Eur. J. Biochem. *48*, 595–601 (1974)

The Interaction of Apoprotein from Porcine High-Density Lipoprotein with Dimyristoyl Phosphatidylcholine

Electron Spin Resonance and Fluorescent Probe Studies

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(Received June 5, 1974)

1. The interactions between the lipid and protein in complexes of dimyristoyl lecithin and porcine apoprotein were studied using electron spin resonance and fluorescent probes, dissolved in the lipid and covalently bonded to the protein.

2. The chain-melting transition of dimyristoyl lecithin is broadened on complexing with porcine apoprotein, indicating that the cooperative interaction between the lipid hydrocarbon chains is modified in the complex.

3. The broadening of the chain-melting transition is seen to be greatest by probes located at or near the glycerol region of the phospholipid.

4. Labels covalently bound to the protein experience only a small perturbation from the lipid chain-melting transition.

5. The results are interpreted as being consistent with a model for the complex, consisting of a lipid-bilayer, partially coated with protein and with some interdigitation of the protein.

There have been a number of studies using spin-labelling and fluorescent labelling to investigate the interactions between lipids and proteins in intact and reassembled serum lipoproteins. In these studies, the labels have been attached to the protein moiety [1,2], or introduced into the lipid regions either by sonication [3] or by addition [4–6]. The results of these studies have been rather inconclusive mainly because of the complex mixtures of lipids present in natural lipoproteins.

In this report we describe the application of spin labelling and fluorescence polarisation to study the interactions between apoprotein of porcine high-density lipoprotein and pure synthetic dimyristoyl lecithin. The probes were dissolved in the lipid and covalently-linked to the protein. The work was performed with the objective of investigating the effect of formation of a lipoprotein complex on the chain-melting transition of the phospholipid in a well-defined system [7]. The results, obtained from probes at different locations in the lipid and protein, have been used to attempt to obtain information about the relative positions of the lipid and protein in the complex.

Abbreviation. Dansyl, 5-dimethylaminonaphthalene-1-sulphonyl.

MATERIALS AND METHODS

Apoprotein and Lipids

The preparation and characterisation of porcine high-density lipoprotein and its apoprotein have been described in detail elsewhere [8]. The total porcine apoprotein used in these studies was prepared from high-density lipoprotein by the ethanol-ether procedure of Scanu *et al.* [9].

Dimyristoyl lecithin was purchased from Koch-Light Ltd (Colnbrook). It was found to be pure by the criteria of thin-layer chromatography and gas-liquid chromatography.

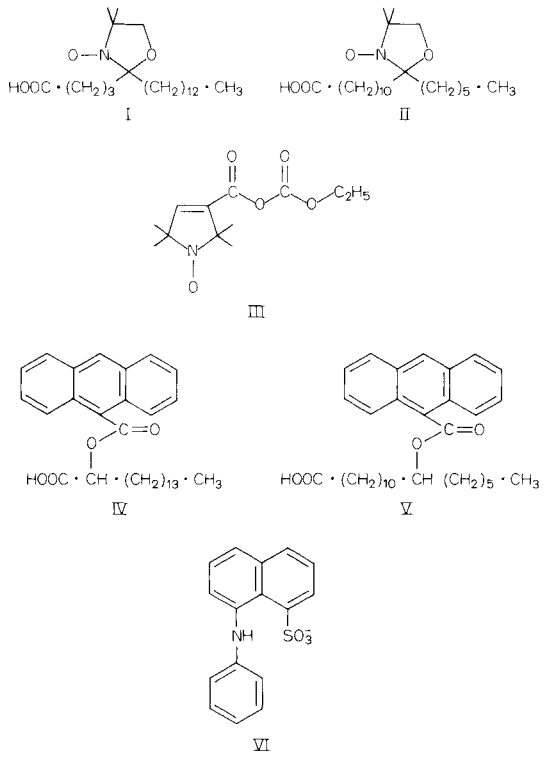

Fig. 1. *Chemical structures of electron spin resonance and fluorescent probes*

Electron Spin Resonance and Fluorescent Probes

The chemical structures of the various probes and labels used in this study are illustrated in Fig. 1.

Probe I was purchased from Syva Inc. (Palo Alto, Calif.). Probe II and label III were synthesised in this laboratory by previously described methods [10,11].

Probes IV and V were synthesised by acylating the appropriate hydroxy acids with the mixed anhydride formed from equimolar proportions of 9-anthroic acid (R. Emanuel) and trifluoroacetic anhydride essentially by the method of Lenard *et al.* [12]. After refluxing in methylene chloride with activated charcoal and three recrystallisations from hexane, the probes were found to be pure by thin-layer chromatography on silicic acid. Probe IV had m.p. 94 °C. Found: C, 78.4; H, 8.53%. $C_{31}H_{40}O_4$ requires: C, 78.1; H, 8.46%. Probe V had m.p. 77–78 °C, (lit: 78–79 °C) [13]. Found: C, 78.5; H, 9.09%. Calculated for $C_{33}H_{44}O_4$: C, 78.5; H, 8.75%.

Elementary analyses were carried out by Weiler and Strauss (Oxford).

1-Anilino-8-naphthalene sulphonic acid (probe VI) was purchased from Eastman Kodak and purified by recrystallisation of its magnesium salt from aqueous ethanol.

Dansyl chloride was purchased from Sigma Chemical Co.

Sample Preparation

Buffer solutions used in the experiments are quoted in the legends to the figures.

Sample containing probes I and II were prepared by dissolving the appropriate lipid and probe in chloroform, evaporating the solvent under reduced pressure and dispersing the resultant mixture in buffer solution. The probe concentration was generally 0.1 mM in 2% (w/v) of phospholipid.

The labelling of apoprotein with probe III was carried out as described previously [2].

Complexes of apoprotein and dimyristoyl lecithin were formed by brief sonication as described elsewhere [7]. Under these conditions porcine apoprotein and dimyristoyl lecithin form complexes of stoichiometry apoprotein–dimyristoyl lecithin, 1:2.4 (w/w) [7]. Complexes containing lipid probes were made up with a small excess of protein whilst those with labelled protein contained a small excess of lipid.

Phospholipid samples containing probes IV, V and VI were prepared by adding 10 µl of an ethanol solution of the probe to 3 ml of a sonicated dispersion of phospholipid. The final concentration was 2 µM probe in 0.05% dimyristoyl lecithin dispersion. At these concentrations, the probe was completely bound to the phospholipids as determined by fluorescence enhancement.

Dimyristoyl lecithin–apoprotein complexes were prepared by adding apoprotein in buffer solution to the sonicated phospholipid dispersion, and incubating at a temperature above the chain-melting transition of the lipid.

Dansylated apoprotein was prepared by incubating a 40-fold excess of dansyl chloride with the apoprotein in buffer solution overnight. The solution was protected from the light whilst standing. The labelled apoprotein solution was then purified from unbound label by passing twice through a column of Sephadex G-25.

Electron Spin Resonance Spectroscopy

Electron spin resonance spectra were obtained using a Varian E-4 spectrometer equipped with an E-257-9 variable temperature accessory.

When fatty acid spin probes such as I and II are dissolved in phospholipid dispersions, they undergo rapid anisotropic rotation about an axis perpendicular to the plane of the lipid surface. The amplitude of the deviation of the anisotropic motion from this axis,

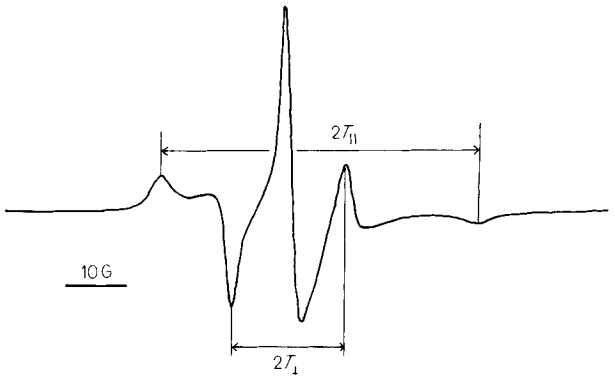

Fig. 2. *A typical electron spin resonance spectrum of probe I in a lecithin dispersion*

is conveniently measured by the order parameter, S_3 (using the notation of Seelig [14], who provides a detailed discussion of the theory).

S_3 is determined experimentally from the equation

$$S_3 = \frac{T_{\parallel} - T_{\perp}}{T_{zz} - T_{xx}}$$

where T_{zz} and T_{xx} are the values of the hyperfine coupling tensor in the z and x directions (assuming axial symmetry) and $T_{\parallel}$ and $T_{\perp}$ are measured as indicated in Fig. 2.

The isotropic hyperfine coupling constant A_N was calculated from the equation:

$$A_N = \frac{1}{3}(T_{\parallel} + 2T_{\perp})$$

for the anisotropic spectra, or measured directly from the low-field and centre lines of isotropic spectra.

Fig. 5 was constructed by measuring the low-field peak height of the electron spin resonance spectrum of apoprotein labelled with probe III at constant modulation amplitude, receiver gain, time constant and scan speed for samples of identical protein concentration. The 100% value was taken as that of the apoprotein sample at 20 °C.

Fluorescence

Fluorescence polarisation was measured on an apparatus built essentially to the design of Weber [15]. The variation of polarisation (or its reciprocal) was recorded automatically as a function of temperature using a dual photomultiplier apparatus linked to an $x-y$ recorder. The absolute values of the polarisations may be in error by up to 20% because of instrumental factors requiring the application of back-off voltages to compensate for light scattering. Comparisons made between samples measured under identical conditions are however considerably more accurate.

Polarisation values at constant temperature were measured using a fluorimeter (Perkin-Elmer-Hitachi MPF 2A).

Fluorescence polarisation of probes V and VI has been shown to be sensitive to phase changes in phospholipid bilayers despite probable anisotropic motion, perturbation of environment and changes of the probe lifetime with temperature [16–18]. The changes in microviscosity in the immediate vicinity of the probe, appear to be sufficient to change the motions and/or the angular distributions of the probes and thus their polarisation.

RESULTS AND DISCUSSION

Spin-Labelling

The graphs of the order parameter (S_3) *versus* temperature for probes I and II in dimyristoyl lecithin dispersions and in dimyristoyl lecithin–apoprotein complexes at pH 7.4 are shown in Fig. 3 and 4.

The observed values of S_3 from all samples, decreased with increasing temperature. Sharp changes in S_3 occurred as a result of the chain melting transition (T_c) in the dimyristoyl lecithin dispersions, at 21–22 °C and 23 °C for probes I and II respectively, (*cf.* 23 °C by differential scanning calorimetry [19]). The plots for heating and cooling were identical for most of the dimyristoyl lecithin and the dimyristoyl lecithin–apoprotein dispersions. In dispersions of dimyristoyl lecithin containing relatively high concentrations of probe II, the chain-melting transition was depressed by some 8–9 °C in the electron spin resonance experiment. (Similar results were reported by Oldfield *et al.* for dipalmitoyl lecithin [20].) The effect was not reproduced in the cooling curve (illustrated). It probably arises from the sequestration of spin probe molecules at temperatures below the T_c, thus causing the probes to sample their own perturbations.

The effect of complexing dimyristoyl lecithin with an excess of apoprotein was to eliminate the effect of the lipid T_c on probe I (Fig. 3). At pH 7.4 we would expect the nitroxide group of probe I to be located in the region of the glycerol backbone in dimyristoyl lecithin [21]. This suggests that the interaction of the apoprotein is very strong in this region of the lecithin. Further evidence for this interaction is that the apparent isotropic hyperfine coupling constants for probe I in the complex are significantly higher than those in dimyristoyl lecithin dispersions at all temperatures (*e.g.* 15.0 and 14.8 gauss respectively at 25 °C). This indicates that the probe experiences a

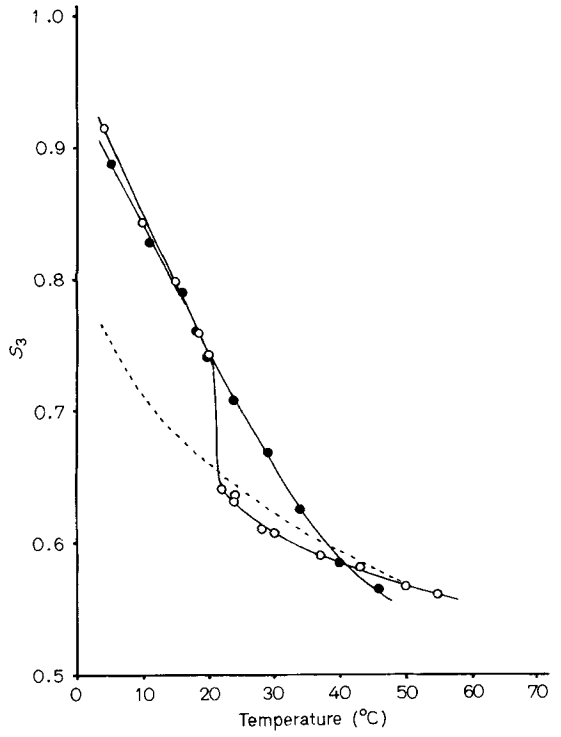

Fig. 3. *Plot of* S_3 versus *temperature for probe I (0.1 mM) in lecithin dispersions and complex (all 2% in lecithin).* Buffer was 5 mM Tris, 1 mM EDTA, 50 mM NaCl, 0.02% NaN_3 pH 7.4. (○) Dimyristoyl lecithin; (●) complex of dimyristoyl lecithin with apoprotein (2:1); (----) Egg-yolk lecithin

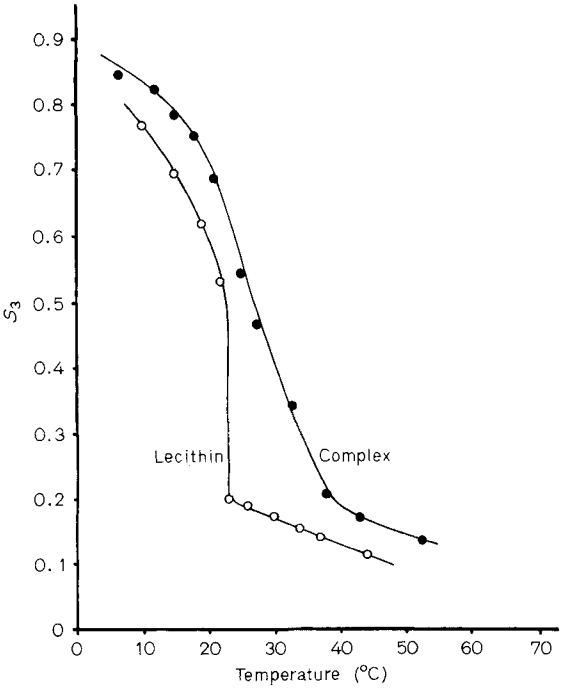

Fig. 4. *Plot of* S_3 versus *temperature for probe II (0.1 mM) in dimyristoyl lecithin and complex (both 2% in lecithin).* Buffer was 5 mM Tris, 1 mM EDTA, 50 mM NaCl, 0.02% NaN_3 pH 7.4. (○) Dimyristoyl lecithin; (●) complex of dimyristoyl lecithin with apoprotein (2:1)

more polar environment in the complex than in the lipid dispersion, possibly as a consequence of opening out the structure of the lipid head group region to the solvent, or perhaps as a result of a change in location of the probe.

Comparison of the temperature dependence of S_3 for probe I in dimyristoyl lecithin and its complex with apoprotein with that for egg lecithin (dotted line in Fig. 3) indicates that the effect of apoprotein on the glycerol backbone region of dimyristoyl lecithin is to extend the higher order associated with the gel phase of dimyristoyl lecithin to higher temperatures.

From its isotropic hyperfine coupling (14.50 gauss at 26 °C), probe II is located in the hydrocarbon chain region of dimyristoyl lecithin [22]. This value is unaffected when dimyristoyl lecithin is complexed with apoprotein. This suggests that the influence of the apoprotein on the region of the nitroxide group of probe II is rather less than on that for probe I.

The effect on the S_3 *versus* temperature plot of probe II when dimyristoyl lecithin is complexed with apoprotein is to broaden the chain-melting transition and to increase slightly the order parameters at all temperatures. The motion of the probe in the complex at temperatures above the broadened chain-melting transition is approximately isotropic indicating that the alkyl chains possess a high degree of mobility.

Fig. 5 shows a graph of log (relative low-field peak height) *versus* temperature for apoprotein labelled with probe III, (spectrum shown in Fig. 6). This type of plot has previously been shown to give straight lines for apoprotein and lipoprotein samples [2].

The effect of complexing the labelled apoprotein with dimyristoyl lecithin was to increase the amount of broad component (indicative immobilised label) in the electron spin resonance spectrum, as seen by the decrease in the overall relative peak height. In addition there was a change in gradient of the plot at about 21 °C (*cf.* T_c for dimyristoyl lecithin at 23 °C [19]). This plot was only slightly modified by increasing the ionic strength of the solution to a value which gives a different size of dimyristoyl lecithin–apoprotein complex [7]. For comparison, the temperature dependence was also measured for a complex of apoprotein with dilauroyl lecithin. This complex exhibited less broad component in its electron spin resonance spectrum than that of dimyristoyl lecithin but more than that of apoprotein alone. In this case the change in gradient was at about 5 °C (*cf.* T_c for dilauroyl lecithin at 2–3 °C [23]).

The conformational changes occurring in apoprotein on complexing with phospholipid and the changes in the electron spin resonance spectra of spin-labelled apoprotein which they produce, are well documented [24]. The theoretical reasons for

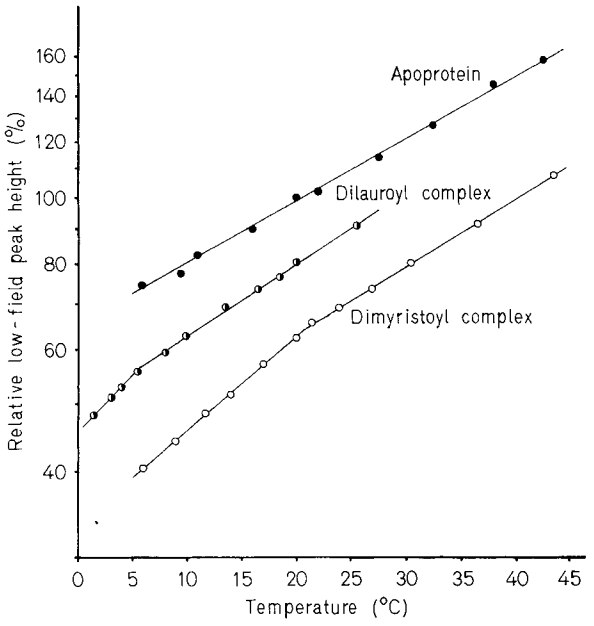

Fig. 5. *Logarithmic plot of low-field relative peak height* versus *temperature for apoprotein spin-labelled with probe III (●) and complexes with dilauroyl (◑) and dimyristoyl (○) lecithin.* Peak height for apoprotein at 20 °C = 100%. Buffer was 10 mM Tris, 1 mM EDTA, 0.04% NaN_3 pH 8.6

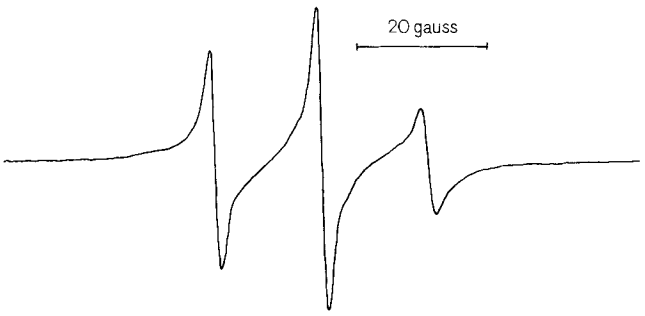

Fig. 6. *Electron spin resonance spectrum of apoprotein spin-labelled with probe III (20 °C)*

the linearity of the plots in Fig. 5 are however not established. Their usefulness lies in the fact that they demonstrate quite clearly that spin labels on apoprotein are sensitive, albeit to a small extent, to the chain-melting transition of the phospholipids in lecithin–apoprotein complexes.

Fluorescence

Probes IV, V and VI were selected to monitor the fluidity of the lipids and complexes at different depths in the bilayer. Probe IV is presumed to be located immediately below the polar head group of the phospholipid with the hydrophobic character of the

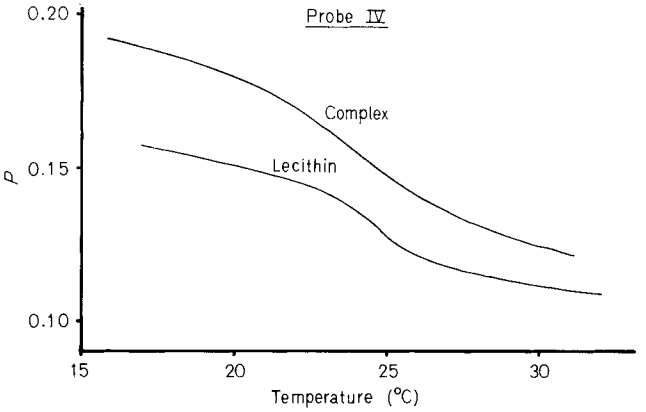

Fig. 7. *Plots of* P versus *temperature for probe IV in dimyristoyl lecithin dispersion and its complex with apoprotein.* Buffer was 10 mM Tris, 1 mM EDTA, 0.02% NaN_3 pH 8.6

anthracene overriding the ionic character of the carboxylate group. Probe V has been shown by X-ray diffraction to be located approximately 0.15 μm from the head group region in lipid dispersions [25]. Probe VI has similarly been shown to be oriented with its polar group in the aqueous interface and its aromatic group in the hydrocarbon chain region [26]. Isotope effects with ^2H_2O [27, 32] and nuclear magnetic resonance data confirm this location [28].

Probes IV, V and VI were all bound to sonicated dispersions of dimyristoyl lecithin with an accompanying enhancement of fluorescence. The emission maxima of probes IV and V on binding to phospholipid were blue-shifted by about 15 nm relative to those of their ethanol solutions, consistent with location in a fluid, apolar environment [29] although the effects of solvent correlation times on fluorescence spectra should not be overlooked [30–32].

The variation of fluorescence polarisation (P) with temperature for dimyristoyl lecithin and its complexes with apoprotein containing probes IV, V and VI is shown in Fig. 7, 8 and 9 respectively. For all of the probes, the polarisation decreases with increasing temperature. The decrease in P, which arises from a decrease in the microviscosity experienced by the probes, is especially marked in the region of 23–25 °C, the chain-melting temperature of dimyristoyl lecithin. Small variations of fluorescence intensity were also observed with temperature.

At a given temperature, the polarisation values measured for probe IV are greater than those for probe V. [Allowing for the differences in fluorescence lifetimes (7.3 ns for probe IV and 12.6 ns for probe V at 30 °C) probe IV can be shown to be the more immobilised.] This is in agreement with what one might expect from the location of these two probes in the lipid bilayer. Direct comparisons between probe VI

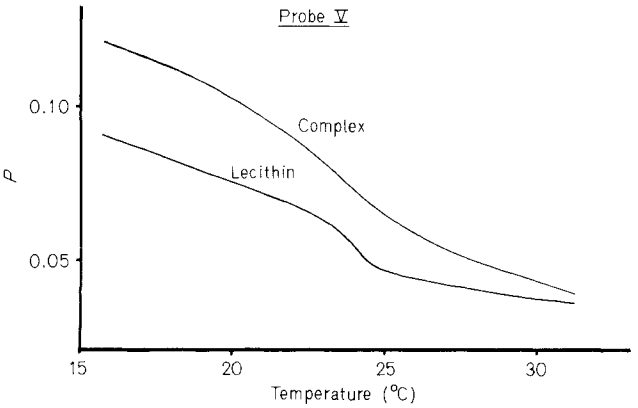

Fig. 8. *Plots of* P versus *temperature for probe V in dimyristoyl lecithin dispersion and its complex with apoprotein.* Buffer was 1 mM Tris, 1 mM EDTA, 0.02% NaN_3 pH 8.6

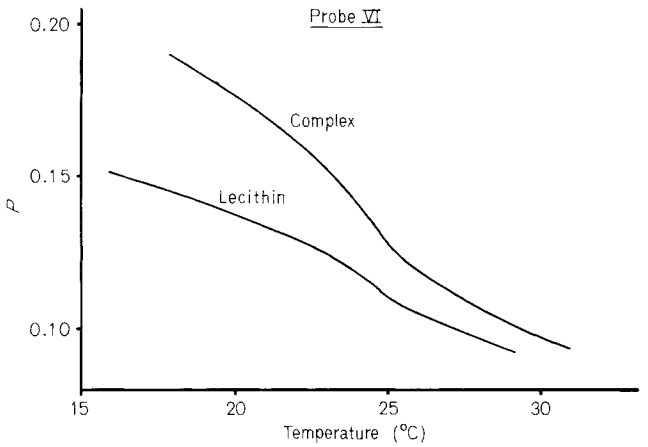

Fig. 9. *Plots of* P versus *temperature for probe VI in dimyristoyl lecithin dispersion and its complex with apoprotein.* Buffer was 1 mM Tris, 1 mM EDTA, 0.02% NaN_3 pH 8.6

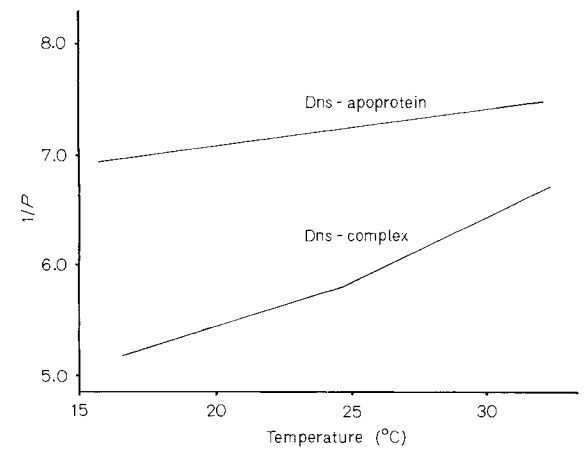

Fig. 10. *Plots of* 1/P versus *temperature for dansylated apoprotein and its complex with dimyristoyl lecithin.* Buffer was 1 mM Tris, 1 mM EDTA, 0.02% NaN_3 pH 8.6. Dns, dansyl

and the others is not justified because it has a different structure as well as a shorter fluorescence life-time.

The chain-melting transitions observed by probes IV, V and VI for dimyristoyl lecithin are broadened when it is complexed with apoprotein. In addition, the polarisation of fluorescence at a given temperature is greater in the complex than in dimyristoyl lecithin alone for all three probes. This suggests that the mobility of the probes is more restricted in the complex than in the phospholipid alone particularly since the fluorescence life-times are changed relatively little on complex formation.

The plots of $1/P$ *versus* temperature for the dansylated apoprotein and its complex with dimyristoyl lecithin are shown in Fig. 10. $1/P$ values obtained from the complex are lower than those from the apoprotein indicating that the label is less mobile in the complex. Both dansyl and label III are probably attached to the protein by acylation of lysine residues. Similar modification (succinylation and acetylation) have previously been shown not to affect the lipid-binding properties of the apoprotein [33].

There is a small but reproducible inflection in the plot of $1/P$ *versus* temperature for the complex. The temperature at which the change in slope occurs is consistent with that for the chain-melting transition of dimyristoyl lecithin. This result reinforces that obtained from the spin-labelled complexes and confirms that the apoprotein is sensitive to the chain-melting transition of the phospholipid.

General Discussion

Since probe molecules both perturb and report on their immediate environments, the extraction of quantitative structural conclusions from their spectroscopic properties is unreliable. The extent of perturbation may well be different for the two types of reporter molecule and is likely to be a function of size, polarity and location. In addition, more fundamental structural changes can occur with excessive probe concentrations [34]. The latter effect has been minimised in our work by the use of low concentrations of probe. The former difficulties are largely overcome by using the two types of probe which, in this study, yield very similar information. In particular, the chain-melting transitions are reported at the same temperatures as observed by differential scanning calorimetry. Both types of probe also indicate increased immobilisation on binding of apoprotein.

In order to interpret the observations in more detail, it is necessary to know the locations of the probes. Whereas this can sometimes be determined with reasonable accuracy in lipid bilayers, it is much less certain in lipid-protein complexes where there

is a possibility of some probe molecules binding to the protein.

While most of the probes used bind to apoprotein in the absence of lipid, the lack of a highly-immobilised second signal from the lipoprotein complex, in the case of the electron spin resonance experiments, suggests that the major part of the signal arises from probes dissolved in the lipid. If we assume that the probes in the lipoprotein complex occupy similar locations to those in the lipid alone then we can conclude that the effect of the protein on probe mobility is greatest near the polar head-group region of the phospholipid. With probe I, which is located in the glycerol region of the phospholipid, the effect of complexing with apoprotein is to broaden out the chain-melting transition completely. The probes located in the hydrocarbon chain region clearly demonstrate that the chain-melting transition of dimyristoyl lecithin is broadened in the complex with apoprotein. A similar result has been obtained by differential scanning calorimetry (M. C. Phillips, unpublished).

The results obtained from the dansylated apoprotein and that spin-labelled with probe III are directly comparable. In both cases a small but significant effect is seen by the reporter group at the chain-melting transition of the lipid.

The fact that the chain-melting transition is broadened but not eliminated in the alkyl chain region, suggests that the interaction of the protein with the lipid reduces, but does not remove the cooperative interaction between the hydrocarbon chains of the phospholipid. This, together with the elimination of the effect of the chain melting transition in the glycerol backbone region, and the small effects seen using covalently-labelled proteins, is consistent with a protein-coat, lipid-core model for the complex with some interdigitation of the protein [7].

The authors are grateful to Mr R. Henry for his assistance in the preparation of samples and for preparing the porcine apoprotein used in this work. C.G.M. was supported by an SRC CAPS award.

REFERENCES

1. Gotto, A. M. & Kon, H. (1969) *Biochem. Biophys. Res. Commun. 37*, 444–450.
2. Barratt, M. D., Leslie, R. B. & Scanu, A. M. (1971) *Chem. Phys. Lipids, 7*, 345–355.
3. Devi, S., Morrisett, T. D. & Gotto, A. M. (1971) *Circulation, suppl. II to vol. 43 and 44*, 11.
4. Keith, A. D., Melhorn, R. J., Freeman, N. K. & Nichols, A. V. (1973) *Chem. Phys. Lipids, 10*, 223–236.
5. Hart, C. J., Leslie, R. B. & Scanu, A. M. (1970) *Chem. Phys. Lipids, 4*, 367–374.
6. Jonas, A. (1972) *J. Biol. Chem. 247*, 7773–7778.
7. Hauser, H., Henry, R., Leslie, R. B. & Stubbs, J. M. (1974) *Eur. J. Biochem. 48*, 583–594.
8. Davis, M. A. F., Henry, R. & Leslie, R. B. (1974) *Comp. Biochem. Physiol. 47*, 831–849.
9. Scanu, A. M. & Edelstein, C. (1971) *Anal. Biochem. 44*, 576–588.
10. Waggoner, A. S., Kingzett, T. J., Rottschaefer, S., Griffith, O. H. & Keith, A. D. (1969) *Chem. Phys. Lipids, 3*, 245–253.
11. Griffith, O. H., Keana, J. F. W., Noalt, D. L. & Ivey, J. L. (1967) *Biochim. Biophys. Acta, 148*, 583–585.
12. Lenard, J., Wong, C. & Compans, R. W. (1974) *Biochim. Biophys. Acta, 332*, 341–349.
13. Waggoner, A. S. & Stryer, L. (1970) *Proc. Natl. Acad. Sci. U. S. A. 67*, 579–589.
14. Seelig, J. (1970) *J. Am. Chem. Soc. 92*, 3881–3887.
15. Weber, G. (1956) *J. Opt. Soc. Am. 46*, 962–970.
16. Vanderkooi, J. M. & Chance, B. (1972) *FEBS Lett. 22*, 23–26.
17. Lussan, C. & Faucon, J. F. (1971) *FEBS Lett. 19*, 186–188.
18. Badley, R. A., Martin, W. G. & Schneider, H. (1973) *Biochemistry, 12*, 268–275.
19. Chapman, D., Williams, R. M. & Ladbrooke, B. D. (1967) *Chem. Phys. Lipids, 1*, 445–475.
20. Oldfield, E., Keough, K. M. & Chapman, D. (1972) *FEBS Lett. 20*, 344–346.
21. Barratt, M. D. & Laggner, P. (1974) *Biochim. Biophys. Acta, 363*, 127–133.
22. Dodd, G. H., Barratt, M. D. & Rayner, L. (1970) *FEBS Lett. 8*, 286–288.
23. Ladbrooke, B. D. & Chapman, D. (1969) *Chem. Phys. Lipids, 3*, 304–356.
24. Leslie, R. B. (1971) in *Plasma Lipoproteins* (Smellie, R. M. S., ed.) pp. 47–85, Academic Press, London.
25. Lesslauer, W., Cain, J. E. & Blasie, J. K. (1972) *Proc. Natl. Acad. Sci. U. S. A. 69*, 1499–1503.
26. Lesslauer, W., Cain, J. E. & Blasie, J. K. (1971) *Biochim. Biophys. Acta, 241*, 547–566.
27. Stryer, L. (1966) *J. Am. Chem. Soc. 88*, 5708–5712.
28. Barker, R. W., Barrett-Bee, K., Berden, J. A., McColl, C. E. & Radda, G. K. (1974) *Soc. Symp. Energy Coupling* (Slater, E. C. & Ernster, L., eds) Elsevier, Amsterdam, in press.
29. Chance, B., Lee, C. & Blasie, J. K. (1971) *Probes of Structure and Function of Macromolecules and Proteins*, vol. 1, Academic Press, New York, N. Y.
30. Mazurenko, Y. T. & Bakshiev, N. G. (1970) *Opt. Spectrosc. 28*, 905–913.
31. Chakrabarti, S. K. & Ware, W. R. (1971) *J. Chem. Phys. 55*, 5494–5498.
32. Radda, G. K. & Vanderkooi, J. (1972) *Biochim. Biophys. Acta, 265*, 509–549.
33. Scanu, A. M. (1967) *J. Biol. Chem. 242*, 711–719.
34. Muesing, R. A. & Nishida, T. (1971) *Biochemistry, 10*, 2952–2962.

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