Biochimica et Biophysica Acta 858 (1986) 135–144
Elsevier

BBA 73124

The interaction of liposomes containing intrinsic erythrocyte membrane proteins with lipid monolayers at air/water and oil/water interfaces

R.J. Davies * and M.N. Jones **

Department of Biochemistry, University of Manchester, Manchester, M13 9PL (U.K.)

(Received November 19th, 1985)
(Revised manuscript received February 24th, 1986)

Key words: Membrane-protein interaction; Lipid monolayer; Air/water interface; Oil/water interface; Membrane vesicle; Surface pressure

The main intrinsic membrane proteins of the human erythrocyte membrane, glycophorin and the anion transporter, were isolated by extraction with Triton X-100 and ion-exchange chromatography. After removal of detergent the extract consisted of proteolipid vesicles with a lipid: protein molar ratio in the range 50–60 and a diameter of the order of 200 nm. The interaction between these vesicles and dipalmitoylphosphatidylcholine (DPPC), cholesterol and cholesterol: DPPC (2:1 molar ratio) monolayers at air/water and *n*-decane/water interfaces has been studied. The vesicles interact with the monolayers, rapidly causing large increases in surface pressure. Limiting values of surface pressure, 39.4–43 $mN \cdot m^{-1}$ at air/water and 31.5–33.4 $mN \cdot m^{-1}$ at the *n*-decane/water interface, were reached at protein levels above 1 $\mu g \cdot ml^{-1}$. At the air/water interface, and probably at the *n*-decane/water, surface pressure increases were limited by monolayer collapse. Compression isotherms and surface potential measurements indicated that material from the proteolipid vesicles entered the monolayer phase. In contrast to proteolipid vesicles, injection of protein-free liposomes beneath the monolayer resulted in smaller, slower increases in surface pressure. Thus, the presence of intrinsic membrane proteins in vesicles greatly facilitated the transfer of material into the lipid monolayer.

Introduction

Monolayers are a convenient experimental model system for the study of protein-lipid and membrane-membrane interactions under controlled conditions [1–6]. Interactions between membrane proteins and lipids are of particular interest in relation to membrane phenomena, and there have been several studies on interactions between intrinsic membrane proteins of the human erythrocyte and lipid monolayers at the air/water interface [7–12]. These studies have largely been concerned with the major intrinsic membrane protein which is involved in anion transport [13]. The incorporation of intrinsic membrane proteins in a functional form in either lipid vesicles or planar lipid bilayers ('reconstitution') generally involves detergent extraction followed by recombination with excess lipid [14–17]. It is, therefore, of some relevance to investigate the mode of interaction between such extracts and lipid monolayers.

In contrast to some of the previous monolayer studies [7–9], the protein extracts prepared here were not fully delipidated during preparation so that the protein should have retained its native conformation in association with 'boundary'

* Present address: Department of Paediatrics, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, U.K.
** To whom correspondence should be addressed.

membrane lipid. The results are thus directly related to 'reconstitution' studies, to the transfer of intrinsic membrane proteins and lipids from vesicular form into monolayers, and they are possibly of some relevance to interactions between biomembranes.

Methods and Materials

Preparation of intrinsic membrane protein extracts

Haemoglobin-free membranes were prepared by hypotonic lysis as described by Dodge et al. [18]. Extrinsic membrane proteins were removed by alkaline EDTA extraction [19]. Membranes were twice suspended in 5 vol. 0.1 mM EDTA (pH 11.2) for 20 min on ice and recovered by centrifugation at $40000 \times g$ for 30 min at 4°C. This procedure led to almost complete removal of bands 1, 2, 4.1, 4.2, 5 and 6. Intrinsic proteins were solubilised by adjusting them to pH 7.5 with HCl and suspending them in 3 vol. 10 mM Tris-HCl/0.67% (v/v) Triton X-100 for 30 min at 4°C. Non-solubilised material was removed by centrifugation at $104000 \times g$ for 2 h at 4°C.

Solubilised material was fractionated by ion-exchange chromatography on Whatman DEAE-cellulose [20]. The column (volume 30 ml, radius 9 mm) was equilibrated with 50 mM Tris-HCl/0.5% (v/v) Triton X-100 (pH 7.5). Non-binding protein (i.e., zone 4.5) was eluted with 2 column volumes of column equilibration buffer and loosely bound material (i.e., bands 1, 2 and 7) was removed with 2 column volumes of 50 mM Tris-HCl/50 mM NaCl/0.5% Triton X-100 (pH 7.5). With extract II, the column was washed overnight with this buffer to try to reduce the lipid : protein ratio of the extract. The fraction containing the anion transporter and glycophorin was eluted with 50 mM Tris-HCl/0.5 M NaCl/0.5% Triton X-100 (pH 7:5).

Triton X-100 was removed from the extracts with a Sephadex G-50 column (1 m × 3.5 cm diameter) equilibrated with 10 mM Tris-HCl (pH 7.5). Up to 30 ml of the extract was applied to the column and eluted at 35 $ml \cdot h^{-1}$ at 4°C. Protein-containing fractions were pooled, concentrated overnight with an Amicon ultrafiltration cell (PM-10 membrane) and reapplied to the column, which had been washed with 1.1 column volumes of buffer. This step was repeated. Extracts were frozen as droplets in liquid nitrogen and stored at −20°C under nitrogen.

Preparation of liposomes

Liposomes were made by vacuum rotary evaporation at 60°C of a cholesterol : DPPC (2 : 1 molar ratio) mixture in 2 : 8 (v/v) ethanol/petroleum ether (b.p. 45–55°C). The dry lipid film was hydrated at 60°C with 10 mM Tris-HCl (pH 7.5) (1.5 ml/mg lipid) and diluted, after phospholipid assay, to 75 $\mu g \cdot ml^{-1}$ total lipid. Liposomes containing Triton X-100 were made by mixing equal volumes of liposomes (150 $\mu g \cdot ml^{-1}$) and 15% (w/v) Triton X-100. The mixture was left at 25°C for 1 h before use. The weight ratio of lipid : detergent in this system is 1 (molar ratio 1 : 0.87) and so it contained about 13-times as much Triton X-100 as the membrane extracts.

Analytical methods

Protein was determined by the method of Wang and Smith [21], Triton X-100 by the method of Garewal [22], phospholipid by a turbidimetric method [23], and cholesterol by the method of Zlatkis and Zak [24]. Qualitative lipid analysis was carried out by thin-layer chromatography (TLC). The protein composition of the extracts was analysed by polyacrylamide gel electrophoresis in sodium *n*-dodecyl sulphate [25], using 7.5% acrylamide gels. Gels were stained for protein (with Coomassie blue) and carbohydrate (periodic acid-Schiff method [26]). For examination by electron microscopy samples were negatively stained with uranyl acetate (2% (w/v) in distilled water) and observed at a magnification of 50000 using a Jeol Jem 100 CX electron microscope.

Determination of interfacial tensions at air/water and n-decane/water interfaces

Interfacial tensions were measured by the Wilhelmy method [27] using an electromicrobalance (Beckman R11C, LM600) coupled to a chart recorder to give a continuous reading of force on the dipping plate. Air/water measurements were made with a roughened microscope coverslip of perimeter 4.833 cm, and with a carbon-coated, platinum plate of perimeter 6.025 cm, prepared by the method of Taylor et al. [27], at the *n*-decane/

water interface. Monolayers were formed in a double-walled, cylindrical glass trough of internal diameter 7.0 cm. Its inner wall was made hydrophobic by exposure to dimethyldichlorosilane vapour. The volume of aqueous substrate in the trough was 50 ml and was measured by reference to an inert indicator needle. Interfaces were cleaned by aspiration via a Pasteur pipette. The system was held at 25 ± 0.5°C by circulating water through the trough wall from a water bath. Monolayers were spread from organic solvent with an Agla micrometer syringe and set to the desired initial surface pressure by removing excess monolayer by aspiration via a fine needle. The spreading of cholesterol monolayers at the *n*-decane/water interface took a great deal of time (over 7 h) because of dissolution of the sterol by the oil phase. All monolayers had a stable interfacial pressure for at least 30 min before injection of material beneath them commenced. Extracts, or other materials, were injected beneath the monolayer with a micrometer syringe and mixed into the substrate with a Teflon stirrer bar.

Compression isotherms were obtained with a Langmuir (Teflon) trough, as previously described [28]. Surface potential isotherms were recorded simultaneously using the ionising electrode method [50]. The air electrode was a piece of ^{241}Am foil (Alpha foil, Amersham International, Amersham) of size 10–12.5 mm and activity 0.24 mCi, held 3 mm above the monolayer. The subphase electrode was an earthed platinum foil. The potential between the electrodes was measured by balancing against a calibrated potential supply using a Vibron 33b electrometer as a null meter. External electrical fields were excluded by an earthed Faraday cage.

Materials

Synthetic L-α-dipalmitoylphosphatidylcholine (DPPC) was from the Sigma Chemical Co., London, and specified as 99% pure. Its purity was checked by high-performance liquid chromatography. Cholesterol was Sigma chromatography grade (99 + % pure) and gave one spot when checked by TLC. Monolayers were spread from 2 : 8 (v/v) ethanol/petroleum ether (b.p. 45–55°C) at the air/water and 1 : 9 (v/v) ethanol/*n*-decane at the *n*-decane/water interface. The *n*-decane was analytical grade (BDH) and was further purified as previously described [29]. Triton X-100 was supplied by the Sigma Chemical Co., London. All other reagents used were of analytical grade, and the NaCl was roasted at 500°C for at least 12 h. Water was triple-distilled, the penultimate distillation being from alkaline permanganate.

Results

Characterisation of membrane extracts

The composition of the extracts used are given in Table I; both contained approximately 30% lipid by weight, the major phospholipid being phosphatidylcholine. The amino acid composition of extract I (data not presented) was like that of similar extracts [30,31] with 53% of the residues being hydrophobic. Fig. 1a shows the Coomassie-stained gel scan of the erythrocyte membrane with the protein bands numbered according to the nomenclature of Fairbanks et al. [32]. Fig. 1b shows the periodic acid–Schiff reagent-stained profile of extract I; the major peak corresponds to the carbohydrate associated with glycophorin and the anion transporter [33]. Fig. 1c shows the Coomassie-stained material in extract I; the major peak corresponds to a molecular weight of approximately 90 000 and accounts for about 85% of the stained material on the gel. Similar scans were obtained for extract II. Extract I was examined by electron microscopy (Fig. 2), which revealed approximately spherical particles of diameter 200 nm. The extracts described here contained very little detergent and consisted of the intrinsic membrane proteins, glycophorin and the anion trans-

TABLE I

ANALYTICAL DATA ON THE MEMBRANE EXTRACTS

Triton X-100 was assayed after being concentrated 20-fold by freeze-drying. The detergent was below the detection limit of the assay and the concentration given is based on twice the detection level of the assay.

	Extract I	Extract II
[Protein], $\mu g \cdot ml^{-1}$	200 ±3.8	145 ±2.6
[Cholesterol], $\mu g \cdot ml^{-1}$	34.7 ± 3.3	29.3 ± 2.7
[Triton X-100], $\mu g \cdot ml^{-1}$	⩽ 5	⩽ 5
[Phospholipid], $\mu g \cdot ml^{-1}$	40.0 ± 1.5	37.1 ± 0.4
Protein/lipid	2.68	2.18

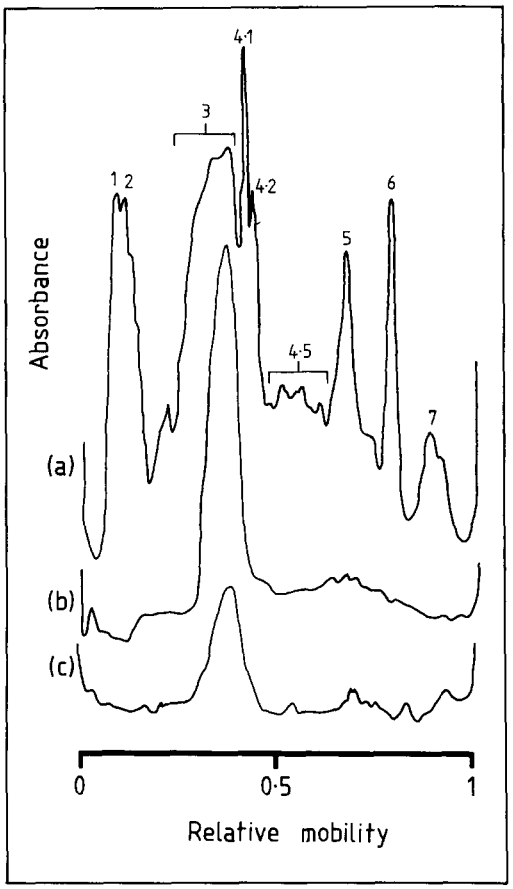

Fig. 1. SDS-polyacrylamide electrophoretograms. Erythrocyte membrane stained with Coomassie blue (a). Extract I, stained with periodic acid-Schiff reagent (b), and Coomassie blue (c).

porter, with associated lipid, and hence can be described as proteolipid vesicles.

Effects of Triton X-100 and liposomes on monolayers at the air/water and n-decane/water interfaces

Before determining the interaction of the extracts with lipid monolayers, control experiments were carried out to assess the effects of Triton X-100 and liposomes on monolayers at the air/water and *n*-decane/water interfaces.

Fig. 3 shows the effect of injection of Triton X-100 beneath monolayers at an initial surface pressure of 10 $mN \cdot m^{-1}$ at these interfaces. The increase in surface pressure followed the sequence cholesterol > DPPC > cholesterol : DPPC (molar ratio 2 : 1) at the air/water interface. For DPPC monolayers the surface pressure increases were larger at the *n*-decane/water than at the air/water interface. The Triton X-100 level in the extracts, even after 20-fold concentration, was $\leqslant 5$ $\mu g \cdot ml^{-1}$. Thus the maximum possible concentration of Triton X-100 in the subphase in measurements with the extracts at their highest level (i.e., 1.5 mg protein per ml) would be $\leqslant 50$ $ng \cdot ml^{-1}$. However, since the surface pressure increases caused by the membrane extracts became constant at above 0.5 $mg \cdot ml^{-1}$ then the maximum detergent concentration would be 17 $ng \cdot ml^{-1}$. This is much lower than the critical micelle concentration of

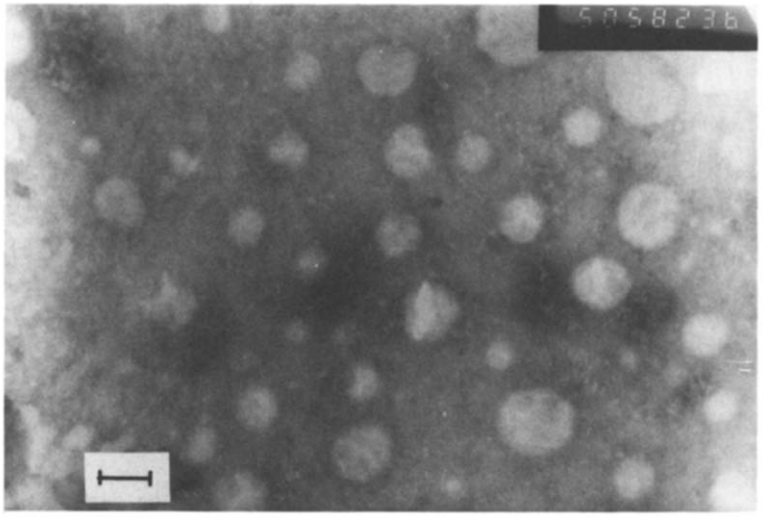

Fig. 2. Transmission electron micrograph of negatively stained preparation of extract II. Magnification × 50000, bar = 200 nm.

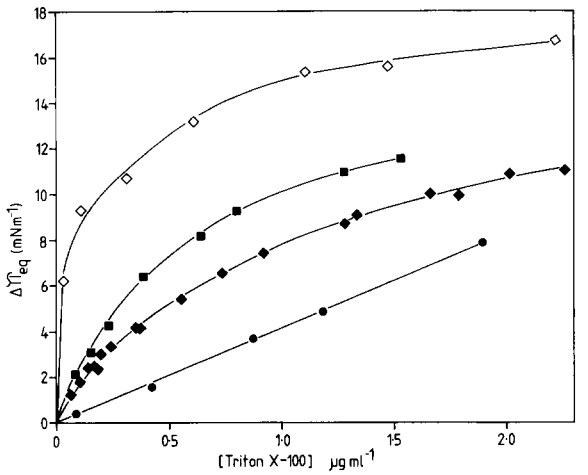

Fig. 3. Penetration of Triton X-100 into monolayers of DPPC (◆), cholesterol (■), and cholesterol : DPPC (2 : 1 molar ratio, ●) at the air/water interface and into DPPC monolayers (◇) at the *n*-decane/water interface. The initial surface pressure of the monolayers was 10 mN · m^{-1}, the aqueous substrate was 20 mM sodium phosphate/0.1 M NaCl (pH 7.4).

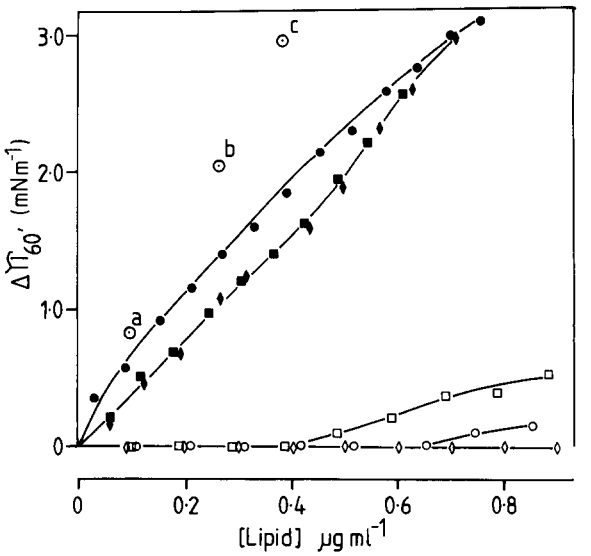

Fig. 4. Interaction of cholesterol : DPPC (2 : 1 molar ratio) liposomes with monolayers of cholesterol (◆, ◇), DPPC (●, ○), and cholesterol : DPPC (2 : 1 molar ratio, ■, □) at air/water (filled symbols) and *n*-decane/water interfaces. Initial film pressures were 10 mN · m^{-1} and surface pressure increases 1 h after injection of liposomes beneath the film are plotted. Points a, b and c are for liposomes incubated with Triton X-100 before injection beneath a cholesterol film at an air/water interface. Substrate as in Fig. 3.

Triton X-100 in water (i.e., 154 μg · ml^{-1} [34]). Triton X-100 at a level of 17 ng · ml^{-1} would lead to an increase in surface pressure at the air/water interface of below 0.5 mN · m^{-1} in cholesterol and DPPC monolayers (Fig. 3), and below 0.2 mN · m^{-1} in cholesterol : DPPC (2 : 1 molar ratio) monolayers. At the *n*-decane/water interface the increase would be approximately 2 mN · m^{-1} with a DPPC monolayer at an initial surface pressure of 10 mN · m^{-1}.

The effect of liposomes, of similar lipid composition to the extracts, on films at air/water and *n*-decane/water interfaces is shown in Fig. 4. (Because cholesterol is far more soluble in *n*-decane than DPPC the film formed at the oil/water interface when the cholesterol : DPPC (2 : 1 molar ratio) is spread will be depleted in cholesterol.) Because the surface pressure increases were very slow, the data in Fig. 4 were taken one hour after injection of the vesicles beneath the monolayer. In the extract experiments (see below) the highest lipid concentrations in the subphase were 0.6 μg · ml^{-1} (extract I) and 0.7 μg · ml^{-1} (extract II). From the liposome data (Fig. 4), the lipid alone in the extracts would be expected to have little effect on surface pressure at either interface. Incubation of the liposomes with levels of Triton X-100 well in excess of those possible in the subphase after injection of the extracts caused a further (approx. 30%) increase in surface pressure.

Effect of the membrane extracts on monolayers at the air/water and n-decane/water interfaces

Fig. 5 shows the effect of extract I on monolayers at the air/water interface as a function of time. Above protein levels of 0.75 μg · ml^{-1} equilibrium pressures were attained after 20 min. Fig. 6 shows the equilibrium surface pressure increases at the air/water interface as a function of protein and lipid concentration. The curves are similar for DPPC, cholesterol and cholesterol : DPPC (2 : 1 molar ratio) monolayers, and plateau at a total surface pressure of 42–43 mN · m^{-1} at protein levels above 1 μg · ml^{-1}. The collapse pressures, in the absence of extract, measured by compression of the monolayer, were 50 mN · m^{-1} (DPPC), 44 mN · m^{-1} (cholesterol) and 46 mN · m^{-1} (cholesterol : DPPC, molar ratio 2 : 1).

Compression (Fig. 7) and surface potential (Fig.

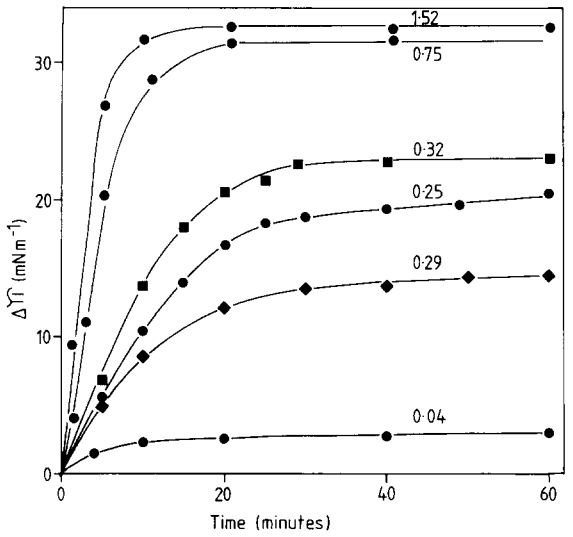

Fig. 5. The kinetics of the interaction of extract I with monolayers of cholesterol (●), DPPC (◆), and cholesterol : DPPC (2 : 1 molar ratio, ■) at an air/water interface. Initial surface pressures were 10 $mN \cdot m^{-1}$ and the number on each curve refers to the initial protein concentration in $\mu g \cdot ml^{-1}$. Substrate as in Fig. 3.

8) isotherms for cholesterol : DPPC monolayers (molar ratio 2 : 1) were determined in the absence and presence of a membrane extract. Isotherms measured after injection of extract were more expanded and about 8-times more compressible, between surface pressures of 15–30 $mN \cdot m^{-1}$,

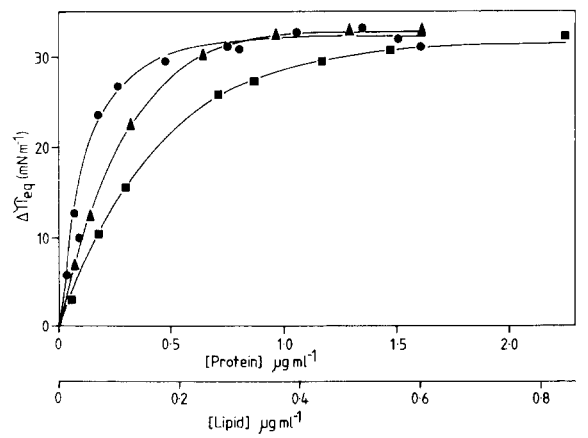

Fig. 6. The equilibrium surface pressure increases reached after interaction of extract I with films of cholesterol (●), DPPC (■), and cholesterol : DPPC (2 : 1 molar ratio, ▲) at an air/water interface. Conditions as for Fig. 3.

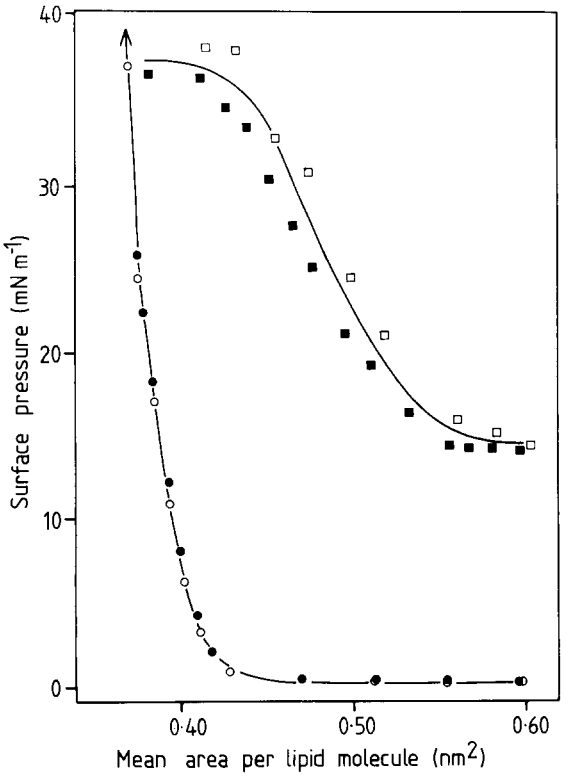

Fig. 7. Compression isotherm at 25°C for cholesterol : DPPC (2 : 1 molar ratio) monolayer before (●, ○) and after (■, □) injection of extract I at an initial protein concentration of 1 $\mu g \cdot ml^{-1}$. Results of two independent measurements are shown. Substrate as for Fig. 3.

than monolayers that had not interacted with the extract. The monolayer collapsed at about 37.5 $mN \cdot m^{-1}$ in the presence of the extract, which suggests that the limiting surface pressures of monolayers exposed to increasing concentrations of extract (Fig. 6) correspond to their collapse pressures. Following injection of extract beneath the monolayer, which was held at a mean area per lipid molecule of 0.60 nm^2, the surface potential increased gradually from 350 mV to a final value close to that of the cholesterol : DPPC (2 : 1 molar ratio) film compressed to a mean area per molecule of 0.38–0.45 nm^2. On compression of the monolayer on the extract-containing substrate the surface potential further increased to a constant value (480 mV) higher than could be explained by an increase in the surface concentration of the lipid alone. A monolayer of cholesterol : dimyristoylphosphatidylcholine (2 : 1 molar ratio) at

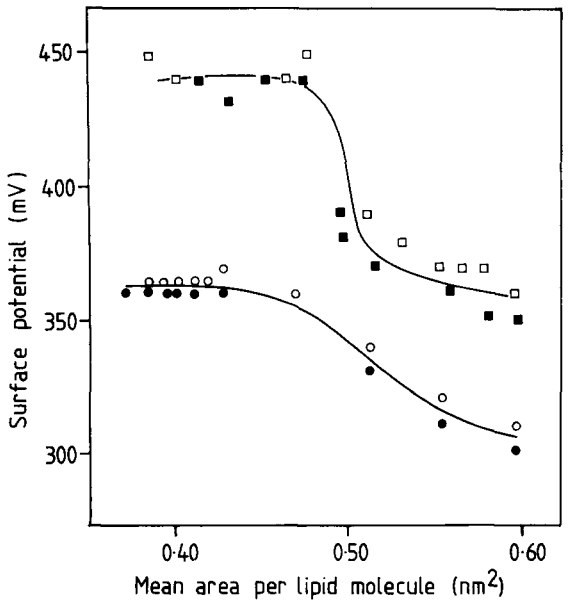

Fig. 8. Surface potential – compression isotherms at 25°C for cholesterol : DPPC (2 : 1 molar ratio) monolayers before (●, ○) and after (■, □) injection of extract I to an initial protein level of 1 $\mu g \cdot ml^{-1}$. Two separate measurements are shown; substrate as for Fig. 3.

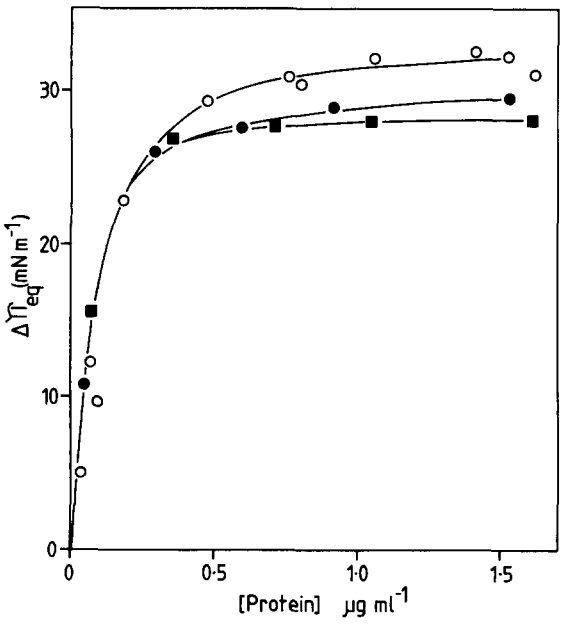

Fig. 9. Interaction of extract I with cholesterol films after probe sonication (●), heating to 70°C for 30 min and then sonicating (■), or untreated (○). Conditions as for Fig. 6.

an average area per molecule of 0.42 nm^2 has a surface potential of about 455 mV [54], indicating that our surface potential values are of the correct magnitude.

Experiments were also carried out to investigate the effect of possible structural changes in the proteolipid vesicles on their interaction with cholesterol monolayers (Fig. 9). Portions of extract I were either heated to 70°C and probe-ultrasonicated or sonicated at room temperature. Sonication altered the visual appearance of the extracts from turbid to opalescent. However, neither of these treatments markedly altered their interaction with the monolayer.

The interaction of the extracts with DPPC monolayers at the *n*-decane/water interfaces was studied, starting at initial film pressures of 10 $mN \cdot m^{-1}$ (extract I) and 20 $mN \cdot m^{-1}$ (extract II). The equilibrium increase in surface pressures at different extract concentrations are shown in Fig. 10, together with the corresponding curves for the air/water interface. With extract I the total surface pressure became constant, at 33.4 $mN \cdot m^{-1}$, above a protein concentration of approx. 1.0 $\mu g \cdot ml^{-1}$. With extract II the total limiting surface pressure was 31 $mN \cdot m^{-1}$. The corresponding total film pressures at the air/water interface were 43 $mN \cdot m^{-1}$ and 39.4 $mN \cdot m^{-1}$, respectively.

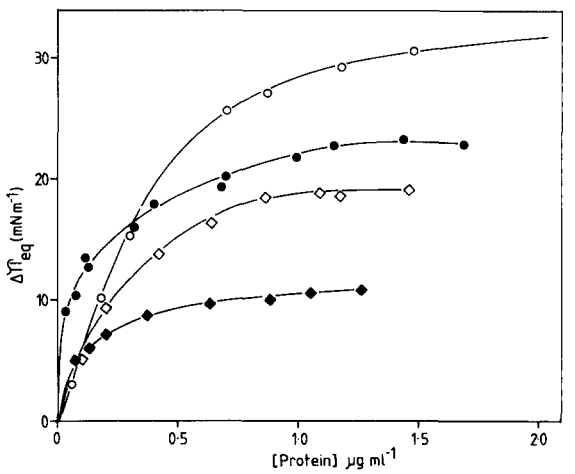

Fig. 10. A comparison of the interaction of extract I (●, ○) and extract II (◆, ◇) with DPPC monolayers at air/water (open symbols) and *n*-decane/water interfaces. Initial film pressures were 10 $mN \cdot m^{-1}$ (extract I) and 20 $mN \cdot m^{-1}$ (extract II). Conditions as for Fig. 6.

Discussion

Electron microscopy revealed that the proteolipid vesicles were approximately spherical. Assuming molecular weights of 90000 for the anion transporter, 31000 for glycophorin and a ratio of 2:1, as in the erythrocyte membrane [35], and assuming that the phospholipid has a molecular weight similar to that of DPPC, then the weight ratios of protein to lipid (phospholipid plus cholesterol) in Table I correspond to lipid-to-protein molar ratios of 51 and 61 for extracts I and II, respectively. These figures are of the same order as the number of 'boundary' lipids that are withdrawn from participation in the gel-to-lamellar phase transitions in a number of protein-lipid systems [12,36]. This lipid could not be removed even by overnight washing of the ion-exchange column with 0.5% (w/v) Triton X-100. The co-elution of the anion transporter ($pI = 6.9$, Ref. 37) and glycophorin ($pI = 4.2$, Ref. 38) from the ion-exchange column [54] may reflect their co-location in a lipid bilayer.

Proteolipid vesicles at the air/water interface

The proteolipid vesicles caused substantial and rapid increases in the surface pressures of DPPC, cholesterol and cholesterol:DPPC monolayers at air/water, and with DPPC films at *n*-decane/water interfaces. These effects were in marked contrast to the much smaller, and slower increases in surface pressure on injection of protein-free liposomes beneath these monolayers. Experiments on the Langmuir trough demonstrated that injection of the proteolipid vesicles in the subphase led to a considerable expansion of cholesterol:DPPC monolayers and a concomitant increase in surface potential. These observations suggest that material from the proteolipid vesicles had penetrated the monolayer. In view of the higher compressibility of protein films in general compared to that of the DPPC:cholesterol monolayer, and the increased compressibility and surface potential of the monolayer after interaction with the extract, it seems that some of the material entering the film was protein. The lower collapse pressure of the monolayer after interaction with the membrane extract ($37.5\ mN \cdot m^{-1}$) may be caused by displacement of protein from the monolayer. It is interesting to note that human erythrocyte band 3 protein, which is the main protein component in our extracts (see Fig. 1), will penetrate cholesterol monolayers at the air/water interface up to surface pressures of $38\ mN \cdot m^{-1}$ [8].

The exchange of lipids between liposomes and monolayers has been considered by Schindler [6] and Jahnig [39]. Liposomes concentrate below a monolayer, probably in the unstirred layer, and exchange occurs between the outer lipid layer of the liposome bilayer and the monolayer. Only a small fraction (less than 20%) of the lipid can leave the outer layer of the vesicle before a decrease in the packing density begins to expose the hydrocarbon core of the bilayer. Lipid movement from the inner to the outer half of the bilayer (flip-flop) is too slow to maintain the packing density and so plays little part in the transfer of lipid into the monolayer. The magnitude of the surface pressure increase is related to liposome size and is independent of liposome/monolayer composition and liposome concentration, provided the liposomes are in excess. The surface pressure increase is thus determined by the amount of lipid each liposome can deliver to the monolayer from its limiting bilayer. The mechanism of lipid transfer between liposome and monolayer in protein-free systems is not clear; exchange may be effected by monomeric lipid in solution [40], or during impact of liposome and film [41].

The main finding in this study indicates that intrinsic membrane protein(s) in the liposome bilayer can greatly facilitate the transfer of material to the film. Both glycophorin [42] and the anion transporter [43] increase the rate of lipid transmembrane flip-flop when incorporated into bilayers by similar methods used in this study. Thus, these proteins would act to maintain equivalent packing densities in inner and outer halves of the limiting bilayer and allow a larger fraction, if not all, of the proteoliposome lipid and some of the protein to be transferred to the monolayer and cause larger equilibrium pressure increases than seen with the protein-free liposomes. It is not possible to quantify the amount of protein entering the monolayer without radiolabelling it.

Probe sonication reduced the size of the proteoliposomes but had no marked effect on their interaction with the monolayer. Since small liposomes

can transfer less lipid than larger liposomes [5,39], sonication should have reduced the size of the surface pressure increase. An increased rate of flip-flop, due to the intrinsic proteins, would tend to make the surface pressure increase liposome-size-independent. Heating the proteoliposomes, which was expected to denature the protein, and possibly allow the lipid to pack in a more stable manner, also had little effect on the behaviour of the extracts. This may be due to the high stability of the anion transporter to denaturation [44].

The inability of pure lipid liposomes to cause large increases in film pressure is in accord with their low surface activity [45,46]. The lower surface pressure increases caused by the liposomes at the oil/water interface may have been due to solubilization of the cholesterol entering the film by the *n*-decane. Incubation of liposomes with Triton X-100 at a molar ratio of 1 : 0.87 resulted in a slightly increased transfer of material into a cholesterol monolayer at the air/water interface. However, the increases in film pressure caused by the Triton X-100 : liposome mixtures was always less than the increases caused by the same concentration of Triton X-100 in the substrate. This suggests that a large amount of Triton X-100 was incorporated into the liposome bilayers. Incorporation of cholate into lipid bilayers increases the rate of lipid flip-flop [47], and Triton X-100 was expected to do likewise. Although the Triton X-100-containing liposomes gave larger surface pressure increases than the pure lipid vesicles, these were not large enough to account for the effects seen with the membrane extracts.

Proteolipid vesicles at the n-decane / water interface

Monolayers at oil/water interfaces have been argued to be better models of one half of a lipid bilayer [48,49]. Because of this, some measurements were repeated at the *n*-decane/water interface to see whether they were any different from the air/water results.

Triton X-100 caused larger surface pressure increases in DPPC monolayers at the *n*-decane/water than at the air/water interface. This is due to the larger fall in free energy on adsorption of amphiphiles to an oil/water compared to an air/water interface [51]. The protein-free liposomes gave smaller surface pressure changes in monolayers at the *n*-decane/water than at the air/water interface. Likewise, both membrane extracts gave limiting equilibrium surface pressure increases some 11 $mN \cdot m^{-1}$ lower at the *n*-decane/water than at the air/water interface. We cannot explain this observation. A simple explanation would be that the total film pressure at the higher protein levels corresponds to the collapse pressure of the monolayer at the *n*-decane/water interface. However, collapse pressures of phospholipid monolayers at oil/water interfaces are not markedly lower than at the air/water interface [52,53].

From the *n*-decane/water measurements, the intrinsic proteins again facilitate transfer of material to the film. From the size of the maximum film pressures reached, there is no apparent difference between monolayers at air/water and oil/water interfaces.

Acknowledgement

We thank the SERC for a studentship for R.J.D.

References

1 Eley, D.D. and Hedge, D.G. (1956) J. Coll. Int. Sci. 11, 445–450
2 Camego, G., Colacicco, G. and Rapport, M.M. (1968) J. Lipid Res. 9, 562–569
3 Quinn, P.J. and Dawson, R.M.C. (1970) Biochem. J. 116, 671–680
4 Ohki, S. and Duzgunes, N. (1979) Biochim. Biophys. Acta 552, 438–449
5 Schindler, H. (1980) FEBS Lett. 122, 77–79
6 Schindler, H. (1979) Biochim. Biophys. Acta 55, 316–336
7 Klappauf, E. and Schubert, D. (1977) FEBS Lett. 80, 423–452
8 Klappauf, E. and Schubert, D. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 1225–1235
9 Schubert, D. and Klappauf, E. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 1171–1177
10 Blank, M., Soo, L. and Cogan, U. (1981) J. Coll. Int. Sci. 83, 448–459
11 Schubert, D. and Boss, K. (1982) FEBS Lett. 150, 4–8
12 Davies, R.J., Goodwin, G.C., Lyle, I.G. and Jones, M.N. (1984) Colloids Surf. 8, 261–270
13 Jennings, M.L. (1984) J. Membrane Biol. 80, 105–117
14 Jones, M.N. and Nickson, J.K. (1980) Biochim. Biophys. Acta 644, 1–20
15 Shelton, R.L. and Langdon, R.G. (1983) Biochim. Biophys. Acta 733, 25–33
16 Kohne, W., Haest, C.W.M. and Deuticke, B. (1981) Biochim. Biophys. Acta 664, 108–120

17 Cabantchik, Z.I., Volsky, D.J., Ginsburg, H. and Loyter, A. (1980) Ann. N.Y. Acad. Sci. 341, 444–454
18 Dodge, J.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochim. Biophys. 100, 119–130
19 Steck, T.L. and Yu, J. (1973) J. Supramol. Struct. 1, 220–232
20 Sogin, D.C. and Hinkle, P.C. (1978) J. Supramol. Struct. 8, 447–453
21 Wang, C.S. and Smith, R.L. (1975) Anal. Biochem. 63, 414–417
22 Garewal, H.S. (1973) Anal. Biochem. 54, 319–324
23 Eibl, H. and Lands, W.E. (1969) Anal. Biochem. 30, 51–57
24 Zlatkis, A. and Zak, B. (1969) Anal. Biochem. 29, 143–148
25 Neville, D.M. (1971) J. Biol. Chem. 246, 6328–6334
26 McGuchin, W.F. and McKenzie, B.F. (1958) Clin. Chem. 4, 476–483
27 Taylor, J.A.G. and Mingins, J. (1970) in A Manual for the Measurement of Interfacial Tension, Pressure and Potential at Air or Non-Polar Oil-Water Interfaces, Unilever Research Laboratories, Port Sunlight, U.K.
28 Davies, R.J., Goodwin, G.C., Lyle, I.G. and Jones, M.N. (1983) Colloids Sur. 8, 29–43
29 Jones, M.N. (1973) J. Coll. Int. Sci. 44, 13–20
30 Steck, T.L. (1978) J. Supramol. Struct. 8, 311–324
31 Ho, M.K. and Guidotti, G. (1975) J. Biol. Chem. 250, 675–683
32 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617
33 Tsuji, T., Irimura, T. and Osawa, T. (1981) J. Biol. Chem. 256, 10497–10502
34 Helenius, A. and Simon, K. (1975) Biochim. Biophys. Acta 415, 29–79
35 Steck, T.L. (1974) J. Cell Biol. 62, 1–19
36 Jones, M.N. and Skinner, H.A. (1982) Annu. Rep. Prog. Chem., Sect. C78, 3–39
37 Bhakdi, S., Bjerrum, O.J. and Knufferman, H. (1976) Biochim. Biophys. Acta 446, 419–431
38 Davies, R.J. (1985) Ph.D. Thesis, University of Manchester, p. 134
39 Jahnig, F. (1984) Biophys. J. 46, 687–694
40 Nichols, J.W. and Pagano, R.E. (1981) Biochemistry 20, 2783–2789
41 Kremer, J.M.H., Kops-Werkhaven, M.M., Pathmanoharan, C., Guizeman, O.L.J. and Wierseria, P.H. (1977) Biochim. Biophys. Acta 471, 177–188
42 Gerritson, W.J. and Henricks, P.A.J. (1980) Biochim. Biophys. Acta 600, 607–619
43 DeKruijff, B., Van Zoelen, E.J.J. and Van Deenen, L.L.M. (1978) Biochim. Biophys. Acta 509, 537–542
44 Makino, S., Nakashima, H. and Shibagaki, K.J. (1981) J. Biochem. 89, 651–658
45 Obladen, M., Klatt, I. and Bartholome, M. (1981) Prog. Respir. Res. 15, 177–187
46 Davies, R. and Morley, C.J. (1984) Prog. Respir. Res. 18, 267–273
47 Kramer, R.M., Hasselbach, H.-J. and Semenza, G. (1981) Biochim. Biophys. Acta 643, 233–242
48 Ohki, S. and Ohki, C.B. (1976) J. Theor. Biol. 62, 389–407
49 Gruen, D.W. and Wolfe, J. (1982) Biochim. Biophys. Acta 688, 572–580
50 Adamson, A.W. (1982) Physical Chemistry of Surfaces, 4th Edn., p. 114, Wiley-Interscience, New York
51 Aveyard, R. and Haydon, D.A. (1973) An Introduction to the Principles of Surface Chemistry, p. 108, Cambridge University Press
52 Mingins, J., Taylor, J.A.G., Pethica, B.A., Jackson, C.M. and Yue, B.Y.T. (1982) Trans. Faraday Soc. I 78, 323–339
53 Phillips, M.C. and Chapman, D. (1968) Biochim. Biophys. Acta 163, 301–313
54 Standish, M.M. and Pethica, B.A. (1967) Biochim. Biophys. Acta 144, 659–665