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STABLE BIOMEMBRANE SURFACES FORMED BY PHOSPHOLIPID POLYMERS

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Phospholipids (phosphatidylcholines) with diacetylene in each acyl chain have been deposited in Langmuir-Blodgett multilayers on a variety of substrates. Upon irradiation the diacetylene groups polymerise and link the phospholipid molecules together with a conjugated chain made up of alternating single, double and triple bonds. Advantage has been taken of this polymerisation process to increase the stability of these lipids layers and to produce stable biomembrane hydrophilic surface. These surfaces may be useful for studies of blood coagulation and protein adsorption. In addition they could also have medical application.

Introduction

Many studies have now been made of the structure and dynamics of biomembranes and it is generally accepted that a lipid bilayer provides a matrix in or upon which proteins and glycoproteins are located [1]. It is significant that within some cell membranes the lipids are asymmetrically distributed. All the phosphatidylserine and phosphatidylethanolamine of blood plasma erythrocyte [2] and platelet cells [3] lie in the inner lamella of the bilayer and all the phosphatidylcholine and sphingomyelin in the outer layer [2,3]. It has been suggested that this asymmetric phospholipid distribution may serve a biological purpose by helping to maintain the delicate balance between haemostasis and thrombosis. Recent results indicate that the outer surface of blood cells is devoid of phospholipids which are active in blood coagulation processes [4].

For a variety of purposes it would be useful to have stable surfaces which contain these polar groupings. For example, studies could be made of the blood coagulation process or the adsorption characteristics of proteins (i.e. prothrombin) on surfaces of different ionic character.

We have recently shown that phospholipid molecules which contain diacetylene groups in the acyl chains can be synthesized and that these phospholipid molecules form cross-linked polymers upon irradiation with ultraviolet light. The polymer chain which is made up of conjugated multiple bonds, absorbs in the visible region of the spectrum and the polymers are strongly coloured [5].

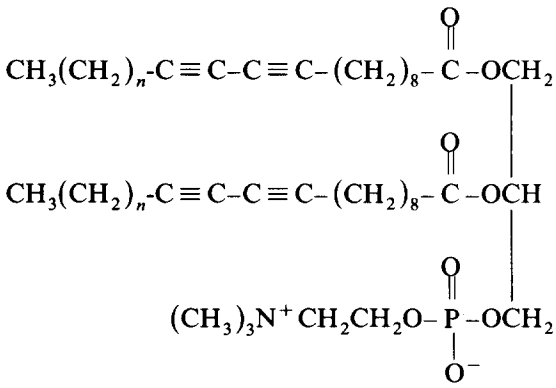
In this paper, we describe the procedures used to obtain Langmuir-Blodgett type multilayers of these phospholipids and the characteristics of the layers after polymerisation. Our aim has been to produce stable layers with a hydrophilic outer surface, i.e. with phosphatidylcholine or sphingomyelin molecules to produce a surface which should be almost identical to the exterior surface of erythrocyte and platelet biomembranes (i.e. of the lipid region).

Using the same technique with diacetylenic phosphatidylethanolamine and phosphatidylserine molecules, stable polymeric surfaces which are similar to the inner lipid surfaces of biomembranes may also be formed.

Materials and Methods

Materials

The synthesis of phospholipids of general structure



has been reported earlier [5].

In this work only the phospholipid with $n = 11$ was used, although phospholipids with larger values of n also make satisfactory multilayers.

Standardised procedures were adopted for preparing the various substrates, glass, quartz, perspex, steel and teflon slides. The slide was immersed in a hot detergent solution (5% RBS 35) for an hour and a half and then thoroughly rinsed with pure water. Hydrophilic slides were dried with a hairdryer and coated as soon as possible. Occasionally, the surfaces of these slides were smoothed with five layers of palmitic or arachidic acid before deposition of the phospholipid. Teflon could also be cleaned satisfactorily by wiping and rinsing with diethyl ether.

Water for the subphase was first distilled and then passed through a Millipore filtration system (Milli-Q). When an ion-containing subphase was required, cadmium chloride (1 g/litre) was added to purified water. In both cases, the pH of the water was found to be 5.5.

The phospholipid was spread on the subphase in a 9:1 hexane-ethanol mixture (Uvasol, Merck and p.a. Merck, respectively). The solution concentration was 0.5 mg/cm^3 or less.

Methods

The film balance used for the production of Langmuir-Blodgett multilayers was of the type described in Ref. 6, but equipped with a Wilhelmy pressure pickup system as described in Ref. 7. The trough measures 18 cm $\times$ 45 cm. A pocket in the bottom of the trough enables slides to be coated over their full length. The depth of the pocket measured from the subphase surface was 4.2 cm. During deposition a digital control unit connected to the barrier drive held the film pressure within 1 mNewton/m^2.

An electronically controlled film lift was used to pass the slides through the surface of the monolayer covered subphase. Up to 18 layers (9 down and 9 up trips) could be deposited completely automatically. The speed of upward and downward movements was independently adjustable and could be varied between 0.3 and 14 cm/min.

Phospholipid multilayers were polymerised by placing them in front of a high intensity ultraviolet light lamp (Mineralight R-52, Ultraviolet Products Inc., CA) for 30 s.

The surface characteristics of a multilayer depend on the orientation of molecules in the final layer. In the case of phospholipids, phosphatidylcholine groups uppermost will render the surface hydrophilic, with acyl chains uppermost, the surface will be hydrophobic. When the substrate is below the surface of the film, phospholipid molecules in the outermost layer will be orientated so that the phosphatidylcholine groups are in contact with water, ie the surface will be hydrophilic. When the substrate is raised either another layer will deposit or, if there is no film on the subphase, the top layer will peel off. In either case the new surface will be composed of phospholipid molecules with their acyl chains outermost and is expected to be hydrophobic. Attempts to obtain hydrophilic surfaces were made in two ways. In the first, the multilayer was polymerised while it was still submerged after deposition of the final layer. The remainder of the phospholipid film was stripped from the subphase before the slide was raised. It was anticipated that the lipid on the surface of the substrate would not be lost or able to reorient after polymerisation.

In the second method, the subphase was stripped of diacetylenic phospholipid and covered with a non-polymerisable amphiphile (DPPC, palmitic acid) before the slide was raised. After the slide had been raised and irradiated, the non-polymeric

material was removed by washing.

The hydrophobic or hydrophilic character of the coated surfaces was checked qualitatively by estimating the contact angle which water makes with the surface.

Results and Discussion

Monolayer properties

Typical surface pressure-area isotherms of the diacetylenic phospholipid are shown in Fig. 1. The decrease in surface pressure which occurs when the film undergoes the main transition may be due to the presence of traces of foreign material. In addition, isotherms measured on pure and $CdCl_2$-containing water were not identical. Isotherms of pure dipalmitoylphosphatidylcholine (Fluka, puriss grade) did not display such a difference.

The area of films held at constant pressure (35–40 mN/cm^2) decreased slowly at first and them more rapidly after 1.5-h. This behaviour was also observed with pure DPPC and is probably caused by the steady accumulation of airborne contaminants on the film. For this reason, no attempt was made to measure precise transfer ratios. The transfer ratio is here defined as the decrease in area of the monolayer on a single pass of the substrate divided by the coated area of the substrate.

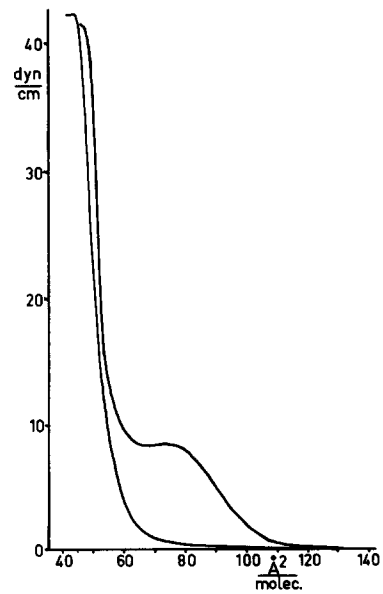


Fig. 1. Isotherms of the diacetylenic phosphatidylcholine in the partially expanded (32.1°C, top curve) and condensed (29.2°C, bottom curve) states.

Multilayer formation

At dipping rates employed for fatty acids (0.3–5 cm/min) the deposition behaviour of diacetylenic phosphatidylcholine and DPPC were similar: only three layers could be deposited on hydrophilic surfaces and two on hydrophobic surfaces. When slides are submerged after deposition of these numbers of layers, the top layer peels off. It redeposits as the slide is withdrawn. Alternate loss and deposition of the third, or second, layer occurred over and over again. The transfer ratios were approx. −0.7 and +0.7 respectively.

We believe this behaviour is due to a weak interaction between phosphatidylcholine groups, it does not occur with saturated or diacetylenic fatty acids. Deposition of further phospholipid layers can be induced by increasing the speed of the downward movement to 10–14 cm/min while the speed of the upward movement is held at 0.5 cm/min or less. Deposition does not occur on the down trip but neither is the surface layer lost. Normal deposition takes place on the up trip. Eventually the transfer ratio drops below one and soon thereafter deposition stops. Using this method we were able to produce deposits with up to 43 layers. All multilayers described in the following sections were prepared in this way.

Surprisingly, when the speed of the downward movement was even further increased, by allowing the slide to drop freely through the surface, deposition restarted. However, the quality of these layers was poor, though they could be improved by reducing the size of the substrate and dipping only when the maximum area of film was on the trough. The reduction in layer quality is probably due to the inability of the servo mechanism to keep pace with the rapid removal of film from the subphase.

Deposition starts on hydrophobic surfaces on the first pass through the monolayer. It does not start on hydrophilic surfaces until the second pass, i.e. the first time the substrate is raised. In this respect, teflon behaved as a hydrophobic surface,

glass, quartz and steel as hydrophilic surfaces. Unexpectedly, perspex also behaved as a hydrophilic surface, deposition not occurring on the first down trip. This holds for the deposition of fatty acids as well as phospholipids.

The conjugated triple bonds of the phospholipid absorb strongly in the ultraviolet region of the spectrum enabling a measure of the consistency of deposition on transparent substrates to be gained from plots of maximum absorbance versus number of layers. A linear relationship was found between these two properties when deposition did not occur on the down trip and measurements were made on the lower 2 cm of the slide.

Multilayer properties

Diacetylenic phospholipid polymer in multilayers is pink although there needs to be about 6 layers on slide before colour is apparent to the eye. The layers become yellow when heated. Such colour changes have been observed before with other polymeric diacetylenes [8]. Fig. 2 shows the visible spectrum of a multilayer at various irradiation times. There is an increase in absorption in both visible and ultraviolet regions as polymerisation proceeds.

Phospholipid multilayers have strongly hydrophobic surfaces if they are polymerised after withdrawal from the subphase. Such surfaces emerge from water absolutely dry and a drop of water rolls freely across them with a contact angle of almost 180°. However, layers irradiated under the subphase or under a coating of non-polymerisable amphiphile have surfaces which are hydrophilic. After immersion in water, a water film adheres to them for several seconds. A drop of water applied to an inclined surface rolls across it slowly and the contact angle lies between 70 and 90°. While these surfaces must be classified as hydrophilic when compared to the strongly hydrophobic ones described previously, they are not totally wettable like clean glass or metal oxides.

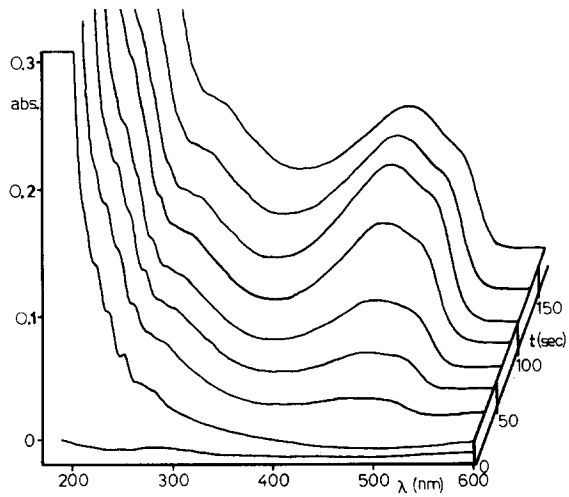


Fig. 2. Visible spectra of 86 layers of diacetylenic phosphatidylcholine at various irradiation times. (43 layers on each side of a quartz slide).

As anticipated, the mechanical and chemical stability of phospholipid films is markedly increased by the cross-linking process. Films were intact after a day's immersion in strong acid or alkali. Ten minutes treatment with a hot, strong detergent solution (5% RBS 35) is needed before films start to peel off the substrate. However, it was noticed that if strong pressure was applied to hydrophilic surfaces they rapidly became hydrophobic.

Conclusion

We have shown that it is possible to coat many materials (glass, quartz, perspex, teflon and steel) with ordered layers of diacetylene containing phosphatidylcholine molecules and that upon irradiation these molecules polymerise. Furthermore, layers can be produced in such a way that the polar groups of the lipid form the outer coated surface. The layers after polymerisation are quite stable in aggressive media and can also, with some precautions, be handled without damage. It is expected that the same technique can be used to deposit other phospholipids and glycolipids so that particular types of charged and zwitterion phospholipid polar groups form the outer surfaces. We are presently synthesizing other types of phospholipids such as phosphatidylethanolamine and phosphatidylserine containing the diacetylene group. In this way, stable polymerised surfaces consisting of the charged polar groups which make up the inner surface of erythrocytes and platelets can be modelled. Stable polymeric surfaces consisting of the carbohydrate groups of certain cell

membranes may be modelled by the biosynthesis of various glycolipid molecules containing these diacetylene groups.

These various stable surfaces may be useful for studies of blood coagulation processes, the adsorption of various types of protein such as fibrinogen, and also for cell-cell contact investigations. The ability to coat a surface such as glass or metal to produce a stable surface having the polar characteristics akin to that of the outer lipid layer of erythrocyte membranes may make such surfaces useful in certain biomedical applications such as the production of biocompatible surfaces.

We shall report our further studies of such stable phospholipid and glycolipid surfaces in future publications.

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