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LANGMUIR-BLODGETT MULTILAYERS OF PHOSPHATIDIC ACID AND MIXED PHOSPHOLIPIDS

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ABSTRACT

Very regular Langmuir-Blodgett multilayers appropriate for use in spectroscopic studies have been made . They are built up from monolayers of phosphatidic acid and mixtures of this compound with saturated phosphatidyl ethanolamine or choline spread on a Ca^{2+} aqueous solution. The influence of Ca^{2+} ions on the transfer is discussed .

I - INTRODUCTION

Thin films can be deposited onto various substrates by means of Langmuir-Blodgett's (1,2) (L.B.) method. Such films consist of superposed monolayers built up by successive dipping and withdrawing of a solid substrate through an insoluble monolayer of an appropriate amphiphilic substance spread on the surface of an aqueous solution and kept at a constant pressure.

If phospholipids are used as amphiphilic substances the structure of a single bilayer - composed by two monolayers bound together by their carbon tails - is analogous to the structure of the cell membranes.

Spectroscopic infra-red or Raman (3) measurements performed on these oriented multilayers might give information about the orientation of phospholipids molecules in the layers and its modification by interaction with other added molecules, for example proteins or antibiotic polyenes.

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This type of information cannot be obtained so easily from the study of liposomes. The study of the "orientation" requires that the transferred material should actually give L.B layers (i.e monolayer deposited one by one on the support under equilibrium conditions) and not any more or less amorphous deposit.

Only a few authors have reported the transfer of L.B. multilayers of phospholipids. J.P. GREEN et al(4) transferred multilayers of lipids, particularly of saturated phosphatidycholines. In their deposition method a 2 cm high glass plate is lowered through the water surface in about 1 second. We tried to reproduce these experiments with dipalmitoylcholine and got on the glass plate some "white spots" which, in no way, can be considered as L.B. multilayers. Indeed, it seems rather obvious that at this rate no monolayer can be transferred.

In the usual transferring conditions (at a rate of 1 cm/mn) only one layer of dipalmitoyl-choline could be transferred, on the first up trip of the glass slide. On the next down trip the layer peeled off the solid substrate and went to the water surface. This fact would mean that the interactions between polar groups and the glass slide are too weak.

Recently, H. NAKAHARA et al.(5) reported the transfer of multilayers of dipalmitoyl-phosphatidyl éthanolamine (D.P.E.) purchased from Kock-Light Laboratories) spread from an ethanol-hexane (1:4 V/V) mixture. However, we found that our sample of D.P.E. (supplied too by Kock Light Laboratories) is only very slightly soluble in the hexane-ethanol mixture, they used, even at a temperature of 45°C. Under these conditions it would be necessary to spread about 35 ml of the saturated solution in order to get a monolayer of 100 cm^2 at a pressure of 25 mN/m (usually 50 μl are required). One can wonder if these authors transferred D.P.E. which was not completely dissolved. We dissolved D.P.E. in the chloroform-ethanol mixture used by DEMEL et al.(6) for the study of some isotherms. Only three monolayers of D.P.E. could be transferred on a glass slide. On the first up trip the molecules are deposited on the solid substrate by their polar heads. Then, on the first immersion, tail-to-tail bonds are created between the molecules of the preceeding layer and the molecules which are transferred. On the second up trip, again head-to-head bonds are created. If the glass slide is again lowered, almost the whole preceeding layer peeles off the solid substrate. This fact should mean that the interactions between polars heads in the multilayers are too weak with respect to the interactions between polar heads and water molecules in the monomolecular film, in spite of the attractive forces between carbon tails.

The above experimental results show that the difficulties we and others met should be imputed to the nature of the polar heads of the phospholipid molecules. We shall therefore first examine the transfer of the phospholipid which has the simplest and smallest polar head, i.e. phosphatidic acid (P.A.).

II - PHOSPHATIDIC ACID MONO AND MULTILAYERS

The neutral Na salt of dipalmitoyl-phosphatidic acid ($P Na_2$) was purchased from FLUKA. PNa_2 is soluble in a chloroform-methanol (1:1 V/V) mixture. It is not always possible to transfer multilayers from a monolayer spread on monodistilled water (pH = 5,3). This lack of reproducibility might be due to the possible absence of traces of Ca^{2+} ions in mono-distilled water, since the importance of bivalent cations for the transfer of L.B. multilayers of fatty acids is known. Indeed, if EDTA, which complexes Ca^{2+} ions, is added to mono-distilled water multilayers cannot be transferred. It was preferable to eliminate Na^+ cations in order to verify the influence of Ca^{++} ions. Therefore, PNa_2 was transformed into phosphatidic acid (P.A.) by mixing it with chlorhydric acid 3 N (Merck Purum). The mixture was dried in vacuum and dissolved in hexane-ethanol (9 : 1 V/V). NaCl thus formed is insoluble in the hexane-ethanol mixture and is easily separated. The purity of the phosphatidic acid was checked by thin-layer chromatography. The transfer of L.B. multilayers of P.A. was examined in the range of pH 1 - 12,4. The water subphase contained $CaCl_2$ 10^{-4} mole/l and HCl or NaOH was used to adjust the pH. We chose a surface pressure of 30 mN/m because at this pressure the monolayer of any dipalmitoyl-phospholipid is in the "condensed state". At pH = 1 and pH = 2, no multilayers could be transferred. This is consistent with the hypothesis that calcium phosphatidate and not P.A. is actually transferred.

From pH = 4 up to pH = 12.4, the multilayers can always be transferred when the content of Ca^{2+} present in the aqueous subsphase is sufficient (10^{-4} M). Up to pH = 9, the transferred multilayers were perfectly transparent and very regular. At pH = 12.4, the transfer was more difficult and the multilayers was somewhat irregular.

In order to understand this behaviour, we studied the properties of the monolayer from which the multilayer was built up.

Figure (1) shows the Π-A isotherms of P.A. spread on a 10^{-4} M $CaCl_2$ solutions. The pH was ajusted with NaOH or HCl. In the observed range $1.1 < pH < 9$, the Π-A curves were practically identical (curve 1). The shape of the Π-A isotherms of P.A. and D.P.E. (curve 2) are quite similar, but the curve 1 is shifted to the left. This is consistent with the fact that the polar groups of P.A. are smaller than those of D.P.E. The D.P.E. isotherm is the same as that reported by DEMEL et al.(6) but

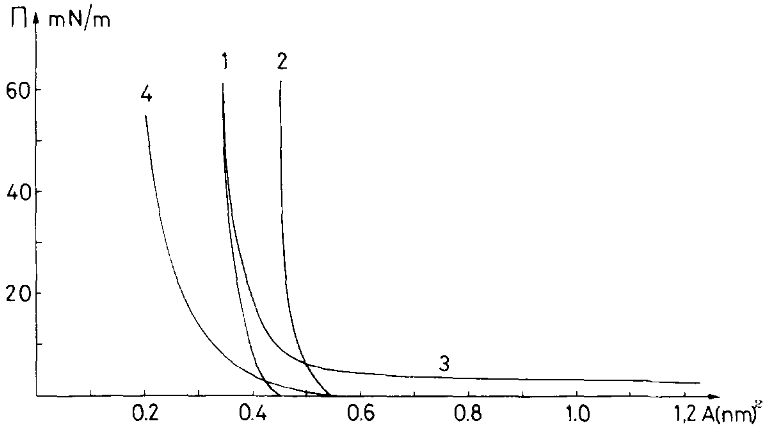

Fig. 1. Π-A isotherms of phospholipids. Curve 1 : P.A. spread on a 10^{-4} M $CaCl_2$ solution (1.1 < pH < 9). Curve 2 : D.P.E. spread on monodistilled water (pH = 5.3). Curve 3 : P.A. force-area curve reported by Van Deenen et al. (pH = 7.4). Curve 4: P.A. spread on a 10^{-4} M $CaCl_2$ solution (pH = 12.4).

rather different from those published by H. NAKAHARA et al(5). The force area curve of P.A. obtained by Van DEENEN et al(7) on a phosphate buffer at pH 7.4, is quite different for the high molecular areas (curve 3). This might be due to the presence of impurities which desorb at high surface pressures.

At pH = 12.4, the Π - A curve is notably modified (curve 4). A partial alkaline hydrolysis of the phosphatidic acid might explain this variation and the difficult transfer mentioned above. Actually we noticed that within one to three months after the preparation of the P.A. solution, multilayers could no more be transferred. This fact would be consistent with the well known acid hydrolysis of P.A. reported by M.J. COULON-MORELEC et al.(8).

The Π - A isotherm of "decomposed" P.A., fig. (2), curve 2, is very different from the Π - A isotherm of P.A., fig. (2), curve 1. It can particularly be noted that the surface pressure begins to increase from a molecular area of about 1.10 nm^2 and that the limit area of the

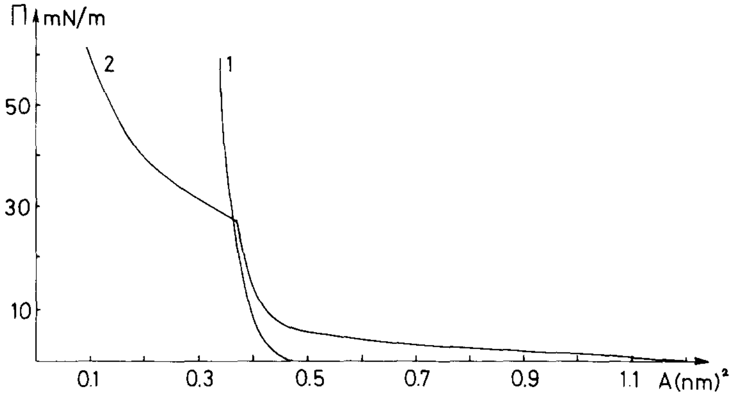

Fig. 2. Curve 1 : Π-A isotherm of P.A.. Curve 2 : Π- A isotherm of decomposed P.A.. In both cases the water subphase contained 10^{-4} mole/l $CaCl_2$, the pH was 5.3.

condensed state is 0,1 nm^2 which is much smaller than the minimum molecular area of P.A.. This could be explained by the breaking of P.A. into two or more smaller molecules which increases the total number of adsorbed molecules at high areas and favours desorption at small areas because of the shorter carbon tails.

Our presumptions are consistent with the fact that on the chromatogram no identifiable separate spots could be observed. This indicates that several compounds were present in the solution. Among these compounds, the phosphoric acid was identified by means of the classical ammonium molybdate - ammonium nitrate test.

III - MIXED MULTILAYERS

Multilayers of D.P.E. dipalmitoyl-choline and egg lecithin can be transferred when they are mixed with phosphatidic acid.

It was observed that the multilayers were very regular with 55 % - 45 % and 40 % - 60 % P.A. - D.P.E. mixtures when transferred from a 10^{-4} mole/l $CaCl_2$ solution at pH = 5,3 with a surface pressure of 25mN/m . The multilayers were somewhat irregular with a mixture poorer in P.A. We checked that 50 % - 50 % P.A.- dipalmitoyl-lecithin or P.A.- egg lecithin mixtures can be transferred in the same conditions as P.A.- D.P.E. mixtures and give very regular multilayers.

IV - EXPERIMENTAL DEVICE

The transfer of L.B. multilayers onto glass slides (2 cm x 3 cm) was performed in a Langmuir trough, from a monolayer spread on the surface of an aqueous solution of appropriate pH and ionic content and kept at a constant pressure. We used a LAUDA film balance for both the transfer of multilayers and the recording of the Π-A isotherms. However, any classical device suitable to measure surface pressure and to keep it constant during the transfer, can be used. The latter requirement can be achieved by any oil piston giving the appropriate pressure. Many authors have obtained Π-A isotherms from surface tension measurements, by means of the WILHELMY plate method. We point out that this method is not suitable for this kind of work. Indeed, the measurement plate generally picks up a part of the monolayer, which modifies its wetting properties, making the measurement incertain.

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