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THE FORMATION OF POLYMERIC MODEL BIOMEMBRANES FROM DIACETYLENIC FATTY ACIDS AND PHOSPHOLIPIDS

DAVID SAMUEL JOHNSTON[a], SUKHPAL SANGHERA[a], ARTURO MANJON-RUBIO[b] and DENNIS CHAPMAN[a]

[a]*Royal Free Hospital School of Medicine, University of London, Department of Biochemistry and Chemistry, 8 Hunter Street, London WC1N 1BP (U.K.) and* [b]*Departmento Bioquimica, Facultad Ciencias de Alicante, Aptdo. 99, Alicante (Spain)*

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Summary

Diacetylenic fatty acid monolayers at the air/water interface and multilayers on suitable supports polymerise when exposed to ultraviolet radiation. It has been found that polymerisation still occurs when monolayers are diluted with cholesterol or gramicidin. The rigid, crystalline nature of the films formed makes them useful biomembrane models. Phospholipids made from the fatty acids were less reactive. Multilayers deposited on hydrophobic supports would polymerise but not monolayers on water.

The ability of certain crystalline diacetylenes to form crystalline polymers was first recognised by Wegner [1]. Since then, other workers have demonstrated that similar polymerisations can take place in surface-active diacetylenes at the water/air interface or in Langmuir-Blodgett multilayers. Day and Ringsdorf [2] and Tieke et al. [3] investigated the polymerisation of the diacetylenic fatty acid:

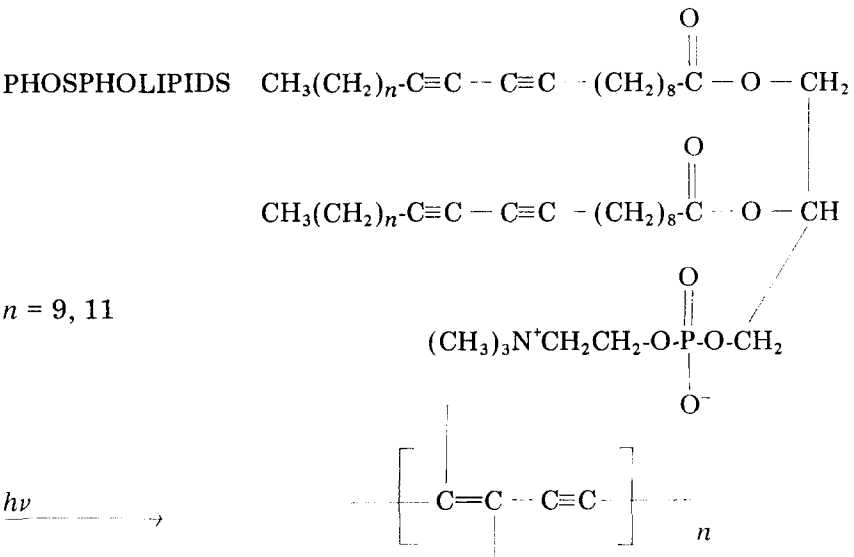
CH_3-$(CH_2)_9$-C≡C—C≡C — $(CH_2)_8$-COOH

When monolayers on water or multilayers were irradiated, polymerisation occurred and coloured brittle films were formed. These compounds are of interest because, in principle, it should be possible to make rigid model biomembranes from them. Cell membranes contain proteins, polypeptides and cholesterol and there is no indication in the literature whether diacetylene films will polymerise when they contain such 'foreign molecules'. We

FATTY ACIDS $CH_3(CH_2)_n\text{-}C{\equiv}C - C{\equiv}C - (CH_2)_8\text{-}CO_2H$

$n = 9, 11$

PHOSPHOLIPIDS



Scheme I. Chemical structure of diacetylenic fatty acids and phospholipids.

have experimented with both fatty acid and phospholipid films. The phospholipids were made by ester-linking diacetylenic fatty acids to phosphatidylcholine. Polymerised mono-, bi- or multilayers would be expected to be more stable and more easily handled than their monomeric counterparts. Their visible spectra might provide information about the packing of molecules in the film. Dyes incorporated into multilayers as the polar head group have been useful in this respect [4].

The details of the syntheses will be described in a full paper. The structures of the acids and phospholipids are set out in Scheme I. They were characterised by their ultraviolet/infrared spectra and R_F values on silica gel thin-layer chromatography plates.

Phospholipid polymer/monomer mixtures (30—40% polymer) were produced by irradiating the monomer in compressed (10 ton) KBr discs. The KBr was removed by water wash.

Surface pressure measurements were made on a Teflon trough fitted with a driven Teflon barrier and Wilhelmy balance. Monolayers were spread from $CHCl_3$ /CH_3OH (9:1, v/v) solutions, all having a concentration of approx. 1 mg/cm^3. The water substrate was triple distilled, initially from alkaline permanganate. A glass heat-exchange coil was submerged in the substrate. Water from a constant temperature bath could be pumped through the coil and in this way it was possible to maintain the substrate temperature constant at values as low as 7° C.

Multilayers were built up on glass and Teflon slides by the conventional

Langmuir-Blodgett procedure. The area of the film was reduced as deposition occurred to maintain a constant deposition pressure. Changes of film area on each passage of the substrate and the meniscus shape were noted. Phospholipids were deposited from pure distilled water.

Polymerisation was initiated by irradiating mono- or multilayers with a Mineralight R-52 ultraviolet (254 nm peak) lamp. This lamp has an energy output of 1200 $\mu W/cm^2$, 15.2 cm from its face. The enclosed trough was purged with N_2 before and during irradiation.

Day and Ringsdorf [2] have reported compression isotherms for the fatty acids shown in Scheme I. At 20°C, the twenty-five carbon acid (n = 11) polymerised readily. For polymerisation of the shorter acid (n = 9), the temperature of the substrate had to be reduced to 2°C. We find that monolayers of either acid containing less than one molecule of cholesterol per molecule of acid and one molecule of gramicidin per four molecules of acid will polymerise at 10°C and form rigid homogeneous films. If the levels of these molecules are further increased polymerisation becomes very slow and limited in extent. The films are red in colour, the colour being less intense the more cholesterol or gramicidin present.

Surface pressure-area isotherms for the two phospholipids are similar to other phosphatidylcholines with unsaturated acyl chains. Phase changes are evident at room temperature. Attempts to polymerise condensed phospholipid films at the water/air interface were unsuccessful. Fatty acid monomer multilayers polymerise readily when deposited on glass or Teflon, phospholipid multilayers will polymerise only when deposited on Teflon.

To get deposition (always Z type) a quite high film pressure had to be employed: 35—40 dyne/cm. With Teflon, the ratio of the change in film area per dip to the substrate area varied between 0.7 and 0.9, with glass it could be as high as 2.0 and never fell below 1.2. Therefore, in the case of glass, simple film transfer from water to substrate does not occur.

Diacetylene polymerisations require the order of a crystalline phase [5] and the phospholipid deposit on Teflon must therefore be a well ordered multilayer. Polymerisation does not easily occur on glass probably because a well ordered multilayer has not been formed. Evidently, there is a stronger

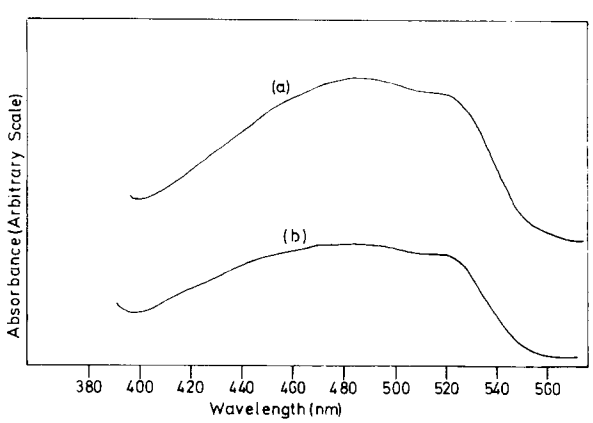


Fig. 1. Visible spectrum of phospholipid polymer multilayer (a) after irradiation and (b) unirradiated.

interaction between hydrocarbon and Teflon than phosphatidylcholine and glass. Other workers also report difficulties depositing lecithins on glass [6].

The criteria for diacetylene polymerisation have been discussed at length by Baughmann and Yee [5]. Polymerisation occurs more readily the more alike the polymer and monomer crystalline phases. The fact that phospholipid polymerises in multilayer form on Teflon but not as a condensed monolayer on water is unusual and warrants further investigation.

Partially polymerised monomer from KBr disc can be spread at the air/water interface. As with pure monomer, this mixture can be deposited on to glass and Teflon substrates by the Langmuir-Blodgett method. The visible spectrum of such a multilayer on glass is shown in Fig. 1. When irradiated, further (probably complete) polymerisation occurs as evidenced by the increased absorption. This is in strong contrast to pure monomer.

Rigid model membranes such as these will be easy to study and may have practical application. Currently, an evaluation of these unique membranes is under way and other, perhaps more reactive, diacetylenic phospholipids are being synthesized.

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