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A monolayer study on cytochrome b_5-phospholipid interactions

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Cytochrome b_5 has been incorporated into phospholipid monolayers at the air/water interface (Langmuir films). Protein incorporation was followed by monitoring changes in surface pressure at constant film area or by measuring film area changes at constant surface pressure. It was possible to deposit proteolipid films on solid substrates using the Langmuir-Blodgett technique. Using the homologous series of phosphatidylcholines, $C_{10:0}$–$C_{22:0}$, it was found that increasing chain-length led to increased cytochrome penetration into the surface film. ^{125}I-labelled cytochrome b_5 was used to quantify the degree of protein uptake into the film. Phospholipid/protein ratios of 32 and 60 were determined for dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine, respectively. A molecular area of 790 Å^2 was calculated for the hydrophobic segment of cytochrome b_5. The results are discussed with reference to other work on protein-phospholipid interactions, in particular to studies on cytochrome b_5-liposome systems.

Introduction

Extensive efforts have been directed to the immobilisation of proteins onto solid surfaces [1]. Where the enzyme has electron-transferring properties, the possibility exists of replacing complex and expensive organic cofactors with a solid metal electrode [2]. It seems likely that enzyme systems derived from cell membranes will function maximally in vitro when conditions of immobilisation closely resemble those in vivo.

The rapidly developing Langmuir-Blodgett technology [3] offers an almost ideal means for immobilising proteins in a controlled manner in monomolecular or multilayer films deposited into solid supports. The protein can be incorporated directly onto the floating monolayer in a Langmuir trough or introduced via the aqueous subphase.

We have chosen to study the incorporation of cytochrome b_5 from the aqueous subphase into phospholipid monolayers. The phospholipids are natural matrices for the incorporation of many proteins and have been studied in monolayer form [4,5]. Cytochrome b_5 is an appropriate amphipathic molecule for monolayer formation and an extensive literature on its physical, molecular and redox properties exists [6,7]. It is an integral membrane protein which can be isolated in a highly purified form and its structure is well-characterised [7]. Cytochrome b_5 has two principal structural domains, a hydrophilic, catalytic segment containing a prosthetic haem group and a hydrophobic segment which is involved in membrane attachment [7–9].

Previous studies on cytochrome b_5-phospholi-

Abbreviations: DPPA, dipalmitoylphosphatidic acid; DPPE, dipalmitoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DDPC, didecanoylPC; DLPC, dilauroylPC; DMPC, dimyristoylPC; DSPC, distearoylPC; DBPC, dibehenoylPC; DOPC, dioleoylPC.

pid interactions have used as model systems either microsomes and phospholipid vesicles [10–19] or phospholipid lamellae [20]. These studies were all confined to phosphatidylcholines, however, because of the poor ability of other phospholipids to form bilayer structures. Using Langmuir monolayers as model systems, a range of phospholipids can be studied. Penetration of protein into the phospholipid monolayer can be measured by the change in area or pressure of the monolayer [21].

Materials and Methods

Materials. All synthetic phospholipids were obtained as the DL-α-isomers from Sigma (Poole, U.K.) and were eof the highest available purity. Each lipid batch was routinely checked for purity and further purification was carried out where necessary. Natural phospholipid extracts, with a heterogenous fatty acid distribution were purchased from Koch Light (Colnbrook, Herts., U.K.). Cytochrome b_5 was extracted from bovine liver (approx. 2 kg per batch) in the presence of detergents by the method of Strittmatter et al. [22]. The purified protein had an A_{280}/A_{413} value of not more than 0.36 and migrated as a single band when subjected to polyacrylamide gel electrophoresis by the method of Weber and Osborn [23]. The molecular weight determined by the latter method was found to be 17000. ^{125}I-labelled protein was prepared by the method of Salacinski et al. [24] using iodogen (Pierce and Warriner, Chester, U.K.) as the oxidising agent. Radioactivity was subsequently determined using a gamma counter (Bioscint, ESI Nuclear, Surrey, U.K.).

Preparation of monolayers. Monolayer experiments were performed in a polypropylene trough ($60 \times 26 \times 4.5$ cm) located on a thermostatically controlled metal base plate. All monolayer experiments were carried out at 20 ± 1°C. In some experiments, a mini trough ($24 \times 15 \times 1$ cm) was used to obtain relatively high protein concentrations in the aqueous subphase. The trough assembly was of the continuous-barrier type described by Blight et al. [25] and was equipped with a Wilhelmy plate/electrobalance feedback arrangement for applying and maintaininag a constant surface pressure on the floating monolayer. Both the film area and surface pressure were continuously recorded. Ultrapure water for the trough was obtained from an Elgastat RO1 system (Elga, High Wycombe, Bucks. U.K.) which comprised reverse osmosis, activated carbon and nuclear grade mixed-bed deionising filters.

Phospholipid solutions (approx. 1 $mg \cdot ml^{-1}$) were prepared in chloroform (Aristar grade, BDH, Poole, Dorset, U.K.) and 60–100 nmol spread on the subphase using a microlitre pipette fitted with a glass capillary. Cytochrome b_5 was injected at multiple sites underneath the monolayer using a microsyringe to ensure thorough dispersion.

In some experiments, lipid-protein monolayers were deposited onto aluminium foil (4×5 cm) previously cleaned in chloroform/methanol (2 : 1, v/v) and ultrapure water. Deposition was carried out at a surface pressure of 40 $mN \cdot m^{-1}$ at a dipping speed of 8 $mm \cdot min^{-1}$. Deposition ratios (area film deposited/area foil dipped) were consistently greater than 0.9.

Results

Single-component monolayers

In this investigation, we have examined the effects on the uptake of cytochrome b_5 by phospholipid monolayers of changing the phospholipid polar headgroup, varying the length of the fatty acid chains and of using natural as opposed to synthetic phospholipids. To this end, the pressure-area isotherms of the range of phospholipids given in Table I were characterised. Both the recorded isotherms as well as the molecular areas calculated from the isotherms were in good agreement with previous work [4,5]. Examples of isotherms are given in Fig. 1a and b and the values given in Table I.

If a solution of cytochrome b_5 in water (35 nmol) is injected into the aqueous subphase, then despite the solubility of the protein, after approx. 30 min a film of protein becomes established at the subphase surface resulting in the isotherm shown in Fig. 1c. A molecular area for the protein cannot be calculated directly from the curve because the partition of protein between the bulk water and the air/water interface is unknown.

Cytochrome b_5-phospholipid monolayers

Using the mini trough to ensure relatively high

TABLE I

UPTAKE OF CYTOCHROME b_5 INTO MONOLAYERS COMPOSED OF VARIOUS PHOSPHOLIPIDS

The effect of headgroup and fatty acid chain-length are shown for synthetic phospholipids (1st and 2nd group) and data for natural phospholipids from various sources are given (final group). Area/molecule is given for pure phospholipid films. $\Delta\pi_m$ is the maximum increase in surface pressure. ΔA_m and ΔA_{40} are the differences in area between pure lipid and the proteolipid films at 0.1 and 40 $mN \cdot m^{-1}$, respectively. PL, phospholipid; BBPC, bovine brain PC; BLPE, bovine liver PE; YPI, yeast phosphatidylinositol.

Phospholipid	Area/molecule (Å^2)		$\Delta\pi_m$ (mN/m)	ΔA_m (cm^2/nmol PL)	ΔA_{40} (cm^2/nmol PL)	PL/protein mole ratio)
	40 mN/m	0.1 mN/m				
DPPA	43	51	9.2	3.2	0.63	84
DPPE	45	55	12.2	4.3	0.88 ± 0.14 [a]	60 [b]
DPPC	47	103	12.4	7.1	1.63 ± 0.15 [a]	32 [b]
DDPC (C_{10})	56	155	9.2	2.2	0.60	88
DLPC (C_{12})	55	129	11.0	6.0	1.14	47
DMPC (C_{14})	55	121	11.3	6.2	1.37	39
DPPC (C_{16})	47	103	12.4	7.1	1.63	32 [b]
DSPC (C_{18})	47	60	14.5	9.8	1.90	28
DBPC (C_{22})	47	58	15.4	10.4	2.02	27
DOPC	83	130	12.1	6.9	–	–
BBPC	85	130	12.1	6.7	–	–
BLPE	70	105	9.8	6.4	–	–
YPI	50	95	4.7	3.4	–	–

[a] Standard deviation from 12 experiments.
[b] Experimentally determined values.

protein concentrations in the subphase, a series of experiments were performed in which monolayers of DPPC and DPPE were compressed to an initial surface pressure of 50 $mN \cdot m^{-1}$, while cytochrome b_5 (35 nmol) was injected into the subphase with a microlitre syringe and allowed to disperse to a final uniform concentration of 0.1 μM. This procedure eliminates the effects of protein diffusion in the subphase. The phospholipid film was then relaxed to a predetermined set pressure. Initially, the monolayer area at this constant pressure was determined by the phospholipid isotherm, but as time elapsed the area increased as a result of cytochrome uptake. The initial rate of area increase as a function of set pressure is plotted in Fig. 2. As found in previous monolayer studies on bovine serum albumin [26,27], spectrin [28] and prothrombin [29], the penetration rate decreased with increasing monolayer pressure. The change in gradient at approx. 6 $mN \cdot m^{-1}$ for DPPC reflects the characteristic gel-liquid phase transition at this pressure and temperature (19°C), and it is seen that cytochrome b_5 penetrates a phospholipid monolayer in the gel phase less rapidly than in the liquid phase. Such an effect has been previously reported for the penetration of colicin A into DPPC monolayers [30].

If the penetration rates in Fig. 2 are replotted against effective molecular area of the phospholipid at the set pressure, then a linear relationship is found (Fig. 3). Mombers et al. [29] made similar observations for the penetration of spectrin into several phospholipids. From the curves in Figs. 2 and 3 it can be seen that cytochrome penetration ceases when DPPC and DPPE monolayers are compressed to effective area/molecule values of 56 and 49 Å^2, respectively, corresponding to surface pressures of 12–13 $mN \cdot m^{-1}$.

These preliminary experiments showed that cytochrome penetration is most rapid at low surface pressure and is significant even at low cytochrome concentrations. Therefore, the remainder of the programme was carried out in the larger trough for practical convenience and greater accuracy, and protein penetration into films initially pressurised to 0.1 $mN \cdot m^{-1}$ was determined. These experiments were carried out either by holding the monolayer area constant as its initial value and

recording changes in film pressure or by allowing the area to expand while holding the pressure constant at 0.1 $mN \cdot m^{-1}$. This surface pressure was chosen since it is the lowest pressure that the control system can maintain accurately. It should be emphasised that even at such a low surface pressure, the area available per headgroup (103 $Å^2$) is only approximately twice that at 40 $mM \cdot m^{-1}$ (47 $Å^2$). If this area represents the projection onto the surface of a cone swept out by a 25 Å tail, then this tail is inclined at an angle of 77° to the surface.

Measurements at constant area

After spreading a phospholipid monolayer and compressing to 0.1 $mN \cdot m^{-1}$, cytochrome b_5 was injected into the subphase at many points under the monolayer. Fig. 4 shows the ensuing increase in monolayer pressure when the surface area is held constant. For DPPC, DPPE and DPPA, an immediate increase in pressure occurs. After about 60–120 min, the pressure increase saturates at maximum values, $\Delta\pi_m$, given in Table I. Also given are the corresponding values for the other phospholipids investigated. For DPPC, a change in the rate of pressure increase occurs at approx. 6 $mN \cdot m^{-1}$ corresponding to the gel-liquid phase transition for this phospholipid (see also Fig. 2).

Measurements at constant surface pressure

Comparison of the $\Delta\pi_m$ values in Table I for DPPC and DPPE with the results given in Fig. 2 show that $\Delta\pi_m$ is essentially the maximum monolayer pressure at which cytochrome b_5 will penetrate into the monolayer. Therefore, the $\Delta\pi_m$ value is likely to be a characteristic of the phospholipid rather than a quantitative measure of the maximum amount of cytochrome that a particular monolayer can accommodate. Furthermore, the kinetics of the pressure change are characteristic of the phospholipid (see Fig. 2). Therefore, diffusion effects in the subphase are likely to be unimportant.

It has been suggested [29,31] that pressure changes are dependent upon the compressibility of the phospholipid. It has been further suggested that changes in film area at constant surface pressure are a more quantitative measure of protein insertion into monolayers. This approach has been extensively used to study lipolytic enzymes (see Ref. 21 for review), but has also been used to study non-lipolytic membrane-associated proteins

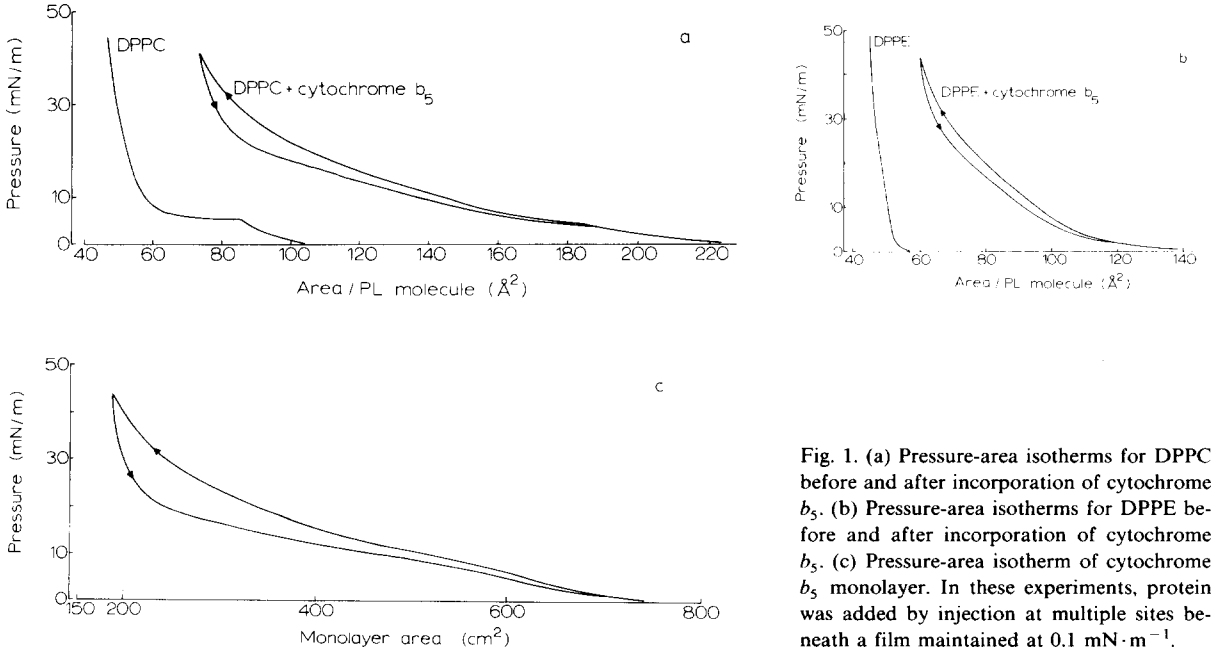

Fig. 1. (a) Pressure-area isotherms for DPPC before and after incorporation of cytochrome b_5. (b) Pressure-area isotherms for DPPE before and after incorporation of cytochrome b_5. (c) Pressure-area isotherm of cytochrome b_5 monolayer. In these experiments, protein was added by injection at multiple sites beneath a film maintained at 0.1 $mN \cdot m^{-1}$.

[28,32,33]. We have adopted the same approach to investigate further the cytochrome b_5-phospholipid system.

Cytochrome b_5 was injected into the aqueous subphase below a phospholipid layer compressed to 0.1 $mN \cdot m^{-1}$ as described above. This time, however, the pressure control system, by expanding the area occupied by the film, maintained the surface pressure constant during the uptake of protein. The increases in surface area with time for DPPC, DPPE and DPPA monolayers are shown in Fig. 5. Again, an immediate effect was seen after addition of cytochrome, but the area change reached a maximum after 30–40 min. The maximum area changes, ΔA_m (expressed in cm^2 per nmol phospholipid) are given in Table I. It was necessary to normalise the area changes to quantity of phospholipid in the monolayer because in the presence of a saturating concentration of cytochrome b_5 the area change depended on the amount of phospholipid in the monolayer.

Fig. 1a and b show the effect on the pressure-area isotherm of allowing cytochrome b_5 penetration to go to saturation at 0.1 $mN \cdot m^{-1}$. It is obvious that film area is larger at all surface pressures and that a certain degree of hysteresis is present. Table I also shows a clear trend in which ΔA_m and ΔA_{40} for a series of PC films both increase with increasing fatty acid chain-length.

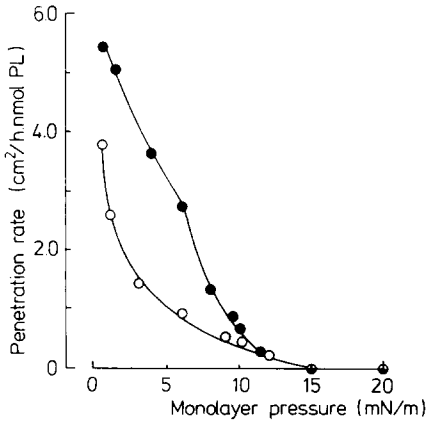

Fig. 2. Rate of penetration of cytochrome b_5 into DPPC (●) and DPPE (○) monolayers maintained at various pressures, following dispersion of the cytochrome in the subphase below a film maintained at 50 $mN \cdot m^{-1}$. PL, phospholipid.

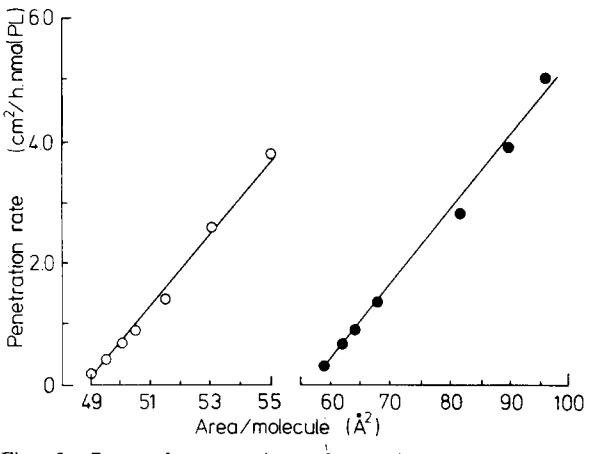

Fig. 3. Rate of penetration of cytochrome b_5 versus area/molecule for DPPC (○) and DPPE (●) monolayers. Data from the experiment of Fig. 2. PL, phospholipid.

Quantification of protein penetration

The effective area of the cytochrome b_5 incorporated into the monolayer cannot be determined directly from the pressure-area isotherms because the partition of protein between the aqueous phase and the amphipathic monolayer is not known. This problem was overcome by labelling the protein with ^{125}I and radioassaying Langmuir-Blodgett monolayers of the mixed films deposited onto aluminium foil.

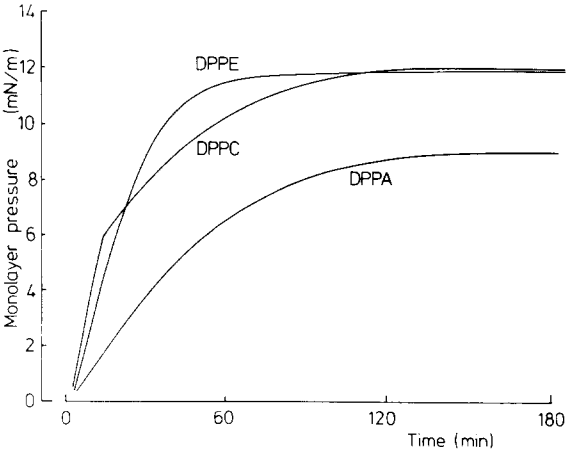

Fig. 4. Increase in monolayer pressure following penetration of cytochrome b_5 into a phospholipid monolayer maintained at constant area. Protein was added by injection at multiple sites beneath a film maintained at 0.1 $mN \cdot m^{-1}$.

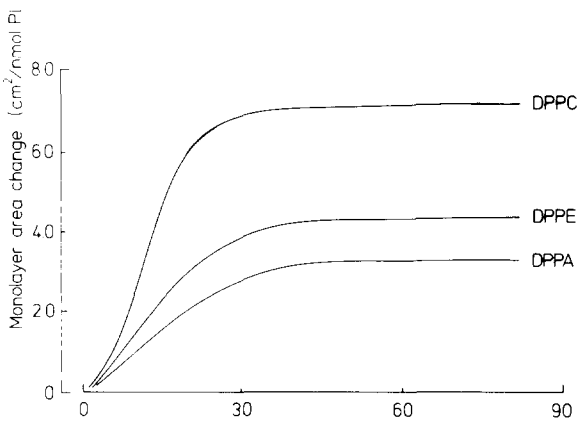

Fig. 5. Increase in monolayer area following penetration of cytochrome b_5 into a phospholipid monolayer. Protein was added by injection at multiple sites beneath a film maintained at 0.1 $mN \cdot m^{-1}$. PL, phospholipid.

For these measurements, the labelled cytochrome was introduced as before into DPPC or DPPE monolayers. After allowing the monolayer to expand to its maximum area while maintaining a constant pressure of 0.1 $mN \cdot m^{-1}$, the film was compressed to 40 $mN \cdot m^{-1}$. The resulting $\pi - A$ characteristic followed the curves given in Fig. 1a and b. From such recordings, the area difference between lipid and protein-lipid films at 40 $mN \cdot m^{-1}$ (ΔA_{40}) was calculated (Table I). Only films of the saturated phospholipid proved to be sufficiently stable at this pressure to permit a reliable determination of ΔA_{40}.

The protein-lipid monolayer was deposited onto an aluminium foil plate of known surface area at a pressure of 40 $mN \cdot m^{-1}$ using the Langmuir-Blodgett technique. The quantity of cytochrome in the deposited film was determined from the specific radioactivity of the protein and from this the total cytochrome in the film was calculated. Since the total phospholipid in the film is also known, the phospholipid/protein ratios in DPPC and DPPE monolayers could be calculated. By depositing from various sites in the film, the latter was found to be macroscopically homogenous with respect to ^{125}I-labelled protein distribution. The values so obtained for the whole film are given in Table I. Furthermore, combining the radioassay with the area change, ΔA_{40}, caused by protein uptake, allows a molecular area to be calculated for cytochrome b_5. The values thus determined lie in the range 720–850 $Å^2$, giving an average of 790 $Å^2$ from six experiments. Importantly, the molecular area was found to be independent of the final phospholipid/protein ratio and was the same for both DPPC and DPPE films.

Assuming a protein area of 790 $Å^2$ to be applicable in all films, the phosholipid/protein ratios can be calculated for all the remaining phospholipids (Table I). Clearly, protein uptake was greater into films made up of phospholipids with the longer chain-length fatty acid. These results suggest that it is protein-phospholipid interactions that determine the degree of uptake of protein into the film, rather than simply the affinity of the protein for the air/water interface.

Discussion

Our results have demonstrated that cytochrome b_5 can be readily incorporated into phospholipid monolayers. The quantity of protein incorporated into the monolayer varied according to the method of protein penetration used and the nature of the phospholipid.

Having found that cytochrome incorporation was a maximum at low surface pressure (0.1 $mN \cdot m^{-1}$), two experimental approaches to protein penetration were compared. In one approach, we determined the pressure change ($\Delta\pi_m$) at constant surface area, whereas in the second approach the area (ΔA_m) at constant surface pressure was determined (see Table I). It was found that, for a homologous series of phosphatidylcholines ($C_{10:0}$–$C_{22:0}$), both the $\Delta\pi_m$ and ΔA_m values showed a good correlation with the value for the effective area/ phospholipid molecule at 0.1 $mN \cdot m^{-1}$. Both $\Delta\pi_m$ and ΔA_m decreased with increased area/phospholipid molecule. Similarly, a decrease in both values was recorded if the area/phospholipid molecule was increased by the introduction on an unsaturated bond. Thus, the $\Delta\pi_m$ and ΔA_m for DOPC ($C_{18:1}$) were lower than those for DSPC ($C_{18:0}$). Similar correlations between area/phospholipid molecule and protein penetration have been previously reported in studies on bovine serum albumin [26,27] and acetylcholine receptor protein [33].

If, however, the area/phospholipid molecule is

altered by changing the polar headgroup the relationship between the area/molecule values and the $\Delta\pi_m$ and ΔA_m values is not obeyed. Moreover, the correlation between $\Delta\pi_m$ and ΔA_m, seen for the homologous series of phosphatidylcholines, is also lost. Thus, if a series of phospholipids were chosen at random, each having different polar headgroups and fatty acid compositions, an anomalous relationship between $\Delta\pi_m$ and ΔA_m would be observed. Indeed, this has been previously reported for bovine serum albumin [26,27] and spectrin [28]. In the light of these findings, many of the conclusions from monolayer studies based solely upon $\Delta\pi$ determinations may appear spurious. A direct relationship between $\Delta\pi$ and protein penetration may only be assumed for a given phospholipid [30]. Calculations can be made, however, which take into account the compressibility of the phospholipid and may provide a quantitative parameter for protein penetration [31].

Direct comparisons can be made between the present results and those from studies of cytochrome b_5-phospholipid vesicles. Our molecular area value of 790 Å^2 yields a radius of 16 Å for the membrane segment of cytochrome b_5, which compares favourably with values obtained by other workers. Thus, Vaz et al. [34] calculated the radius to be 16 Å from studies of rotational diffusion of the protein. Visser et al. [35] recorded a value of 17 Å using a gel filtration method. In comparison, the radius of the catalytic segment was found to be 11 Å by X-ray diffraction studies [36].

Also in excellent agreement with reports from vesicle studies is the DPPC/cytochrome b_5 ratio of 32. Dufourcq et al. [11] obtained a value of 35 by protein and lipid analyses. Bendzko and Pfeil [19] using differential scanning calorimetry calculated the minimum DPPC/cytochrome b_5 ratio to be 35 ± 4. Interestingly, assuming a radius of 16 Å, these workers calculated that 33 DPPC molecules would be required to form a annular ring around the lipophilic segment of cytochrome b_5. The agreement between the values we have found for phospholipid/protein ratios and those found in vesicle studies suggest that our proteolipid films are reasonably homogeneous. Indeed, we have found no difference between films which are formed on a subphase in which the protein is completely homogeneous (mechanically stirred) and those which are formed by our usual multiple-point injection technique.

The phospholipid/protein ratios (experimental and calculated) for the other phospholipids varied greatly. For DPPE and DPPA, the values were 60 and 84, respectively, whereas the values for phosphatidylcholines ranged from 88 in DDPC ($C_{10:0}$) to 27 in DSPC ($C_{22:0}$). This range of values may arise from a combination of two factors. For the phosphatidylcholine series, values of 32 and below probably indicate a stable, closely packed ring of phsopholipid molecules around the membrane segment of the protein (see above). Values above 32 corresponding to phosphatidylcholines with fatty acid chain-lengths less than C_{16} may arise from a lower stability of the protein in these monolayers of shorter phospholipids. The high ratios for DPPE and DPPA may reflect reduced interaction between the protein and the phospholipid polar headgroups. Electrostatic interactions between the choline headgroup of phosphatidylcholine and specific amino acid residues in the protein have been implicated in the binding of cytochrome b_5 to phosphatidylcholine vesicles [7]. Our findings may have implications for the architecture of membranes, i.e., membrane asymmetry. Cytochrome b_5 is thought to reside exclusively in the cytoplasmic side of endomembranes [37–39]. However, conclusive evidence is not yet available for the specific location of phosphatidylcholines in endomembranes [40]. The Langmuir monolayer technique could facilitate research in this area, particularly in the study of lamellophobic phospholipids, such as phosphatidylethanolamines [41], which do not readily form vesicles.

Recent studies on cytochrome b_5-phospholipid interactions have revealed two possible conformations for the protein. Cytochrome b_5 may exist in a tightly bound, non-transferable form or a loosely bound, transferable form [12,15,17,18]. From the molecular area determined here, it is very likely that the membrane segment of cytochrome b_5 assumes the 'helical hairpin' structure as described by Dailey and Strittmatter [15]. Furthermore, the methods used in the present study for maximal incorporation of cytochrome b_5 is very similar in approach to those used to achieve the tightly bound form of the protein (i.e., the cholate dial-

ysis method or the entrapment of protein during vesicle formation). Thus, our results would appear to contradict those of Takagaki et al. [18] who proposed that the tightly bound form of cytochrome b_5 has a membrane segment with a single-stranded helical structure.

Finally, our work shows clearly that cytochrome b_5 will not penetrate phospholipid monolayers compressed to more than 15 $mN \cdot m^{-1}$. At first sight, this is at variance with the studies [10–19] of cytochrome b_5 incorporation into vesicles, in which it has been inferred that membrane pressures lie in the range 30–35 $mN \cdot m^{-1}$, a conclusion based on comparison of the physicochemical properties of vesicles and monolayers [42,43]. From our studies and others, a DPPC monolayer compressed to 30 $mN \cdot m^{-1}$ has an area per headgroup of 52 $Å^2$. On the other hand, in a theoretical argument based on calculations from NMR data, Huang and Mason [44] show that in 200 Å diameter vesicles of egg phosphatidylcholine, the area per headgroup in the outer leaflet of the bilayer is 74 $Å^2$, because of the severe curvature of the membrane. The work of Sheetz and Chan [45] also demonstrates a much loosened structure for these vesicles. The vesicles used for cytochrome b_5 penetration studies were of this small size (a value of 250 Å diameter is quoted by Enoch et al, [46]), and therefore had relatively large area per phospholipid headgroup. Thus, it appears that when considering incorporation of proteins into membranes it is the 'free area' (cf. free volume in bulk materials) that is the important factor, with membrane pressure being of secondary importance.

Further evidence for this view is provided by Inoko [20] who showed that cytochrome b_5 was not incorporated into phospholipid lamellae unless the 'pretransition' temperature (34°C, as opposed to 41.4°C for the main melting transition) was exceeded. The pretransition is related to a change in the hydrocarbon chain packing density [47] which would again result in an increased area per headgroup at the higher temperatures. It should be noted also that on a pressure-area characteristic, such as Fig. 1a, the pretransition would occur in the steeply rising part of the curve, at a pressure higher than about 6 $mN \cdot m^{-1}$, the main liquid-gel transition. This is consistent with a maximum incorporation pressure of 15 $mN \cdot m^{-1}$ for our monolayers which corresponds to a headgroup area of 58 $Å^2$.

We conclude that monolayer experiments can reflect membrane processes if the area per phospholipid molecule is taken as the basis for comparison, particularly, if proteins such as cytochrome b_5 which span half of the native bilayer are being considered. We believe that, for any model membrane system it is the available area per headgroup which will determine protein penetration characteristics, and possibly other physiochemical properties. For example, small DPPC vesicles (which have a large available headgroup area) have a main transition temperature of 37°C, compared with 41.4°C for lamellae. A unique advantage of the Langmuir monolayer as a model system is the facility with which molecular area can be precisely controlled.

Because of the very low curvature in Langmuir monolayers, the effective area/phospholipid headgroup may be very similar to that in endoplasmic reticulum. Thus, in conjunction with vesicle studies, monolayers may provide much useful information concerning protein endomembrane interactions. It may be possible to solve some of the dilemmas concerning cytochrome b_5-membrane interactions, such as the effects of cholesterol (see Refs. 45 and 49). Moreover, now that methods are available for spreading protein-lipid vesicles directly onto an air/water interface [21,50], the possibility exists that monolayers, prepared from endogenous membranes, may be used to study the membrane specificity of cytochrome b_5 (see Refs. 37–39).

References

1 Mosbach, K. (1976) Methods Enzymol. 44, 1–7
2 Hill, H.A.O. and Higgins, I.J. (1981) Phil. Trans. R. Soc. Lond. Ser. A 302, 267–273
3 Gaines, G.L. (1965) Insoluble Monolayers at Lipid-Gas Interfaces, Interscience, New York
4 Phillips, M.C. and Chapman, D. (1968) Biochim. Biophys. Acta 163, 301–313
5 Van Deenen, L.L.M., Houtsmuller, U.M.T., De Haas, G. and Mulder, E. (1962) J. Pharm. Pharmacol. 14, 429–444
6 Von Jagow, G. and Sebald, W. (1980) Annu. Rev. Biochem. 49, 281–314
7 Strittmatter, P. and Dailey, H.A. (1982) in Membrane Transport (Martinosi, A.N., ed.), Vol. 1, pp. 71–82, Plenum Press, New York

8 Ito, A. and Sato, R. (1968) J. Biol. Chem. 243, 4923
9 Spatz, L. and Strittmatter, P. (1971) Proc. Natl. Acad. Sci. USA 68, 1042–1046
10 Strittmatter, P., Rogers, M.J. and Spatz, L. (1972) J. Biol. Chem. 247, 7188–7194
11 Dufourcq, J., Bernon, R. and Lussan, C. (1976) Biochim. Biophys. Acta 433, 252–263
12 Enoch, H.G., Fleming, P.J. and STrittmatter, P. (1979) J. Biol. Chem. 254, 6483–6488
13 Leto, T.L., Roseman, M.A. and Holloway, P.W. (1980) Biochemistry 19, 1911–1916
14 Poensgen, J. and Ullrich, V. (1980) Biochim. Biophys. Acta 596, 248–253
15 Dailey, H.A. and Strittmatter, P. (1981) J. Biol. Chem. 256, 3951–3955
16 Nordlund, J.R., Schmidt, C.F. Holloway, P.W. and Thompson, T.E. (1982) Biochemistry 21, 2820–2825
17 Takagaki, Y., Radhakrishnan, R., Gupta, C.M. and Khorana, H.G. (1983) J. Biol. Chem. 258, 9128–9135
18 Takagaki, Y., Radhakrishnan, R., Wirtz, K.W.A. and Khorana, H.G. (1983) J. Biol. Chem. 258, 9136–9142
19 Bendzko, P. and Pfeil, W. (1983) Biochim. Biophys. Acta 742, 669–676
20 Inoko, Y. (1980) Biochim. Biophys. Acta 599, 359–369
21 Verger, R. and Pattus, F. (1982) Chem. Phys. Lipids 30, 189–227
22 Strittmatter, P., Fleming, P., Connors, M. and Corcoran, D. (1978) Methods Enzymol. 52, 97–101
23 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
24 Salacinski, P.R.P., Maclean, C., Sykes, J., Clement-Jones, V.V. and Lowry, P. (1981) Anal. Biochem. 117, 136–146
25 Blight, L., Cumper, C.W.N. and Kyte, V. (1965) J. Colloid. Sci. 20, 393–399
26 Quinn, P.J. and Dawson, R.M.C. (1969) Biochem. J. 115, 65–75
27 Quinn, P.J. and Dawson, R.M.C. (1970) Biochem. J. 199, 21–25
28 Mayer, L.D., Nelsestuen, G.L. and Brockman, H.K. (1983) Biochemistry 22, 3156–322
29 Mombers, C., De Gier, J., Demel, R.A. and Van Deenen, L.L.M. (1980) Biochim. Biophys. Acta 603, 52–62
30 Pattus, F., Martinez, M.C., Dargent, B., Cavard, D., Verger, C. and Lazdunski, C. (1980) Biochemistry 22, 5698–5703
31 Van Zoelen, E.J.J., Zwaal, R.F.A., Reuvers, F.A.M., Demel, R.A. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 464, 482–492
32 Bougis, P., Rochat, H., Pieroni, G. and Verger, R. (1981) Biochemistry 20, 4915–4920
33 Popot, J.L., Demel, R.A., Sobel, A., Van Deenen, L.L.M. and Changeux, J.P. (1977) C.R. Acad. Sci. Paris Ser. D 285, 1005–1008
34 Vaz, W.L.C., Austin, R.M. and Vogel, H. (1979) Biophys. J. 26, 415–426
35 Visser, L., Robinson, N.C. and Tanford, C. (1975) Biochemistry 14, 1194–1199
36 Matthews, F.S., Argos, P. and Levine, M. (1971) Cold Spring Harbour Symp. Quant. Biol. 36, 387–395
37 Remacle, J. (1980) Biochim. Biophys. Acta 597, 564–576
38 Elhammer, A., Peterson, E. and Dallner, G. (1983) Biochim. Biophys. Acta 730, 76–84
39 Okada, Y., Frey, A.B., Guenther, T.M., Oesch, F., Sabatini, D.D. and Kreibich, G. (1982) Eur. J. Biochem. 122, 393–402
40 Op den Kamp, J.A.F. (1979) Annu. Rev. Biochem. 48, 47–71
41 Stier, A., Finch, A.E., Greinert, R., Hohne, M. and Muller, R. (1982) in Liver and Aging (Kitani, K., ed.), pp. 3–17, Elsevier Biomedical Press, Amsterdam
42 Demel, R.A., Guertz Van Kessel, W.S.M., Zwaal, R.F.A., Roelofsen, B. and Van Deenen, L.L.M. (1975) Biochim. Biophys. Acta 406, 97–107
43 Blume, A. (1979) Biochim. Biophys. Acta 557, 32–44
44 Huang, C. and Mason, J.T. (1978) Proc. Natl. Acad. Sci. USA 75, 308–310
45 Sheetz, M.P. and Chan, S.I. (1972) Biochemistry 11, 4573–4581
46 Enoch, H.G., Flemming, P.J. and Strittmatter, P. (1972) J. Biol. Chem. 252, 5656–5660
47 Brady, C.W. and Fein, D.B. (1977) Biochim. Biophys. Acta 464, 249–259
48 Roseman, M.A., Holloway, P.W. and Calacro, M.A. (1978) Biochim. Biophys. Acta 507, 552–556
49 Tajima, S. and Sato, R. (1979) Biochim. Biophys. Acta 550, 357–361
50 Schindler, H. (1979) Biochim. Biophys. Acta 555, 316–336