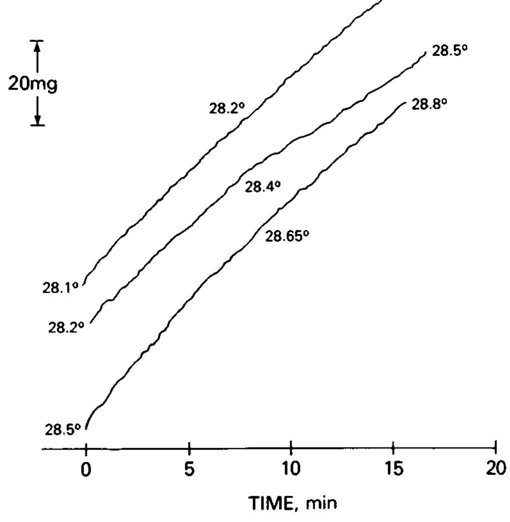

FIGURE 2 Water evaporation measurements using temperature scanning and damping of mechanical vibrations are illustrated. Recorder tracings of three consecutive independent temperature scans of DMPC dispersions (0.2 mg/ml) are given. Detector set at $x = 0.5$ cm to accommodate the single scan on chart.

DISCUSSION

The major conclusion from the evaporation studies is that surface bilayers of DMPC retard the evaporation of water, but only at 28.4°C the temperature at which the surface bilayer occupies the entire surface area. Thus at temperatures 0.1°C higher or lower than 28.4°C, the rate of evaporation is essentially that of a lipid-free surface despite the fact that condensed DMPC film is present.

This result concurs with our previous results based on surface concentration and surface pressures, that surface bilayers form at 29°C (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985). The small difference in temperature for surface bilayer formation between the evaporation study and the earlier studies very likely arises because evaporation leads to ~0.5°C cooling of the surface (Barnes and Hunter, 1982).

To compare the water permeability of the surface bilayer with permeabilities of typical bilayers we use the specific resistance of the film at 28.4°C, calculated by using Eqs. 4 and 5. For the DMPC surface bilayer the specific resistance is ~1 s/cm, while for phosphatidylcholine bilayer membranes it is of the order of 200 s/cm (Huang and Thompson, 1966; Graham and Lea, 1972; Graziani and Livne, 1972; Fettiplace, 1978; Boroske et al., 1981).

These properties of the surface bilayer raise two questions: (*a*) Why does the evaporation rate decrease only in the very narrow temperature interval where the surface bilayer occupies the entire surface, and (*b*) If the surface bilayer is structurally identical with all other bilayers, why is the specific resistance so much smaller than that of the typical bilayer? The following model for the structure of the DMPC film provides a reasonable basis for answering these questions.

The surface concentration-temperature relation for DMPC (Gershfeld and Tajima, 1979; Tajima and Gershfeld, 1985) indicates that between the temperatures of 26° and 37°C, the film structure passes continuously from a monolayer to the surface bilayer at 29°C followed by a continuous decrease in film density to that of a condensed monolayer at 37°C. This sequence appears to conform to criteria for higher order surface phase transitions (Tajima and Gershfeld, 1985). The structure of the film in the temperature interval where the surface concentration varies between monolayer and surface bilayer is likely to be a hybrid of both states in keeping with other higher order transitions (Ubbelohde, 1965). To model the permeability properties of these hybrid states, we assume that at temperatures above and below 29°C, monolayer and bilayer coexist as a patchwork array; the relative amount of bilayer to monolayer decreases as the temperature is either raised or lowered from 29°C, the temperature where only bilayer exists. The specific resistance of the surface film, r_{film}, will depend on the relative areas occupied by monolayer and bilayer; r_{film} may be calculated from Eq. 8, for parallel resistances

$$1/r_{\text{film}} = \alpha/r_{\text{m}} + (1 - \alpha)/r_{\text{b}}. \tag{8}$$

r_{m} and r_{b} are the specific resistances of monolayer and bilayer, respectively, and α is the fraction of the surface occupied by monolayer.

Because DMPC forms liquid expanded monolayers in the temperature interval of 25°–37°C (Phillips and Chapman, 1968; Gershfeld and Tajima, 1977) we use the value of r_{m} that has been found for other liquid expanded films. A variety of liquid expanded films have been studied, and the results indicate that they do not retard the evaporation of water (Rosano and LaMer, 1956; Jarvis et al, 1962). Our results with DMPC at 27°C, where the surface concentration is equivalent to a liquid expanded monolayer (cf. Fig. 1, and Tajima and Gershfeld, 1985, Fig. 2), also indicate that r_{m} is indistinguishable from that for a lipid-free surface. The specific resistance for the water surface has been estimated to be 2×10^{-3} s/cm (Archer and LaMer, 1955); this value is used for r_{m}. For r_{b} we use 200 s/cm, the value for a typical lecithin bilayer.

Using Eq. 8, based on the patchwork model of DMPC films, we calculate values of r_{film} as a function of α, the fraction of the surface occupied by monolayer; these values are listed in Table I. Our model indicates that even if 99.8% of the surface is occupied by bilayer, the presence of only 0.2% of the surface as monolayer is sufficient to reduce the specific resistance of a bilayer surface film from 200 to 1 s/cm. Moreover, the model indicates that the surface bilayer will reduce the rate of evaporation to that for typical bilayers only when the great preponderance of the surface (>99.99%) is covered by the bilayer; this state exists only in a very narrow temperature interval at 29°C.

TABLE I

CALCULATION OF r_{film} AS A FUNCTION OF α*

α	0	10^{-4}	2×10^{-3}	10^{-2}
r_{film} (s/cm)	200	20	1.0	0.2

*The values of r_{film}, specific resistance of a mosaic surface film consisting of patches of monolayer (*m*) and bilayer (*b*), as a function of α, the fraction of surface area occupied by monolayer were calculated using Eq. 8 with $r_{\text{m}} = 2 \times 10^{-3}$ s/cm and $r_{\text{b}} = 200$ s/cm (see text).

Thus, as Fig. 1 indicates, evaporation reduction will occur only in a very limited temperature range at 29°C.

This model also provides an estimate of the temperature control required to achieve evaporation rates that are equivalent to those for typical bilayers. From the temperature dependence of the surface concentration for DMPC dispersions (Tajima and Gershfeld, 1985) we estimate the temperature dependence of the monolayer surface coverage; for temperatures near 29°, $|\Delta\alpha/\Delta T| \approx 0.1$/deg, both below and above 29°C. Thus, to achieve appreciable values for r_{film}, e.g., 20 s/cm, requires values of α smaller than 10^{-4}, and therefore temperature control that is better than ±0.001°C. Our present experimental arrangement, under the best of circumstances, allows for temperature control of the order of ±0.05°C, leading to the maximum possible value for r_{film} of 0.4 s/cm, the same magnitude as observed. However, even these low values are more than two orders of magnitude greater than for the lipid-free system.

Given the general sensitivity of all planar bilayer systems to mechanical vibrations, it is not surprising that evaporation retardation of the surface bilayer improves significantly when the system is mechanically insulated from extraneous vibrations.

In summary, our experimental results are consistent with a model for the DMPC film structure in which a bilayer coexists with lipid expanded monolayer over the entire range of temperatures where the surface concentrations exceed monolayer surface densities. Accordingly, the narrow temperature interval of evaporation resistance at 29°C, the temperature where only surface bilayer is present, is the result of the large ratio of specific resistances $r_{\text{b}}/r_{\text{m}} \approx 10^5$. The presence of even extremely small amounts of monolayer greatly decreases the effectiveness of the surface bilayer in retarding evaporation. The model also predicts that to obtain a specific resistance characteristic of typical planar bilayers requires temperature control of ±0.001°C. Thus, at a singularity in temperature when surface concentration correlates with bilayer density and where partial molar surface entropies are equal to those for bilayers (Tajima and Gershfeld, 1985), we have demonstrated resistance to water movement for DMPC films.

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