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A NEW METHOD FOR THE DEMONSTRATION OF PHASE TRANSITIONS IN LIPID FILMS

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SUMMARY

Monolayers of L-α-lecithin (β, γ-dimyristoyl), L-α-lecithin (β, γ-dipalmitoyl) and egg phosphatidylethanolamine were formed on a water surface at a sufficiently low temperature. The rate of evaporation of water was measured as a function of increasing temperature.

In agreement with previous reports, the monomolecular films had a marked influence on the rate of evaporation of water, and the passage of water molecules through the monolayer was retarded.

Using films in which phase transitions can be observed at a specific transition temperature [L-α-lecithin (β, γ-dimyristoyl) at 23 °C and L-α-lecithin (β, γ-dipalmitoyl) at 41 °C], an unexpected decrease of water permeability was measured at this particular temperature. The retardation suggests that water transport through the film is hindered. It is thought that these results are best explained by an enhanced interaction between the water molecules and the chains of the lipid molecules above the phase transition temperature.

INTRODUCTION

The phase transitions of phospholipids in biological membranes may be responsible for many membrane functions [1]. There are many explanations for these features but not much is known about the molecular mechanisms involved.

Phase transitions of phospholipid films have been demonstrated by different methods such as ESR [2], NMR [3], optical methods (fluorescence, absorption, light scattering), calometry [5], X-ray diffraction [6] and surface pressure measurements [7]. In this paper a new method is described which allows the continuous recording of water evaporation over a temperature range from 20–50 °C.

MATERIALS AND METHODS

Phospholipids were purchased from: Applied Science Laboratories, Inc. (A Grade) and Calbiochem (phosphatidylethanolamine (from egg) (A Grade). The lipids were analysed by thin-layer chromatography on Silica gel with chloroform–methanol–NH_4OH–water as solvent. 4 μg of impurities could be detected if 4 mg starting material was used. In each case single spots and traces consisting of free fatty acids were obtained. Using L-α-lecithin (β, γ-dimyristoyl) a trace of lysophosphatide was observed in addition. The lipids were used without additional purification. Chloroform was used as a spreading solvent (1 mg/ml) for all lipids examined. Twice-distilled, deionized water was used. A film of P_2O_5 was prepared by dipping a glass-plate with platinum electrodes into acetone containing 20% phosphoric acid; the film was then dried for about 24 h in a desiccator. The drying agent (KC-Trockenperlen) was purchased from Roth, Heidelberg.

Principles and apparatus

When a water surface is covered by a monolayer of phospholipid, the water evaporation is strongly inhibited by the lipid barrier [8]. The effect depends on the surface tension. Therefore, the water permeability should be influenced by thermal phase transitions. From pressure–area isotherms it is known that phase transitions in monolayers can also be induced by varying the molecular density in the layer. By choosing a suitable fixed value for the area per molecule the possibility of producing a phase transition is restricted to the variation of temperature, which means that phase transitions can no longer be obtained by variation of the surface pressure.

The amount of material required to form a monolayer is known from pressure–area diagrams. The following cross-sectional areas for the different molecules were used for the computation of the monolayer: 60 ± 3 Å^2 for L-α-lecithin (β, γ-dimyristoyl) [9], 50 ± 3 Å^2 for L-α-lecithin (β, γ-dipalmitoyl) and 58 ± 3 Å^2 for egg phosphatidylethanolamine. (These values were measured using a self-made Langmuir trough.) In order to register the water permeability, dry air (moisture content $\leqslant$ 2 ppm, rate about 200 cm^3/min) was blown over the film. The apparatus used is illustrated in Fig. 1.

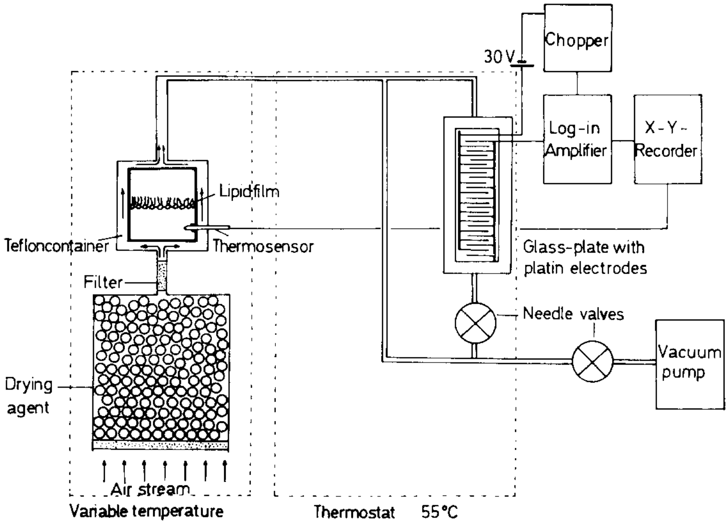

Fig. 1. Schematic representation of the experimental arrangement for the measurement of the rate of water evaporation.

Before measuring the rate of evaporation of water, several monolayers were spread with a Hamilton syringe and subsequently the contaminants were removed by suction. Then the vacuum pump was turned on for 1 h to establish an equilibrium. A teflon container with a surface area of 3 cm^2 was used for the spreading experiments. The temperature of the subphase (pH 7) was measured with a thermosensor TM 15 (Mettler Instruments AG). It is difficult to state the precise temperature because a difference in temperature was established between surface and underlying water [10]. The greater the rate of the evaporation, the cooler the surface film of water becomes. The moisture of the gas was analysed using a self-made electrolytic cell. A water-absorbing film of P_2O_5 deposited between two platinum electrodes served as detector. Small impurities can cause considerable variations in the current. For that reason extremely pure chemicals were used for the film on the glass plate. A filter (Du Pont) was applied to eliminate the contaminants.

By applying a voltage of 30 V to the electrodes, a current which is directly proportional to the moisture can be measured. This current was periodically chopped and gained by a lock-in amplifier (Lock-in amplifier TE 9000 Tekelec). The current is directly and linearly related to the absorption of water and the magnitude of the measured current may be calculated from Faraday's law. A calibration of the current was not tried.

RESULTS

The rate of evaporation rises with increasing temperature. Figs 2 and 3 show some curves plotted by a X–Y-recorder. It is obvious that the current depends on the temperature. Curves 2a and 3a give the rate of evaporation of a free water sur-

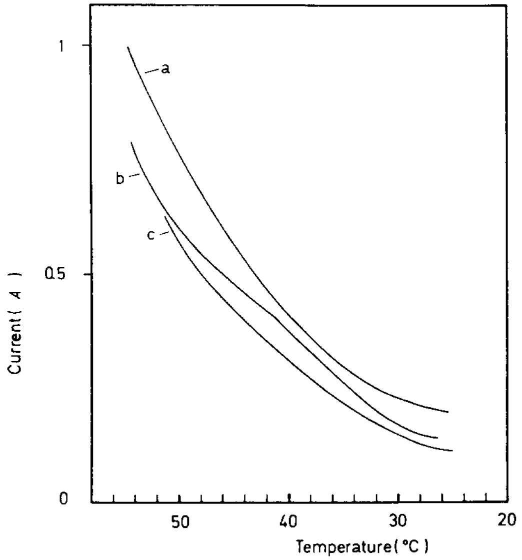

Fig. 2. The rate of evaporation of water as a function of temperature. (a) free water surface; (b) water surface with film of L-α-lecithin, (β,γ-dipalmitoyl); (c) water surface with film of phosphatidylethanolamine.

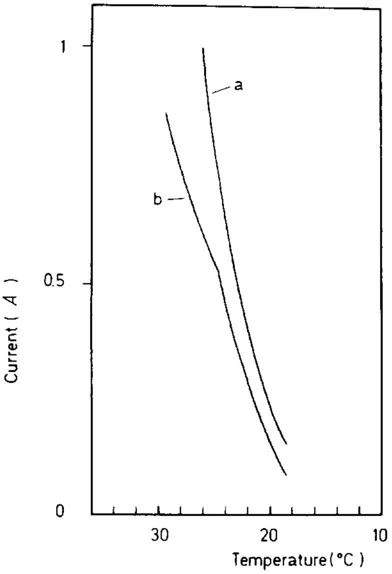

Fig. 3. The rate of evaporation as a function of temperature. (a) free water surface; (b) water surface with film of L-α-lecithin (β,γ-dimyristoyl).

face. It is well known that water surfaces covered by lipid films exhibit decreased evaporation (see Curves 2b, 2c, 3b). Furthermore, the temperature diagrams of water surfaces covered with L-α-lecithin (β-γ-dipalmitoyl) and L-α-lecithin (β-γ-dimyristoyl) show a discontinuity at the point of a phase transition [4]. Phosphatidylethanolamine (from egg) has no phase transition (Hegner, D., unpublished) and the temperature diagram does not indicate any significant effect (Fig. 2c).

DISCUSSION

The decrease of the evaporation above the transition temperatures [L-α-lecithin (β,γ-dipalmitoyl) $\geqq$ 41 °C, L-α-lecithin (β,γ-dimyristoyl) $\geqq$ 23 °C] is difficult to explain because the properties of the film in this region are not well understood.

It is known that at the phase transitions of the phospholipids the distance between the polar head groups can increase [4] and the hydrocarbon chains are in high motion [11].

The mechanism of water transport through monolayers has been discussed by La Mer [8]. According to this author, a monomolecular film causes an energy barrier which is effected much more by the hydrocarbon chains than by the polar groups. Water molecules can penetrate this film by a one-step mechanism.

Our results might be in accordance with this theory. Owing to the gradually increasing rotation of the CH_2-groups the chains interfere with the motion of the water molecule at the transition temperature and the evaporation decreases.

At the present state of the investigation a quantitative interpretation of these preliminary results is not possible. Undoubtedly, the purity of the lipid film has a marked influence on the rate of evaporation of water. In addition, the magnitude of the surface pressure can be calculated from the amount of lipid. The pressure increased with rising temperature while the area of the film was kept constant.

From the relatively small difference between the rate of evaporation of water with and without a lipid film (about 30% at 20 °C) it can be concluded that the film was not free from impurities [8]. The experiments of La Mer on fatty acids show that removal of impurities can influence this difference markedly already at impurity concentrations of 0.1% [9]. We believe our measurements to be influenced by impurities which are mainly contaminants of the striking air stream. From earlier experiments with less pure L-α-lecithin (β, γ-dipalmitoyl), which gave results identical with those published here, it can be concluded that the effect of these impurities on our results is not very serious.

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