Biochimica et Biophysica Acta, 510 (1978) 216—229

BBA 78060

THE EFFECT OF SYNTHETIC POLYMERS ON THE ELECTRICAL AND PERMEABILITY PROPERTIES OF LIPID MEMBRANES

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(Received November 17th, 1977)

Summary

1. The effect of two series of hydrophilic and hydrophobic polymers on the stability, conductivity and permeability towards water and leucine of black lipid membranes and liposomes is reported.

2. The changes in properties of these membrane preparations is related to bulk phase viscosity and dielectric measurements together with monolayer studies.

3. The hydrophobic polymers dramatically increase membrane stability, had no effect on conductivity, but increased the permeability coefficient of leucine.

4. The hydrophilic polymers produced minor, but significant changes to membrane properties.

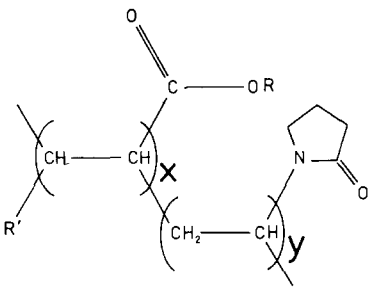
5. It is concluded that not only basic polymers but also neutral and acidic macromolecules can interact strongly with lipid membranes.

Introduction

Many workers have incorporated synthetic macromolecules into lipid bilayer membranes in an attempt to mimic protein interactions that occur in biological membranes. Most of these studies have utilized basic polypeptides [1—3]. Such experiments have been useful in gaining an understanding as to how basic membrane proteins can interact with membrane lipids, for example, myelin protein A1 [4]. However, a large number of membrane proteins, possibly the majority, are not strongly basic [5—8]. Consequently, we decided to study the interaction of neutral and negatively charged polymers with lipid bilayers. Polyglutamic acid and polyleucine have little effect on membranes containing neutral or negatively charged lipids and in an attempt to study macromolecules

* Subsequent to the completion of the bilayer lipid membrane portion of this work, C.R. Dawson was tragically killed on December 26, 1975. The death of this young man is a great loss to his scientific colleagues.

SCHEME I



AP, R = H
R′ = H

LP, R = $C_{12}H_{25}$
R′ = CH_3

with properties intermediate between those of polyglutamate and polyleucine, two series of amide-rich copolymers were synthesized. The hydrophilic series (AP) consisted of copolymers of acrylic acid and vinylpyrrolidone and the hydrophobic series (LP) were copolymers of laurylmethacrylate and vinylpyrrolidone. This paper reports the properties of phosphatidylcholine/cholesterol membranes which contain these macromolecules.

Materials and Methods

Cholesterol (British Drug Houses) was recrystallized twice from ethanol and stored under N_2. *n*-Decane (British Drug Houses) was dried and purified on an alumina column. Tris (Sigma) and NaCl (British Drug Houses) were Analar grade. Triply distilled mercury (Johnson-Matthey, London) was used as supplied. Sodium [^{32}P]phosphate (5 Ci/mol), *n*-[1-^{14}C]decane (3 Ci/mol) and [26-^{14}C]cholesterol (58 Ci/mol) were purchased from The Radiochemical Centre, Amersham. Egg yolk phosphatidylcholine was extracted from eggs (free range Rhode Island Red) according to a modification of the methods developed by Dawson [9] and Wren [10]. All extraction stages and chromatography steps were performed at room temperature and under N_2. Silicic acid, Mallinkrodt SilicAR CC-4 special (Camlab, Cambridge) was used in the final chromatography step. The phosphatidylcholine was homogeneous to thin-layer chromatography [11]. Analysis of the methyl esters formed subsequent to the alkaline hydrolysis of phosphatidylcholine gave the following acyl composition: palmitate, 37%; stearate, 11%; oleate, 37% and linoleate, 15%, yielding an average molecular weight of 781.

Preparation of samples for scintillation counting. A 10 ml portion of scintillator consiting of 0.2% (w/v) 2,5-bis-5′-*tert*-butylbenzoxyazol-2-ylthiophen in Triton/toluene (1 : 2, by vol.) was added to various quantities of aqueous and chloroform extracts. The counts of radioactivity were corrected for quenching by internal standards. Efficiency of radioactivity counting was ^{3}H, 52%; ^{14}C, 70%; ^{32}P, 37% and in the presence of chloroform (80 μl), ^{14}C, 67%; ^{32}P, 30%.

Preparation of [^{32}P]phosphatidylcholine. Although various synthetic [^{32}P]-

phosphatidylcholine preparations are available, it was decided to label the egg yolk phosphatidylcholine preparation that was used routinely in our laboratory. A chicken was given an interperitoneal injection with sodium ortho[^{32}P]phosphate (0.5 ml, 0.5 mCi, 5 Ci/mol). The phosphatidylcholine from eggs laid between the 2nd and 7th days were extracted as described above. After purification on an alumina column the activity of crude lipid was 0.2 μCi/mg. After further purification on a silica column, all ^{32}P activity was associated with phosphatidylcholine, as judged by thin-layer chromatography. The ^{32}P-labelled phosphatidylcholine was stored in chloroform under N_2 at 258 K.

Preparation and analysis of polymers. The hydrophobic polymer series (LP) consists of copolymers of lauryl methacrylate and vinylpyrrolidone and was synthesized by the method of Bauer et al. [12]. The composition of the LP series was determined by ^{1}H NMR.

The hydrophilic polymer series (AP) consist of copolymers of acrylic acid and vinylpyrrolidone and were synthesized by free radical initiation, using H_2O_2. Quantitative analysis of the monomer incorporation into the hydrophilic copolymers was achieved by non-aqueous titration, using dimethylsulphoxide [13]. Iodination of the polymers was achieved via covalently attached *p*-anisidine moieties [14].

Viscosity measurements were made using an Ubbelholde No. 3 viscometer.

Dielectric constant measurements were made using a Wayne Kerr Universal bridge B221 in conjunction with a permittivity cell (D121).

Apparatus for black lipid membranes. All permeability studies were performed with vertical membranes and, by necessity, the composition studies required horizontal membranes. In order to use the same Teflon aperture support in both studies, a system was designed such that the same support would fit into both sets of apparatus (Figs. 1 and 2). Teflon pieces and O-rings were cleaned overnight in a soxlet apparatus containing chloroform. Perspex sections were cleaned in haemosol and then washed copiously with water. Throughout this study NaCl (150 mM) buffered to pH 7.4 by Tris · HCl (10 mM) was used as the bathing solution. Solutions of lipids in decane were applied to prepainted apertures via a 10 μl Hamilton syringe, according to the method Bunce and Hider [15]. The hydrophobic polymers (LP) were added directly to the normal, decane brush mixtures but all the hydrophilic polymers were found to be insoluble in decane. Consequently a search for alternate solvents was made as briefly described in Results.

Ag/AgCl electrodes were used for electrical measurements. The electrical resistance of the membranes was measured by a mV meter (C 10 Meter, WPA Saffron Walden, England), the output being continuously recorded on a servoscribe chart recorder. Capacitance measurements were made with a Wayne Kerr bridge (Type B221) operating at a fixed frequency of 1.5 kHz. A variable low capacitor was also included in the circuit to protect the membrane from rapid voltage changes when the bridge was switched between ranges. For estimates of membrane thickness the method described by White and Thompson [16] was adopted. The membranes were photographed using transmitted light and the areas estimated by projection onto graph paper. The standard deviation of the measurement of the areas was less than 2% of the area adopting this technique.

Permeability studies utilized a two-chamber system in which a continuous

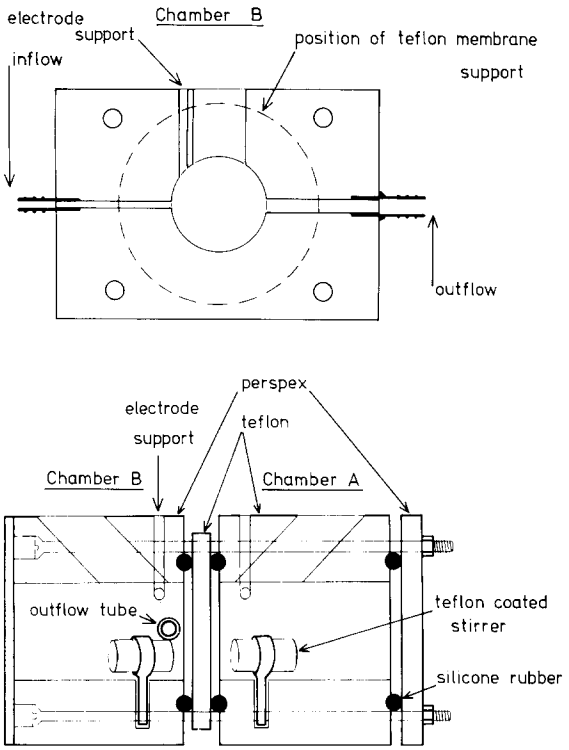


Fig. 1. Apparatus for the study of vertical black lipid membranes illustrating the relative positions of the stirrer magnets, inflow and outflow pipes with respect to the lipid membrane. The diameters of the inflow and outflow pipes of chamber B (perspex) were 1 and 3 mm, respectively. The thickness of the support, unless otherwise stated, was 2 mm and one side was dimpled such that the thickness in the vicinity of the aperture was 1 mm. The aperture diameter was 1 mm. In both systems the cells were separated from the central Teflon aperture support by silicone-rubber O-rings and bolted together by brass bolts. The capacity of the chambers A and B were both 8 ml and the clearing time of the rear compartment is such that 80% of a ^3H_2O pulse was cleared from the cell within 10 min.

flow of buffer was maintained in one chamber (Fig. 1). Chamber A (Teflon) was attached to an optically flat perspex window which permitted viewing of the membrane by a microscope (35—70 ×). The membrane was viewed either by reflected or transmitted light. A drop-forming spoon, as originally reported by Petkau and Chelack [17] was placed at the end of a gravity-fed perfusion system, the perfusate being collected in a fraction collector. Both chambers contained Teflon-covered magnets driven at 60 rev./min [18]. The cell and perfusing system were placed in a constant temperature air cabinet (310 K). At the start of permeability studies, subsequent to the membrane becoming black and when the stirrers and perfusion were operating, radioactive isotopes (high activity 2 μl) were added to chamber A. The permeability of membranes was monitored continuously at a steady state. A membrane potential of 40 mV was utilized for these studies and the capacitance was monitored in order to establish the absence of myelin formations (see Results) and other abnormal behavior. A typical profile of the rate appearance of ^3H_2O in the perfusate is illustrated in Fig. 3. Permeability measurements were only utilized from membranes which

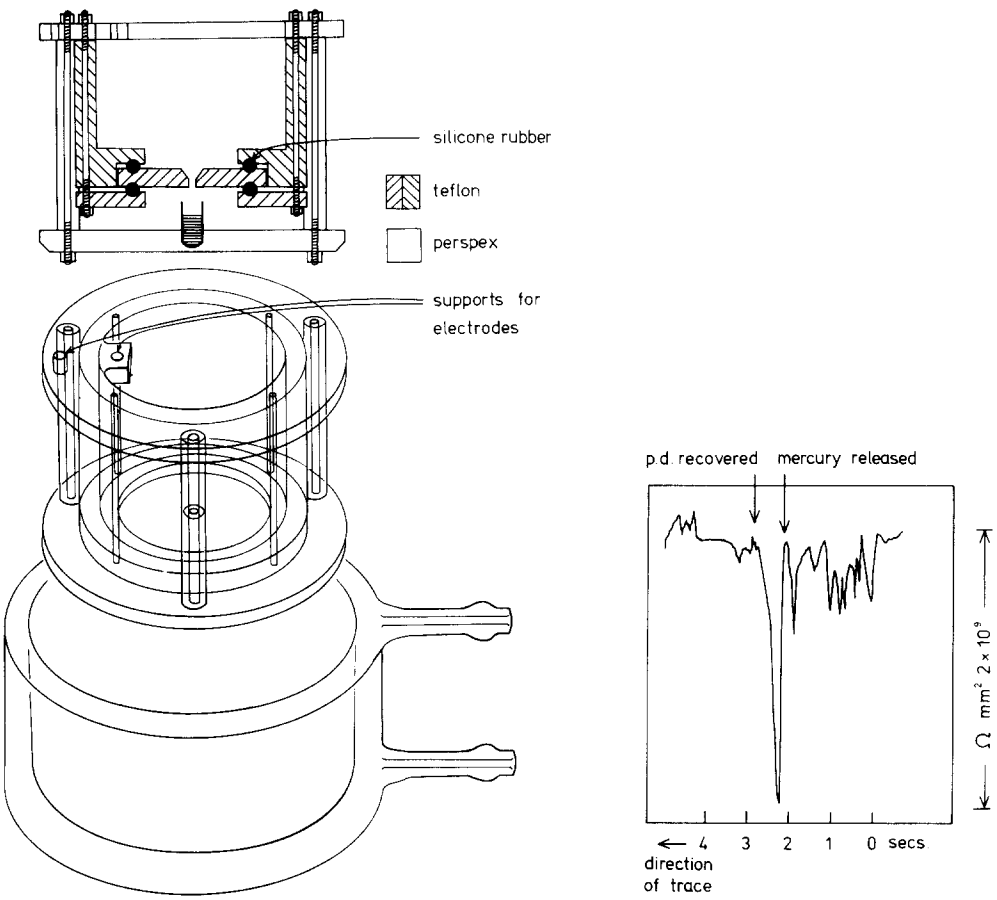


Fig. 2. (a) Apparatus for the study of horizontal black lipid membranes. The aperture was supported over a small glass beaker, which contained chloroform (80 μl) and was topped up with bathing fluid. The entire assembly was placed in a glass cell which was maintained at 310 K by circulating water from a constant temperature bath. The glass cell was placed on the base of the microscope, which was used for observation of the membrane from above. Each mercury drop, together with its membrane coating was collected in a glass beaker, which was subsequently removed using forceps, after the bathing solution had been siphoned off. The beaker was immediately deposited in a scintillation vial. (b) Record of the potential difference across horizontal membrane (V_m) during the passage of a mercury drop. The bilayer was not broken, but V_m dropped to zero for a short time period as the mercury drop actually passed through the bilayer. The trace is noisy as these experiments were conducted without an effective Faraday cage.

survived, in a steady-state condition, for longer than 45 min.

Composition studies also utilized a two-chamber system (Fig. 2a). The bathing solutions were electrically insulated from one another, thus enabling the measurements of membrane capacitance and resistance. The entire system was filled with bathing fluid by siphoning and the solution was allowed to stand for 10 min before applying lipid solution to the aperture. A 1 μl syringe containing mercury was held by a micromanipulator (Prior Instruments, England) and positioned approx. 2 mm above the bilayer and 50° to the vertical, with the aid of the microscope.

As the mercury passed through the membrane, interference patterns were

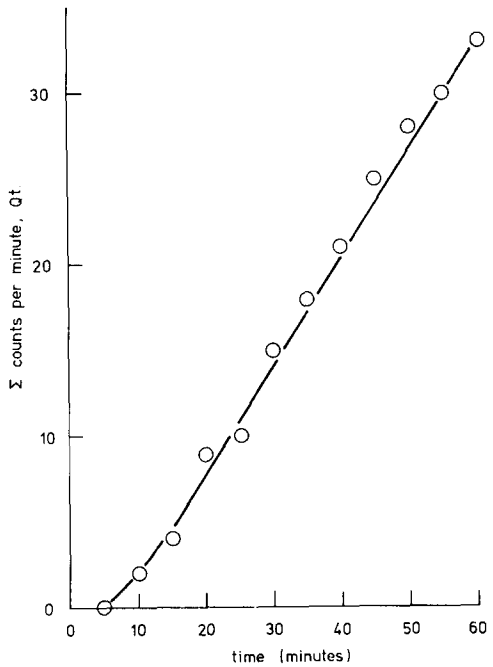


Fig. 3. Profile of the rate of appearance of ^3H_2O in perfusate from chamber B, when the two chambers are separated by a Teflon disc supporting a phosphatidylcholine/cholesterol/decane membrane (area 0.57 mm^2). Concentration of ^3H_2O in chamber A was $28.6 \cdot 10^3$ cpm/ml.

often seen and the area of the bilayer decreased as material was withdrawn from the torus. Further drops were not passed through the same membrane until a time period sufficient to give a stable membrane resistance (5 min) had elapsed. When mercury drops pass through a membrane, the potential difference approaches zero but then rapidly recovers to its original value (Fig. 2b). The capacitance/area measurements and membrane resistance before and after such experiments were found to be constant. No isotopes were detected in chloroform when mercury was dropped through the teflon aperture, after membranes (containing radioisotopes) had been made and deliberately broken. The mass of each mercury drop used in composition studies was approx. 0.6 mg. By counting a known quantity of a radioactive sample under standardized conditions, it was possible to convert the disintergrations per min into the number of molecules present in the sample.

Liposomes. Partially sonicated liposomes were subjected to osmotic shock and the rate of subsequent swelling in the presence of leucine was measured by changes in the intensity of scattered light at 450 nm [19]. The initial rate of shrinkage which is related to the permeability of liposomes towards water was monitored using a stopped-flow technique. Hydrophobic polymers were added to the chloroform solution of lipids, before lipsome formation. Hydrophilic polymers were added to the aqueous buffer (100 μg/ml) used to form the liposome preparation.

Monolayers. Monolayers were formed on a teflon Langmuir-Adams trough [20]. Limiting areas per molecule and per polymer segment were estimated by

extrapolating the upper linear portions of the force area curves [21]. Hydrophobic polymers were added to the system in hexane solution and the hydrophilic polymers were added in aqueous solution to the monolayer subphase forming a final concentration of 100 μg/ml. All experiments were performed at 310 K.

Results

Properties of membranes prepared from egg yolk phosphatidylcholine and cholesterol

As previously reported by Bunce and Hider [15], the ratio of phosphatidylcholine/cholesterol was found to be approximately equimolar at 37°C, and there was little evidence for the existence of microlenses. A similar observation concerning microlenses in phosphatidylcholine membranes has been made by White and Thompson [16].

The electrical properties of horizontal and vertical membranes were indistinguishable. Values of capacitance/area of 3.4 ± 0.4 nF/mm^2 for horizontal and 3.6 ± 0.4 nF/mm^2 for vertical membranes were obtained. These values were usually constant throughout the lifetime of the membranes. By assuming the dielectric constant of the hydrocarbon portion of the membrane to be 2.0 at 310 K [22], the membrane thickness was calculated as 5.1 ± 0.5 nm. Values of the membrane resistance were not so constant, falling in the range of $0.2 \cdot 10^9$–$4.0 \cdot 10^9$ Ω nm^2, for both membrane preparations. This range of values agrees well with other workers using egg yolk phosphatidylcholine, cholesterol, decane mixtures [23,24]. With some of the vertical membranes the capacitance rapidly increased after approx. 60 min, whereas the resistance remained relatively constant. This type of behavior has been reported previously for phosphatidylcholine/*n*-decane membranes [25], the authors suggesting that a myelin type growth process was occurring.

The permeability coefficient of water for the basic phosphatidylcholine/cholesterol membrane system was estimated as 0.68 ± 0.24 μm/s (mean ± S.D. of four independent determinations). This value is lower than that reported by other workers using ^3H_2O, for instance Cass and Finkelstein [26] report a value of 8 μm/s, Huang and Thompson [27], a value of 4.4 μm/s and Hanai et al. [28] a value of 2.3 μm/s. Cass and Finkelstein [26] demonstrated that additional resistance to ^3H_2O movement is introduced as the aperture thickness is increased. In the apparatus shown in Fig. 1, it was estimated that an unstirred layer of approx. 1 mm thickness is present. By correcting for this layer [26] the water permeability coefficient of the bilayer becomes 0.9 μm/s. The thickness of the unstirred layer was determined directly by measuring the ^3H_2O flux through the aperture in the absence of a membrane. The resulting P_{H_2O} of 6 μm/s corresponds to an unstirred layer of 0.5 nm. A possible explanation for the lower water permeability reported in this paper as compared with those reported by other workers [26–28] is the use of a continuous flow system, it would appear that the difference cannot be accounted for by the presence of unstirred layers.

The effect of membrane potential on the permeability coefficient of water was found not to vary significantly over the range 0–150 mV.

Due to the relatively small area of black lipid membrane preparations, the continuous flow system is only suitable for measuring the permeability of small molecules, e.g. aliphatic alcohols and amides. The permeability coefficient of ethanol, for instance, was determined as 0.23 μm/s, and that of L-leucine was approx. 10^{-2} μm/s, the limit of the system.

Properties of membranes containing hydrophobic polymers

The hydrophobic polymer (LP11), a copolymer of laurylmethacrylate and vinylpyrrolidone (mol ratio 4 : 1, mol. wt. 30 000) was readily incorporated into decane brush mixtures. Black lipid membranes were found to form easily from such mixtures containing phosphatidylcholine/cholesterol/LP11 (2 : 1 : 1, by wt). The electrical resistance and capacitance values of the resulting membranes fell into the same range as those lacking the polymer. Composition studies with ^{125}I-labelled LP11 using the horizontal membrane system demonstrated that the phosphatidylcholine to polymer weight ratio in the bilayers was approx. 5 : 2 (Table I).

A study of the viscosity of decane solution of LP11 at 310 K demonstrated a large increase in viscosity at polymer concentrations, demonstrated to exist in black lipid membrane preparations (Fig. 4). An intrinsic viscosity of 9 ml/g was determined for LP11 (Fig. 4b) the shape of the curve indicating the presence of a random coil structure. The related copolymers of stearylmethacrylate and vinylpyrrolidone have also been demonstrated to exist in a random coil configuration in the bulk phase [29].

The dielectric constant of these solutions was not much larger than that of decane, a concentrated solution (decane/LP11 (5 : 2, by wt.)) possessing a

TABLE I

THE EXTENT OF INCORPORATION OF ^{125}I-LABELLED POLYMERS IN BLACK LIPID MEMBRANES

Mercury drops were passed through horizontal membranes and the membrane portion trapped on the surface of the drop was counted in a gamma counter. The number of polymer molecules trapped by each drop was calculated from the specific radioactivity of the polymers. The mean number of phosphatidylcholine molecules trapped on a mercury drop in the presence and absence of LPII was $5 \pm 3 \cdot 10^{15}$ (mean of 10 ± S.D.).

	Hydrophobic polymer LPII			Hydrophilic polymer APII		
	counts/ 10 min	Number of mercury drops	Number of polymer molecules/ drop	counts/ 10 min	Number of mercury drops	Number of polymer molecules/ drop
Specific activity of polymer (100 μg)	59 800	—	—	225 000	—	—
Brush mixture (10 μl)	14 900	—	—	920	—	—
Bathing solution (10 μl)	1.4	—	—	30	—	—
Mercury drops passed through bilayer	223	2	$3.5 \cdot 10^{13}$	210	1	$1.2 \cdot 10^{13}$
	192	2	$3.1 \cdot 10^{13}$	650	1	$3.9 \cdot 10^{13}$
	204	2	$3.2 \cdot 10^{13}$	180	1	$1.1 \cdot 10^{13}$
				420	1	$2.4 \cdot 10^{13}$
Mercury drop passed through prepainted aperture	0	1	—	2.0	1	$1.0 \cdot 10^{11}$

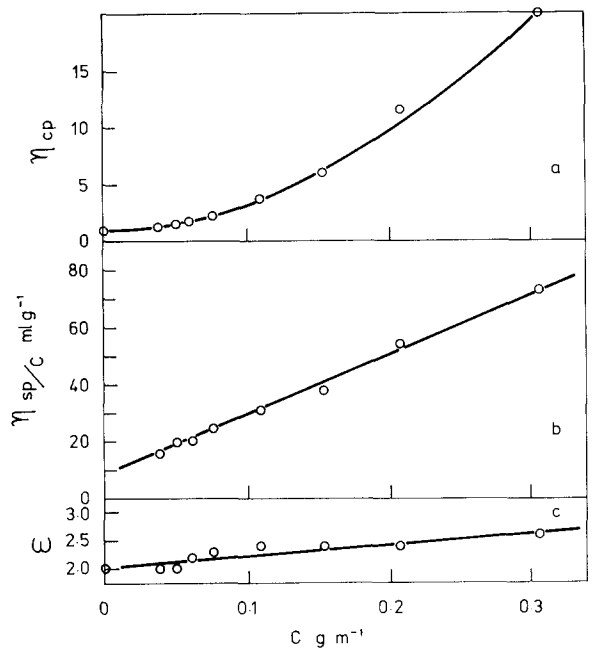


Fig. 4. The variation of (a) viscosity, (b) specific viscosity, and (c) dielectric constant, with concentration of LP11 in decane at 310 K.

value of 2.6 as opposed to 2.0 for decane (Fig. 4c). This small increase in the dielectric constant would indicate that cholesterol/phosphatidylcholine membranes containing LP11 are thicker than those without the polymer; 6.8 nm as compared to 5.1 nm for polymer-free membranes, assuming the existance of a homogeneous hydrocarbon region. With a brush mixture composition lecithin/LP11 (1 : 1, by weight), stable membranes with reproducible capacitances (4.3 ± 0.3 nF/mm^2) result. It would appear that these membranes are rather thinner than those found in the presence of cholesterol, 5.2 nm as opposed to 6.8 nm.

As membrane rupture is caused by many external variables, direct comparison of bilayer lifetimes is difficult. However, it was clear that in the presence of LP11, the lifetime of the membranes was much longer than in its absence. If permitted, membranes would regularly survive for periods in excess of 6 h [30]. Indeed, in contrast to the relatively unstable phosphatidylcholine/decane membranes, phosphatidylcholine/LP11/decane membranes were extremely stable. A more direct method of assessing whether LP11 increased membrane stability was obtained by measuring the applied potential at which bilayer rupture occurs. Electrical breakdown is a measure of electromechanical stability, as rupture results from electrostatic compression, which itself depends on the mechanical properties of the membrane [31]. Membranes (phosphatidylcholine/cholesterol/LP11 (2 : 1 : 2, by wt.) were subjected to increasing applied voltages in increments of 40 mV and were found to rupture in the range 325—950 mV as compared to 150—200 mV in the absence of the polymer.

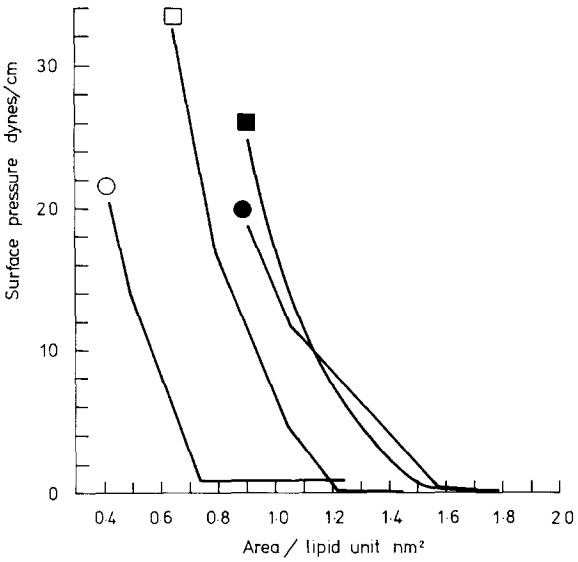


Fig. 5. Force vs. area curves for mixed monolayers of phosphatidylcholine, cholesterol and LP11. Expressed in terms of lipid molecules. The lipid unit is taken as a phosphatidylcholine molecule in the absence of cholesterol, and as a two molecule phosphatidylcholine/cholesterol unit in the presence of cholesterol. ○, phosphatidylcholine alone; □, phosphatidylcholine/cholesterol (2 : 1, by wt.); ●, phosphatidylcholine/LP11 (5 : 2, by wt.); ■, phosphatidylcholine/cholesterol/LP11 (10 : 5 : 4, by wt.). Each curve presented is typical of at least six independent determinations performed for three different quantities of surface-active material.

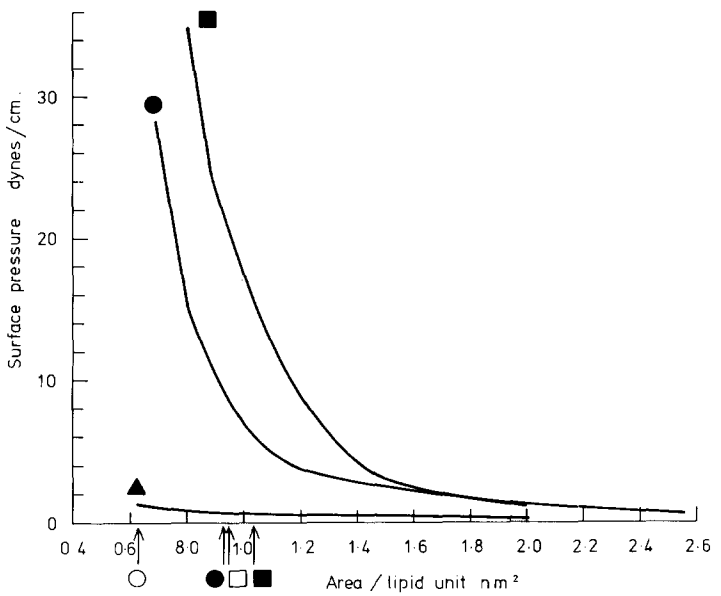


Fig. 6. Force vs. area curves for mixed monolayers of phosphatidylcholine, cholesterol and AP11. The lipid unit is taken as a phosphatidylcholine molecule in the absence of cholesterol, and as a two molecule phosphatidylcholine/cholesterol unit in the presence of cholesterol. The concentration of AP11 in the subphase was 1 mg/ml. ●, phosphatidylcholine in the presence of AP11; ■, phosphatidylcholine/cholesterol (2 : 1, by wt.) in the presence of AP11; ▲, AP11 alone; ○ and □ indicate area per lipid unit for phosphatidylcholine and phosphatidylcholine/cholesterol, respectively.

TABLE II

LIMITING AREAS OF LIPID UNITS AND POLYMER SEGMENTS IN MIXED MONOLAYERS OF PHOSPHATIDYLCHOLINE, CHOLESTEROL AND LPII

Limiting areas were taken from Fig. 6.

Monolayer composition (by wt.)			Limiting area per lipid unit (nm^2)	Limiting trough area per 5.62 μg phosphatidylcholine	Estimated area corresponding to LPII (cm^2)	Limiting area per lauryl segment (nm^2)
Phosphatidylcholine	Cholesterol	LPII				
1	—	—	0.63	29	—	—
2	1	—	0.95	43	—	—
5	—	2	1.30	55	26	0.45
10	5	4	1.22	54	11	0.23
—	—	1	—	—	—	0.06
—	1	—	0.45	—	—	—
—	5	2	0.52	—	6	0.11

Despite the increased thickness of phosphatidylcholine/cholesterol/LP11 membranes and their enhanced stability, they possess a permeability coefficient for water which is virtually the same as for those membranes not containing the polymer, 0.7 ± 0.2 μm/s as compared to 0.8 ± 0.2 μm/s. This rather surprising observation was confirmed using liposomes. In contrast to the results with water, however, LP11 increased the rate of permeability of phosphatidylcholine liposomes towards leucine by approx. 30%.

Monolayer studies clearly demonstrate that LP11 interacts with the hydrocarbon portion of the lipids as the polymer induces a large increase in the area per phosphatidylcholine molecule and phosphatidylcholine/cholesterol unit (Fig. 5). LP11 occupies less space in the phosphatidylcholine/cholesterol monolayers than it does in phosphatidylcholine only monolayers (Table II) indicating that LP11 has greater difficulty in penetrating the tightly packed phosphatidylcholine/cholesterol units [32]. The limiting area per lauryl segment of LP11 also confirms this finding (Table II).

As the limiting area for normal alkyl chains is between 0.20 and 0.22 nm per molecule [21] it would appear that all lauryl segments contribute to the area of the monolayer in both the phosphatidylcholine and phosphatidylcholine/cholesterol preparations. This would imply that the polymer is fully extended in a two-dimensional matrix. In contrast, in the absence of phosphatidylcholine LP11 is not able to unfold completely (Table II). The area per lauryl segment in the phosphatidylcholine only monolayers is surprisingly large, indicating a partially expanded state.

Properties of membranes containing hydrophilic polymers

In contrast to the hydrophobic polymers it was not possible to dissolve hydrophilic polymers into a decane-based "brush mixture". Diamyl ether [33] and related methyl ethers, together with aprotic solvents, were used in an attempt to generate a brush mixture which was capable of dissolving the hydrophilic polymers. Although none of these solvent mixtures proved suitable,

membranes possessing decreased electrical resistance could be generated by adding freeze-dried polymer directly to the decane-based brush mixture. Once formed in the presence of hydrophilic polymers, bilayers were very stable, often persisting for periods in excess of 20 h. Similar results were obtained with polymers possessing monomer ratios vinylpyrrolidone/acrylic acid of 10 : 1 and 1 : 1 but not 1 : 0 and 0 : 1. The phosphatidylcholine/AP11 weight ratio in the bilayer region was found to fall in the range 2 : 1 to 10 : 1 (Table I). There was no observable differences in the water permeability of phosphatidylcholine/cholesterol black lipid membranes and liposomes in the presence and absence of AP11.

Not surprisingly AP11 did not form an insoluble monolayer. At high surface pressures, similar vinylpyrrolidone copolymers are known to dissolve in the subphase [34]. However, the presence of AP11 in the subphase increased the limiting area per lipid unit, the polymer having a greater effect on monolayers containing only phosphatidylcholine (Fig. 6).

Discussion

The results concerning the hydrophobic polymer LP11 can best be interpreted in terms of this polymer being incorporated into the hydrocarbon matrix of the bilayer membranes. The possibility that LP11 is largely concentrated in microlenses can be discounted as no such structures were visible in reflected light, furthermore it would be difficult to explain the stability enhancement if the bulk of the polymer was localised in microlenses. That phosphatidylcholine/cholesterol/LP11 membranes are thicker than the corresponding phosphatidylcholine/LP11 preparations, indicates that the polymer side chains cannot be readily accommodated in the tight cholesterol/phosphatidylcholine packing. Instead, the polymer appears to be largely concentrated at the midplane of the membrane. This differential penetration is also observed in the monolayer studies (Fig. 5). The monolayer studies also demonstrate that LP11 only unfolds in media of low dielectric giving further confirmation of the concept that LP11 accumulates in the hydrophobic region of the bilayer. The enhanced stability of LP11-containing membranes as shown by increased membrane lifetimes and dielectric break-down studies is probably associated with an extended conformation of LP11 in the membrane hydrocarbon phase. Shieh and Packer [35] have reported a similar stabilisation of black lipid membranes by the addition of polystyrene. The "microviscosity" of the hydrocarbon region of egg yolk lecithin liposomes is 73 cP at 310 K which is similar to that found in a variety of biological membranes [36]. However, there is a gradient of "microviscosity" across the membrane, ranging from 210 cP near the surface to 3 cP in the centre [37]. The intermingling of the polymer chains at the high concentrations of polymer, observed in the bilayer might be expected to increase the viscosity of the midplane region. This enhanced viscosity offers an explanation for the increased time period required for thinning (drainage of decane) and for the absence of membrane expansion of lecithin bilayers when LP11 was present.

An increase in membrane viscosity need not lead to an associated decrease in membrane permeability as only the translational modes of the lipid mole-

cules would be decreased, the rotational and vibrational modes being relatively unaffected. Thus the increased permeability towards leucine of liposomes containing LP11 can be interpreted in terms of the high concentration of relatively short lauryl side chains. The finding that LP11 does not influence the water permeability of phosphatidylcholine/cholesterol membranes can be interpreted in terms of Trauble's concept that the movement of small molecules across lipid membranes is largely a result of rotational movement of individual bonds of lipid molecules [38].

Despite the hydrophilic nature of the AP-polymer series, these polymers associate with phosphatidylcholine/cholesterol membranes, as shown by both the black lipid membrane and monolayer studies. Papahadjopoulos et al. [39] have demonstrated a correlation between the extent of protein penetration of the hydrocarbon matrix and conductivity and by analogy, a proportion of the hydrophilic polymers used in this study might be expected to penetrate the hydrocarbon region of the membranes. The ratio of AP11 to phosphatidylcholine molecules detected in black lipid membranes is in the region of 1 : 200. Assuming that the polymer is uniformly distributed over both sides of the membrane each polymer molecule will be associated with an area of approx. 50 nm^2. Thus the surface of the membrane might be expected to be largely covered by these hydrophilic polymers.

From this study it is clear that not only basic polymers and proteins have a profound effect on lipid bilayers, but also acidic and neutral polymers can interact strongly, producing changes in the permeability and stability of these membranes. It is conceivable that the type of polymer-induced, viscous hydrocarbon medium as typified by LP11 membranes would be the physical basis for the protein "integral skeleton" postulated for the extremely strong camel erythrocyte membrane [40].

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