Biochimica et Biophysica Acta, 436 (1976) 283–294

BBA 77321

LIPID-PROTEIN INTERACTIONS IN MEMBRANE MODELS

FLUORESCENCE POLARIZATION STUDY OF CYTOCHROME b_5-PHOSPHOLIPIDS COMPLEXES

JEAN-FRANÇOIS FAUCON, JEAN DUFOURCQ, CLAUDE LUSSAN and ROLAND BERNON

Centre de Recherche Paul Pascal, Domaine Universitaire, 33405, Talence (France)

(Received October 29th, 1975)

SUMMARY

According to previous authors, cytochrome b_5 ,when extracted from bovine liver by a detergent method, is called cytochrome *d*-b_5. On the other hand, the protein obtained after trypsin action, which eliminates an hydrophobic peptide of about 54 residues, is called cytochrome *t*-b_5.

Fluorescence polarization of the dansyl phosphatidylethanolamine probe inserted into phospholipid vesicles is very sensitive to the binding of proteins, and so is a useful method to study lipid-protein interactions.

The chromophore mobility, *R*, decreases markedly when dipalmitoyl phosphatidylcholine vesicles are incubated with cytochrome *d*-b_5, whereas *R* does not change for cytochrome *c* and cytochrome *t*-b_5. This can be interpreted as a strengthening of the bilayer, only due to the interaction of the hydrophobic peptide tail.

Interaction of dipalmitoyl phosphatidylcholine vesicles with cytochrome *d*-b_5 occurs either below or above the melting temperature of the aliphatic chains (41 °C). Even for a high protein to lipid molar ratio (1 molecule of protein for 40 phospholipid molecules), the melting temperature is apparently unaffected.

Phosphatidylserine and phosphatidylinositol do not interact at pH 7.7 with cytochrome *d*-b_5, because electrostatic forces prevent formation of complexes. At low pH, the interaction with the protein occurs, but the binding is mainly of electrostatic nature.

INTRODUCTION

Pure lipid systems have widely contributed to a better understanding of the structure and properties of biological membranes. However, a new step in this field should be reached by studying model systems composed of the two essential components of natural membranes: lipids and proteins. Until now, some work has been done in this way, but mainly on interactions of phospholipids and extrinsic or soluble proteins and synthetic polypeptides [1–3]. Only a few results have been obtained on intrinsic proteins firmly anchored in the lipid matrix [4–6].

Cytochrome b_5 from the respiratory chain of the liver endoplasmic reticulum was first studied by Strittmatter et al. [7] and Enomoto and Sato [8]. They showed that the detergent-extracted protein (cytochrome d-b_5) interacts with natural microsomes. On the other hand, the tryptic hydrophilic core (cytochrome t-b_5) cannot bind to the membrane. Interaction of cytochrome b_5 with pure phosphatidylcholines was better understood after the work of other authors [9–13].

In the above-mentioned experiments, we have characterized the complexes, which are hollow vesicles, the proteins being on the outer face, and shown that the lipid to protein molar ratio can be controlled. Moreover, by studying the intrinsic fluorescence of cytochrome b_5, we showed that tryptophan residues of the hydrophobic tail are deeply embedded in the aliphatic chains region of the lipid bilayer [14].

We have already shown that the polarization of fluorescence of the dansyl phosphatidylethanolamine probe is sensitive to order-disorder transition of phospholipid aliphatic chains [15, 16] and to the effect of various cations on neutral or charged phospholipid vesicles [17].

In this paper, fluorescence polarization of the above-named probe is used in order to detect interactions of cytochrome b_5 with various phospholipids found in liver microsomes, and to study their effects on the lipid bilayer organization.

MATERIALS AND METHODS

Reagents. Gel filtration products were obtained from Pharmacia, deoxycholate from Sigma, Triton X-100 from Eastman Kodak, trypsin from Boehringer GmbH and liquid scintillation products from Intertechnique. [^{14}C]phosphatidylcholine was purchased from New England Nuclear, dipalmitoyl phosphatidylcholine from Nutritional Biochemicals Corporation and cytochrome c from Sigma.

Preparation of cytochrome d-b_5 and cytochrome t-b_5. Cytochrome d-b_5 was extracted from bovine liver microsomes, by means of detergents, following a method similar to that used by Ozols [18], and published elsewhere [11].

The tryptic core, cytochrome t-b_5, was prepared by action of the enzyme directly on pure cytochrome d-b_5 [11]. Its characteristics were identical to those of cytochrome t-b_5 obtained by other authors who used the trypsin on microsomes [19].

Apocytochrome d-b_5 was prepared according to Strittmatter and Ozols [20] by an acetone/acid extraction of the heme.

Preparation of the hydrophobic tail. Trypsin and cytochrome d-b_5 were incubated together (400 mol protein/mol trypsin) at room temperature during 18 h in 20 mM Tris/acetate buffer (pH 8.9)/1 mM EDTA. After gel filtration on Sephadex G-50, aggregates of the hydrophobic tail were eluted on the void volume while cytochrome t-b_5 was eluted as previously described [11].

Extraction and purification of natural phospholipids. Phosphatidylinositol was extracted and purified from baker's yeast following the method of Trevelyan [21]. Phosphatidylserine was prepared according to Rouser et al. [22].

Preparation of reconstituted systems. Phosphatidylserine and phosphatidylinositol dispersions labelled with 1 % dansyl phosphatidylethanolamine [16] were sonicated in 20 mM Tris/acetate buffer (pH 7.7)/0.2 mM EDTA under nitrogen at 0 °C for 15 min. For dipalmitoyl phosphatidylcholine, sonication was done at 45 °C. Large undispersed particles and titanium from the sonicator were removed by cen-

trifugation at 100 000 $\times g$ for 1 h. Then filtration on Sepharose 4B followed to isolate single-shelled vesicles. The phosphatidylcholine concentration was determined from ^{14}C activity of incorporated labelled phosphatidylcholine. In all cases, the phospholipid concentration was about 0.15 mg/ml. Reconstituted systems were prepared in situ by adding the protein solution to vesicles, the mixture being incubated as indicated in the figure legends.

Apparatus. All centrifugation experiments were carried out with an MSE 65 high speed centrifuge. The ultraviolet and visible spectra of cytochromes were recorded with a Cary 16 spectrophotometer. Radioactivity measurements were done on an SL 30 Intertechnique multichannel liquid scintillation spectrometer. The sonication was done with an Annemasse 150 T sonicator.

Fluorescence polarization and excited state lifetime determinations were done with apparatus built in this laboratory.

The isoelectric point of cytochrome *d*-b_5 oligomers was measured by electrofocusing, with ampholines pH range 3–6. The obtained value, pI = 4.8, is in good agreement with that measured by Hulquist et al. [23] for cytochrome b_5 from human erythrocytes, pI = 4.9.

RESULTS

Interactions of cytochrome d-b_5 with dipalmitoyl phosphatidylcholine

The degree of polarization P is a function of the excited state lifetime τ of the chromophore, and of the microviscosity of its environment. Weber [24] has recently proposed a general theory of fluorescence depolarization by anisotropic brownian rotations, applied to membrane models by Shinitzky et al. [25]. He showed that, in first approximation, the function

$$R = \frac{1}{6\tau}\left[\frac{P^{-1}-\frac{1}{3}}{P_o^{-1}-\frac{1}{3}}-1\right]$$

can be considered as varying as the rotation rate of the probe, P_o being the limiting degree of polarization. For dansyl phosphatidylethanolamine, we have already determined P_o and found it equal to 0.37 [16].

It is well known that the order-disorder transition temperature of dipalmitoyl phosphatidylcholine aliphatic chains is 41 °C. This phospholipid interacts with cytochrome *d*-b_5, as we have recently shown [11], either below or above 41 °C. Fig. 1 gives the values of the excited state lifetime of dansyl phosphatidylethanolamine inserted in dipalmitoyl phosphatidylcholine single-shell vesicles versus the protein to lipid molar ratio. Only a very weak decrease of τ is observed, less than 10 %. So, in the following calculations, we kept it constant, at τ = 14.5 ns. From this result and the measured P values, the R function was calculated versus the protein to lipid molar ratio for different proteins: cytochrome *d*-b_5, cytochrome *t*-b_5 and cytochrome *c* (Fig. 2). First of all, cytochrome *c* and cytochrome *t*-b_5 do not markedly change the R value. On the other hand, the incubation of vesicles with cytochrome *d*-b_5 is followed by an important decrease of R, when the mixture is incubated either below or above 41 °C. However, these two curves are very different, the effect on R being stronger when the sample is incubated at 43 °C.

The fluorescence intensity of the dansyl phosphatidylethanolamine probe is plotted on Fig. 3 versus concentrations of cytochrome *d*-b_5, cytochrome *t*-b_5, and cytochrome *c*. It can be noticed that the intensity decreases in the same way for the

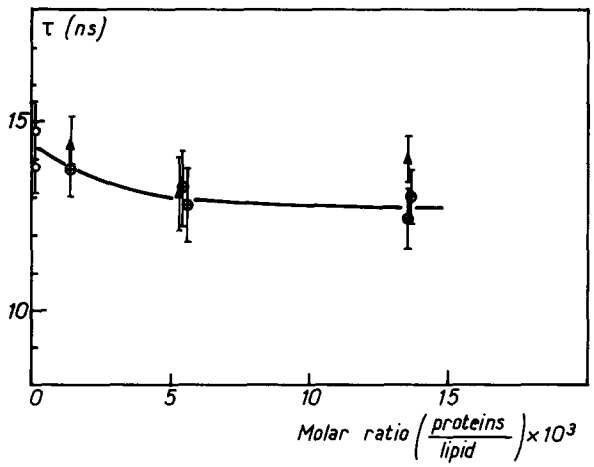

Fig. 1. Excited state lifetimes of the dansyl phosphatidylethanolamine probe inserted into dipalmitoyl phosphatidylcholine vesicles (see Methods). Mixtures were incubated at 20 °C for30 min. ○, dipalmitoyl phosphatidylcholine/cytochrome *d*-b_5 mixture; ▲, dipalmitoyl phosphatidylcholine/cytochrome *c* mixture.

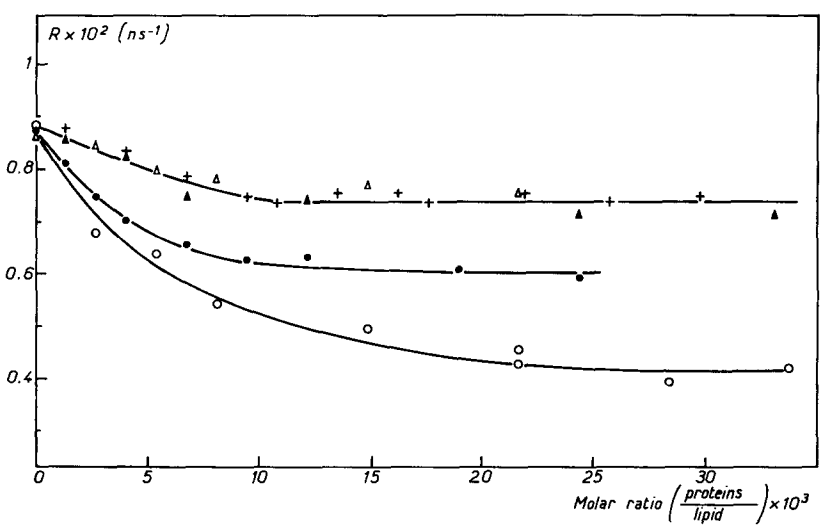

Fig. 2. Effect of protein concentration on the mobility *R* of the dansyl phosphatidylethanolamine probe inserted into dipalmitoyl phosphatidylcholine vesicles (see Methods). The lifetime of the chromophore excited state was 14.5 ns. The incubation time with protein was 30 min. △, cytochrome *c* incubated with vesicles at 43 °C; ▲, cytochrome *c* incubated with vesicles at 20 °C; +, cytochrome *t*-b_5 incubated with vesicles at 20 °C; ○, cytochrome *d*-b_5 incubated with vesicles at 43 °C; ●, cytochrome *d*-b_5 incubated with vesicles at 20 °C.

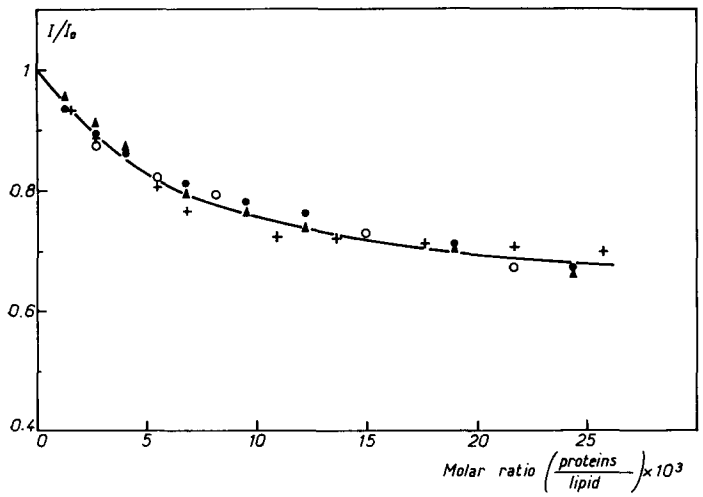

Fig. 3. Protein concentration effect on the fluorescence intensity of the dansyl phosphatidylethanolamine probe inserted into vesicles of dipalmitoyl phosphatidylcholine. The incubation times with protein were 30 min. △, cytochrome *c* incubated with vesicles at 43 °C; ▲, cytochrome *c* incubated with vesicles at 20 °C; +, cytochrome *t*-b_5 incubated with vesicles at 20 °C; ○, cytochrome *d*-b_5 incubated with vesicles at 43 °C; ●, cytochrome *d*-b_5 incubated with vesicles at 20 °C. I_0 is the intensity of the labelled vesicles without proteins, and I is the intensity when proteins are added.

three different proteins. This is not in contradiction with the lifetime measurements, since the variation of fluorescence intensity is not, in this case, directly proportional to the lifetime, because an inner filter effect occurs when proteins are added. Indeed, the heme absorbs notably at 366 nm (excitation wavelength) and slightly above 450 nm (emission wavelength). In order to eliminate this effect, the heme was removed in two different ways. (i) Apocytochrome *d*-b_5, prepared as described under Methods, binds to dipalmitoyl phosphatidylcholine, and has almost the same effect on P as cytochrome *d*-b_5 itself, but the observed intensity does not vary at all. (ii) Trypsin action on reconstituted systems followed by gel filtration on Sepharose 4B eliminates the hydrophilic moiety bearing the heme, cytochrome *t*-b_5 [11]. This removal leads to the same P value as obtained on the whole system, dipalmitoyl phosphatidylcholine cytochrome *d*-b_5.

This latter result also proves that the perturbation of the probe is only due to the peptide remaining bound to the phospholipid bilayer, and, as we know, this peptide is the hydrophobic tail of the protein [14]. This is confirmed by direct-interaction of partially purified hydrophobic peptide with dipalmitoyl phosphatidylcholine, which is followed by a similar increase of P compared to the whole cytochrome *d*-b_5.

Effect of cytochrome d-b_5 on the phase transition of dipalmitoyl phosphatidylcholine

Since cytochrome *d*-b_5 interacts strongly with dipalmitoyl phosphatidylcholine, it can be thought that this interaction can have an effect on the phase transition of this lipid (41 °C). Fig. 4 shows that even for a high concentration of protein (about one cytochrome *d*-b_5 for 40 phospholipid molecules) the shape of the curve log R =

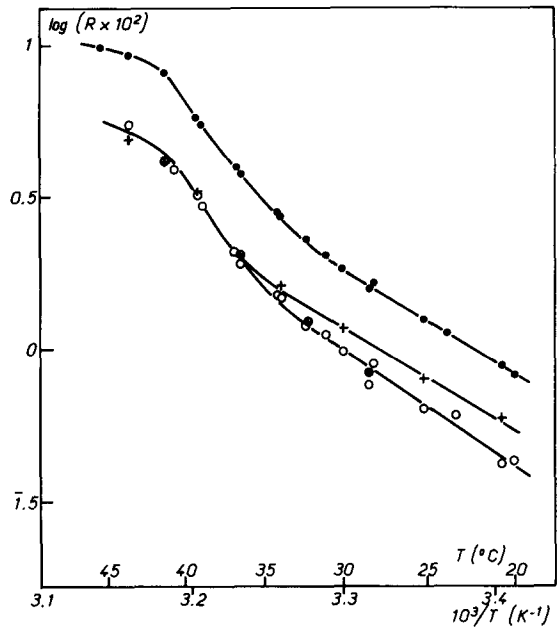

Fig. 4. Temperature effect on the mobility of dansyl phosphatidylethanolamine probe inserted into dipalmitoyl phosphatidylcholine vesicles. ●, dipalmitoyl phosphatidylcholine vesicles alone; ○, cytochrome *d*-b_5 (1 mol for 40 phospholipid molecules) incubated at 43 °C; +, cytochrome *d*-b_5 (1 mol for 40 phospholipid molecules) incubated at 20 °C, temperature increase; ⊕, cytochrome *d*-b_5 (1 mol for 40 phospholipid molecules) incubated at 20 °C, temperature decrease. *R* has been calculated assuming that the chromophore excited state lifetime varies as the fluorescence intensity.

f(1/*T*) is very similar to that of dipalmitoyl phosphatidylcholine alone. When the phospholipid/cytochrome *d*-b_5 mixture is incubated at 43 °C, then centrifuged to remove unbound protein, the effect of temperature is reversible and, apparently, the melting temperature is not affected. When the mixture is incubated at 20 °C, without removal of free protein, the reversibility is not observed. At higher temperatures the *R* values are larger than in the preceding case, but when temperature rises above 41 °C, the incubation conditions of the former experiment being recovered, the two curves become identical. This result can be compared to those obtained in Fig. 1, where the effect on *R* is smaller when the incubation is carried out at 20 °C.

All the above results, obtained with the dansyl phosphatidylethanolamine, probe, have been confirmed by measuring the degree of polarization of perylene, another very different chromophore.

Interaction of cytochrome d-b_5 and cytochrome c with phosphatidylserine and phosphatidylinositol vesicles

Liver endoplasmic reticulum contains phosphatidylcholine, and also charged phospholipids like phosphatidylserine and phosphatidylinositol. So it is interesting to study the possibilities of interaction of these two phospholipids with cytochrome *d*-b_5.

The degree of polarization *P* of the probe is not affected by addition of cyto-

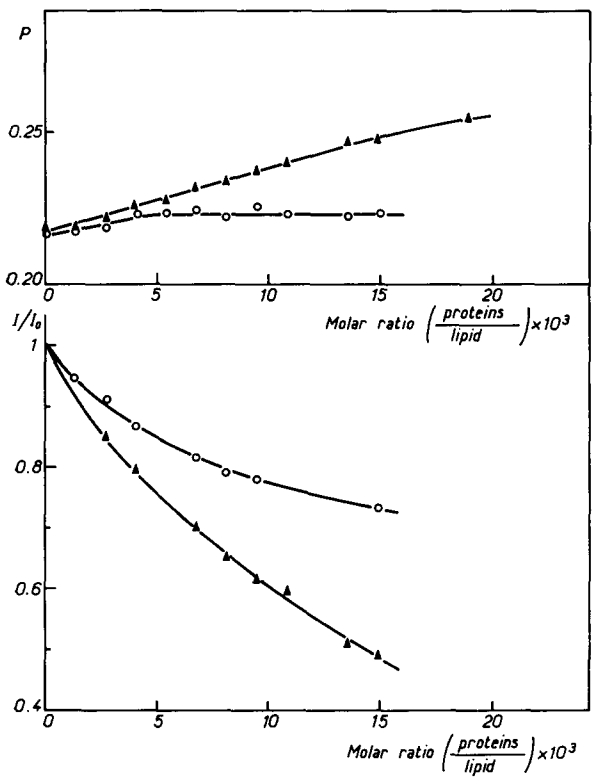

Fig. 5. Effect of protein concentration on the degree of polarization P and the fluorescence intensity of the dansyl phosphatidylethanolamine probe inserted into phosphatidylserine vesicles. Mixtures were incubated at 20 °C for 30 min. ▲, phosphatidylserine/cytochrome *c*; ○, phosphatidylserine/cytochrome *d*-b_5. I_0 is the intensity of labelled vesicles without proteins, and I is the intensity when proteins are added.

chrome *d*-b_5 to phosphatidylserine vesicles, as seen in Fig. 5a. On the other hand, addition of cytochrome *c* leads to a large and regular increase of P. Fig. 5b shows the fluorescence intensity decrease of the probe upon addition of these two proteins. For cytochrome *d*-b_5, the effect is exactly the same as that observed in Fig. 3 and can be attributed to an inner filter effect. Addition of cytochrome *c* leads to a larger intensity decrease, which probably arises from energy transfer between the heme and the dansyl chromophore. This shows that, in the latter case, the distance between the probe and the heme is shorter than in the experiments with cytochrome *d*-b_5. The observed effects on P and the fluorescence intensity can be attributed to the fact that, at the experimental pH (7.7), phosphatidylserine ($pK \simeq 4$) [26] and cytochrome *c* ($pI = 10.7$) [27] are oppositely charged, and so do interact, whereas electrostatic repulsion would prevent interaction for cytochrome *d*-b_5 ($pI = 4.8$). Similar results have been obtained with phosphatidylinositol, either for P or fluorescence intensity variation (Fig. 6).

In order to change the net charge of both components, reconstitution experiments have been carried out by varying the pH in the range 4–8. For phosphatidyl

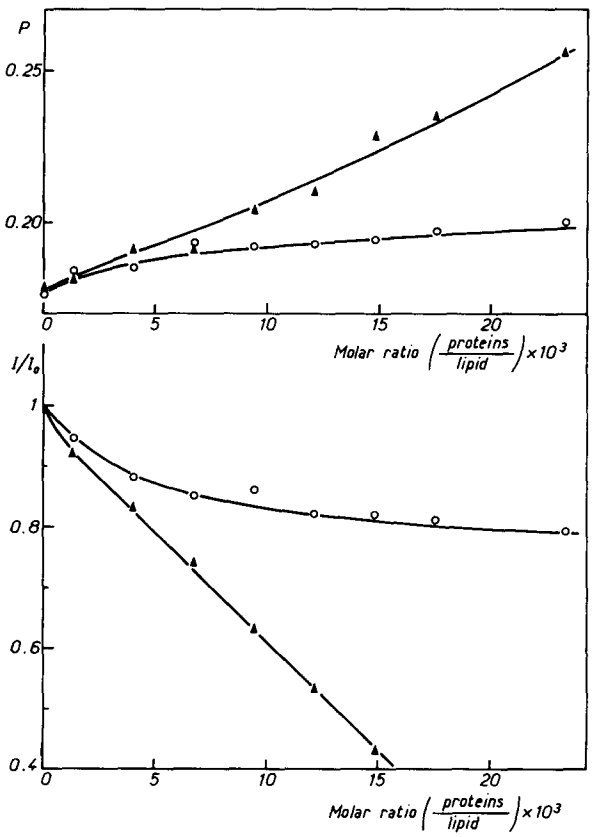

Fig. 6. Effect of protein concentration on the degree of polarization P and the fluorescence intensity of the dansyl phosphatidylethanolamine probe inserted into phosphatidylinositol vesicles. Mixtures were incubated at 20 °C for 30 min. ▲, phosphatidylinositol/cytochrome c; ○, phosphatidylinositol/cytochrome d-b_5. I_0 is the intensity of labelled vesicles without proteins, and I is the intensity when proteins are added.

serine vesicles alone, as seen in Fig. 7, P remains constant from pH 8 to pH 5, then increases below this pH value. This observed change can probably be related to a decrease of the net negative charge of the phospholipid, followed by a strengthening of the bilayer, when the pH value approaches that of the pK of phosphatidylserine. For the phosphatidylserine/cytochrome c mixture, the variation of P with pH is similar to that observed on the pure phospholipid, but the P values are much higher. This indicates that a strong interaction occurs over the whole pH range, which agrees with results obtained by Vanderkooi et al. [28] on cytochrome c-cardiolipin interactions. The phosphatidylserine/cytochrome d-b_5 mixture gives a very different shape P versus pH curve (Fig. 7). Around pH 7, the obtained P value is very close to that of pure phosphatidylserine vesicles, as described in Fig. 5. As pH is lowered from 7 to 5, a drastic increase of P indicates that an interaction occurs. This can be roughly correlated to the variation of the net charge of the protein at the neighbourhood of its isoelectric point. However, it is necessary to point out that at at low pH unfolding of

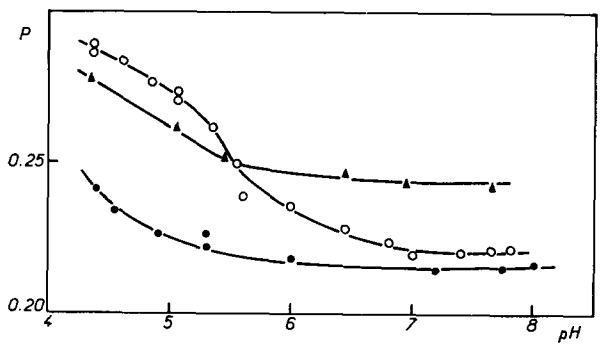

Fig. 7. Effect of pH on the degree of polarization P of the dansyl phosphatidylethanolamine probe inserted in phosphatidylserine vesicles. Mixtures were incubated at 20 °C for 30 min. ●, phosphatidylserine vesicles alone; ▲, phosphatidylserine vesicles/cytochrome c (1 protein for 75 phospholipid molecules); ○, phosphatidylserine vesicles/cytochrome d-b_5 (1 protein for 75 phospholipid molecules).

the protein occurs, since a large change in intensity and frequency of the Soret band at 413 nm is detected, starting at pH 5, and centered at pH 4. It is also interesting to notice that an ionic strength increase to 1.5 M KCl is followed by an important decrease of P, the final value being very close to that observed for pure phosphatidylserine at this pH. This can be interpreted as the dissociation of the complex. The same effect is observed with the cytochrome c-phosphatidylserine system.

Similar results are obtained in the case of phosphatidylinositol, as seen in Fig. 8. P does not change at all in the pH range investigated either for phosphatidylinositol alone or for its complex with cytochrome c; this agrees with the fact that the pK of the phospholipid is between 2 and 3 [29]. At low pH, cytochrome d-b_5 forms a complex in a similar way, as previously shown in Fig. 7 for the case of phosphatidylserine.

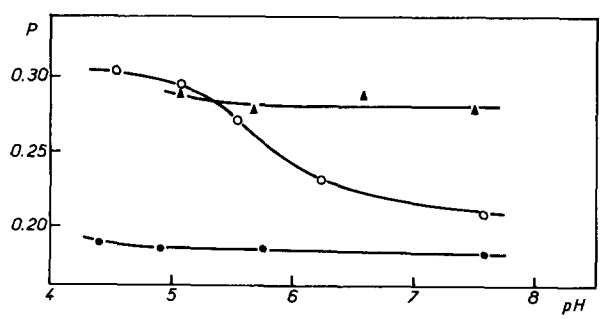

Fig. 8. Effect of pH on the degree of polarization P of the dansyl phosphatidylethanolamine probe inserted in phosphatidylinositol vesicles. Mixtures were incubated at 20 °C for 30 min. ●, phosphatidylinositol alone; ▲, phosphatidylinositol/cytochrome c (1 protein for 35 phospholipid molecules); ○, phosphatidylinositol/cytochrome d-b_5 (1 protein for 35 phospholipid molecules).

DISCUSSION

On interaction of cytochrome b_5 with dipalmitoyl phosphatidylcholine, a decrease of the mobility of the dansyl phosphatidylethanolamine probe is observed, which could be due to a strengthening of the bilayer. By using fluorescence polarization, a similar interpretation was proposed by Barrat et al. [30] on the interaction of apoprotein from high density porcine lipoprotein with dimyristoyl phosphatidylcholine.

However, quantitative structural conclusions from probe studies are always very difficult to ascertain. When lipid-protein systems are investigated, the main difficulties are the following: (i) the probe can induce either a general or a local perturbation of the bilayer; (ii) the respective affinities of the probe and of lipids for the protein can be different; (iii) the protein binding, by changing the bilayer structure can induce a change in the probe position.

Experimentally, the use of small probe concentrations allows the avoidance of a general perturbation of the bilayer since, as has been shown [16], the phase transition temperatures of phospholipids are unaffected under such conditions. On the contrary, a local perturbation of the probe envirionment probably occurs, but it is not sufficient to account for all the obtained results, which are in total agreement with previous ones obtained by gel filtration [11] and intrinsic fluorescence [14] experiments. Moreover, it must be pointed out that fluorescence polarization of two very different probes, dansyl phosphatidylethanolamine and perylene, gives similar results on interactions of cytochrome b_5 with dipalmitoyl phosphatidylcholine. About the possible probe affinity for the protein, a limiting case, the transfer of the dansyl phosphatidylethanolamine chromophore to the protein in solution, can be ruled out, since this could not be detected after gel filtration experiments, which remove the free cytochrome *d*-b_5. So, if specific probe-protein interactions exist, they occur only within the phospholipid bilayer. Finally, whatever the probe localization may be within the bilayer, the detection of the phase transition of dipalmitoyl phosphatidylcholine interacting with cytochrome b_5 proves that the probe is sensitive to what happens in the bulk phospholipids.

Consequently, if it is not possible to attribute with certainty the observed variations of the probe mobility to structural modifications of the bilayer, it is sure that fluorescence polarization of the dansyl phosphatidylethanolamine probe is at least a suitable method for the detection of cytochrome b_5-phospholipid interactions.

In this study, the phase transition temperature of dipalmitoyl phosphatidylcholine in reconstituted systems is apparently not changed even at high protein to lipid molar ratios, which is in agreement with previous results obtained by intrinsic fluorescence of cytochrome b_5 [14]. So it seems that a general perturbation of the bilayer can be ruled out, since it should lead to a change in the melting temperature. The results obtained by NMR [11] on reconstituted systems, which show that most of the phospholipids are dynamically unperturbed, strongly support this conclusion. This agrees with the model for hydrophobic lipid-protein interactions, which was first proposed by Jost et al. [31] and used by Dehlinger et al. [32] for interpreting ESR experiments on cytochrome b_5 bound to microsomal lipids. Papahadjopoulos et al. [33], studying different kinds of lipid/protein mixtures by differential scanning calorimetry, reached the same conclusion for hydrophobic interactions.

The difference observed on the probe mobility R when incubating mixtures of dipalmitoyl phosphatidylcholine and cytochrome d-b_5 below or above the melting temperature can be explained (i) by a weaker interaction when the phospholipid is in crystalline state, due to the difficulty of the hydrophobic tail to penetrate within the phospholipid bilayer and (ii) by a kinetic effect, the binding rate being slower when the phospholipid chains are in a rigid state as it has been proposed by Pownall et al. [34] from requirement of lipid fluidity for the formation of lipoproteins. Binding of the protein studied by gel filtration on the same system versus temperature [11] supports the second possibility. Mollay and Kreil have shown that the binding rate of melittin to phosphatidylcholines is strongly dependent on the bilayer fluidity [35].

In the microsomal membrane containing phosphatidylserine and phosphatidylinositol, it is interesting to notice that, at physiological pH, no binding occurs between cytochrome d-b_5 and the negatively charged phospholipids, while binding exists with phosphatidylcholine. This would have some far-reaching implications on the structure of the natural membrane. However, at low pH it is possible to obtain complexes between cytochrome d-b_5 and phosphatidylserine or phosphatidylinositol, but these complexes behave in a very similar way to those of cytochrome c. They are mainly due to electrostatic forces, and the hydrophobic tail does not interact with the lipids in the same way as in the case of binding of cytochrome d-b_5 to phosphatidylcholines [10, 14] or microsomes [7, 8].

ADDENDUM

Feinstein et al. [36] recently studied the degree of polarization of perylene inserted into phosphatidylserine bilayers in the presence of cytochrome b_5. They observed no effect on the probe mobility due to the presence of the protein. This result is totally in agreement with those we obtained with the same system and dansyl phosphatidylethanolamine as chromophore at physiological pH. However, our interpretation is totally different, since we conclude that there is no formation of any complex in agreement with intrinsic fluorescence results [14]. On the contrary, Feinstein et al., making the assumption that binding occurs and that the probe is statistically distributed, conclude that cytochrome b_5 does not change the microviscosity of bulk phospholipids.

ACKNOWLEDGEMENTS

We thank F. Hare for fluorescence lifetime measurements, Mr O. Babagbeto for his assistance in protein extraction and Mrs J. Favede for purification of natural phospholipids.

REFERENCES

1 Shechter, T., Gulik-Krzywicki, T., Azerad, R. and Gros, C. (1971) Biochim. Biophys. Acta 241, 431–442
2 Kimelberg, H. K. and Papahadjopoulos, D. (1971) J. Biol. Chem. 246, 1142–1148
3 Butler, K. W., Hanson, A. W., Smith, I. C. P. and Schneider, H. (1973) Can. J. Biochem. 51, 980–989
4 Vanderkooi, G. (1974) Biochim. Biophys. Acta 344, 307–345

5 Segrest, J. P., Gulik-Krzywicki, T. and Sardet, C. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3294–3298
6 Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G. and Metcalfe, J. C. (1974) Proc. Natl. Acad. Sci. U.S. 71, 622–626
7 Strittmatter, P., Rogers, M. J. and Spatz, L. (1972) J. Biol. Chem. 247, 7188–7194
8 Enomoto, K. and Sato, R. (1973) Biochem. Biophys. Res. Commun. 51, 1–7
9 Sullivan, M. R. and Holloway, P. W. (1973) Biochem. Biophys. Res. Commun. 54, 808–815
10 Dufourcq, J., Bernon, R. and Lussan, C. (1974) C. R. Acad. Sci. Serie D Paris 278, 2565–2568
11 Dufourcq, J., Bernon, R. and Lussan, C. (1976) Biochim. Biophys. Acta, in the press
12 Robinson, N. C. and Tanford, C. (1975) Biochemistry 14, 369–378
13 Rogers, M. J. and Strittmatter, P. (1975) J. Biol. Chem. 250, 5713–5718
14 Dufourcq, J., Faucon, J. F., Lussan, C. and Bernon, R. (1975) FEBS Lett. 57, 112–116
15 Lussan, C. and Faucon, J. F. (1971) FEBS Lett. 19, 186–188
16 Faucon, J. F. and Lussan, C. (1973) Biochim. Biophys. Acta 307, 459–466
17 Lussan, C. and Faucon, J. F. (1974) Biochim. Biophys. Acta 345, 83–90
18 Ozols, J. (1974) Biochemistry 13, 426–434
19 Ozols, J. and Strittmatter, P. (1964) J. Biol. Chem. 239, 1018–1023
20 Strittmatter, P. and Ozols, J. (1966) J. Biol. Chem. 241, 4787–4792
21 Trevelyan, W. E. (1967) J. Lipid Res. 8, 281–282
22 Rouser, G., Kritchevsky, G., Heller, D. and Lieber, E. (1963) J. Am. Oil Chem. Soc. 40, 425–454
23 Hultquist, D. E., Dean, R. T. and Douglas, R. H. (1974) Biochem. Biophys. Res. Commun. 60, 28–34
24 Weber, G. (1971) J. Chem. Phys. 55, 2399–2407
25 Shinitzky, M., Dianoux, A. C., Gitler, C. and Weber, G. (1971) Biochemistry 10, 2106–2113
26 Abrahamson, M. B., Katzman, R. and Gregor, H. P. (1964) J. Biol. Chem. 239, 70–75
27 Theorell, H. and Akesson, A. (1941) J. Am. Chem. Soc. 63, 1804–1811
28 Vanderkooi, J., Erecinska, M. and Chance, P. (1973) Arch. Biochem. Biophys. 154, 219–229
29 Wills, M. C., Tinker, D. O. and Pinteric, L. (1971) Biochim. Biophys. Acta 241, 483–493
30 Baratt, M. D., Badley, R. A., Leslie, R. B., Morgan, C. G. and Radda, G. K. (1974) Eur. J. Biochem. 48, 595–601
31 Jost, P. C., Griffith, O. H., Capaldi, R. A. and Vanderkooi, G. (1973) Proc. Natl. Acad. Sci. U.S. 70, 480–484
32 Dehlinger, P. J., Jost, P. C. and Griffith, O. Y. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2280–2284
33 Papahadjopoulos, D., Moscarello, M., Eylar, E. H. and Isac, T. (1975) Biochim. Biophys. Acta 401, 317–335
34 Pownall, H. J., Morrisett, J. D., Sparrow, J. T. and Gotto, A. M. (1974) Biochem. Biophys. Res. Commun. 60, 779–786
35 Mollay, C. and Kreil, G. (1973) Biochim. Biophys. Acta 316, 196–203
36 Feinstein, M. P., Fernandez, S. M. and Sha'afi, R. I. (1975) Biochim. Biophys. Acta 413, 354–370