Proc. Natl. Acad. Sci. USA
Vol. 76, No. 11, pp. 5645–5649, November 1979
Biophysics

Lateral mobility of an amphipathic apolipoprotein, ApoC-III, bound to phosphatidylcholine bilayers with and without cholesterol

(membrane lateral diffusion/fluorescence recovery after photobleaching)

WINCHIL L. C. VAZ*†, KENNETH JACOBSON*‡, EN-SHINN WU§, AND ZENON DERZKO¶

*Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York 14263; §Department of Physics, University of Maryland, Baltimore, Maryland 21228; and ¶Department of Biophysical Sciences, State University of New York, Buffalo, New York 14226

Communicated by David Harker, July 9, 1979

ABSTRACT **The technique of fluorescence recovery after photobleaching was used to investigate the lateral mobility of a fluorescein-labeled amphipathic apolipoprotein, ApoC-III, bound to multibilayers prepared from dipalmitoyl phosphatidylcholine, egg phosphatidylcholine, and a 1:1 (molar ratio) mixture of egg phosphatidylcholine and cholesterol. In dipalmitoyl phosphatidylcholine bilayers the lateral diffusion coefficient (D) for the protein is about 2×10^{-9} cm^2 sec^{-1} at 20°C and about 9×10^{-8} cm^2 sec^{-1} at 45°C. Plots of D versus temperature in this system show a transition between about 30 and 35°C. Arrhenius activation energies for the diffusion in this case between 15 and 30°C and between 35 and 45°C are 28.5 and 7.0 kcal mol^{-1}, respectively (1 calorie = 4.18 joules). In egg phosphatidylcholine bilayers, D is about 3×10^{-8} cm^2 sec^{-1} at 20°C and the Arrhenius activation energy for diffusion is 8.1 kcal mol^{-1} between 15 and 35°C in this system. In bilayers prepared from an equimolar mixture of egg phosphatidylcholine and cholesterol D at 20°C is about 1.4×10^{-9} cm^2 sec^{-1} and the Arrhenius activation energy for the diffusion of the protein in this system between 15 and 35°C is 15.1 kcal mol^{-1}. Light-scattering and fluorescence-polarization results indicate that binding of this protein does not affect the gel-to-liquid crystalline phase transition of bilayer membranes but does mediate a major, reversible aggregation of the vesicles at about 33°C. These results lend support to the view that ApoC-III resides in the head-group region of the bilayer and suggest that its lateral diffusion coefficient represents an upper bound for integral membrane proteins.**

The mobility of membrane components has drawn considerable attention in the recent literature (1, 2). Several reports on the lateral (3–7) and rotational diffusion (8, 9) of components in the plane of the membrane in model membrane systems have appeared. In model membranes, as opposed to biomembranes, it is possible in principle to examine simple membrane compositions—for example, one protein and one lipid membrane—and subsequently to increase the complexity of the system at will. In this manner, it is possible to learn about the influence of a given membrane component on the diffusion of other components in the membrane and also, by comparison to natural membrane diffusion results, to learn about the role that nonmembrane components in the cell play in the regulation of membrane component mobility.

In the present communication we report the use of the technique of fluorescence recovery after photobleaching (FRAP) to study the lateral diffusion of an amphipathic apolipoprotein, ApoC-III, in bilayer membranes formed from dipalmitoyl phosphatidylcholine ($Pam_2PtdCho$), egg-yolk phosphatidyl-choline (E-PtdCho), and an equimolar mixture of E-PtdCho and cholesterol (Chol). The results have been communicated in a preliminary form (10). FRAP has been used so far to study a wide range of natural membrane (11–21) and model membrane systems (3–7). It offers a convenient method for studying lateral diffusion and is particularly attractive from the points of view of experimental and theoretical simplicity (19, 22).

ApoC-III is an amphipathic peptide having a molecular weight of about 9000 and a known amino acid sequence (23, 24). Its interaction with lipid membranes has been extensively studied (24). It interacts with E-PtdCho (25) and $Pam_2PtdCho$ (26) bilayer vesicles without disrupting their structure, but it disrupts the vesicular structure of dimyristoyl PtdCho unilamellar vesicles (26). The association of ApoC-III with PtdCho bilayer membranes is characterized by an association constant of about 2×10^6 M (ref. 26; unpublished data) and a lipid/protein stoichiometry of about 50:1 (25). Its interaction with PtdCho bilayers involves a combination of electrostatic and hydrophobic interactions (27).

MATERIALS AND METHODS

ApoC-III was isolated according to the procedure of Brown *et al.* (28) and was labeled with fluorescein by reaction of the protein (1 mg/ml) in 0.1 M sodium borate buffer at pH 9.0 with a 100-fold molar excess of fluorescein isothiocyanate (Baltimore Biological Laboratory Division, Becton-Dickinson) for 1 hr at 25°C. After reaction, the labeled protein (dye/protein ratio = 2) was separated from unreacted dye by filtration through a column of Sephadex G-25 using Dulbecco's Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline.

The phospholipids used in this study were isolated or synthesized by Tom Isac in the laboratory of D. Papahadjopoulos. $Pam_2PtdCho$ was synthesized as described by Robles and Van Den Berg (29) and E-PtdCho was isolated from egg yolks as described by Papahadjopoulos and Miller (30). The phospholipids contained no detectable impurities as determined by thin-layer chromatography on silica gel H with chloroform/methanol/7 M ammonia, 230:90:15 (vol/vol), as solvent.

Slides for FRAP experiments were prepared by evenly spreading a film of 2.5 mg of the lipid (in chloroform solution) over a 1-cm^2 area of a cleaned glass slide. The solvent was evaporated under reduced pressure. The dried lipid films were hydrated by dropping a coverslip with a hanging drop of 10 μl of the fluorescein-labeled protein solution (50 μg/ml) over the film. This procedure was done at 25°C for E-PtdCho, at 37°C for E-PtdCho/Chol, and at 45°C for $Pam_2PtdCho$. After 15 min for the lipid film to become hydrated, the slides were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: FRAP, fluorescence recovery after photobleaching; $Pam_2PtdCho$, dipalmitoyl phosphatidylcholine; E-PtdCho, egg-yolk phosphatidylcholine; Chol, cholesterol; D, diffusion coefficient; $\tau_{1/2}$, recovery halftime.

† Present address: Max Planck-Institut fuer biophysikalische Chemie, Abteilung Spektroskopie, D-3400 Goettingen-Nikolausberg, G.F.R.

‡ To whom reprint requests should be addressed.

pressed with a heavy weight, with care taken to avoid lateral motion of the coverslip over the hydrated lipid film as reported (3). The slides were then sealed with paraffin wax and incubated at 37°C for 48 hr (these long incubations require use of a pure paraffin to avoid contamination of the sample by fluorescent impurities). After the incubation, the slides were stored at room temperature. Slides older than 6 days after preparation were not used for the FRAP experiments.

The preparation of slides from $Pam_2PtdCho$ presents certain problems leading to some degree of irreproducibility. Large, well-formed, multibilayer domains are only formed on the slides when all elements (reagents and equipment) in the preparation, including the hydrating protein solution, are at 45°C (i.e., above the $Pam_2PtdCho$ phase-transition temperature in its hydrated state). However, this high temperature is deleterious to the protein stability. It was observed that often, and particularly if the protein solution had been stored for longer than about 15 min at 45°C, protein binding to the lipid was incomplete as identified by large amounts of the added fluorescent protein located in aqueous pockets within the specimen. When this was the case, it was found helpful to carefully wash the lipid bilayers on the slide with protein-free buffer by drawing it under the coverslip with a filter-paper wick. Preparation of E-PtdCho and E-PtdCho/Chol systems did not present these difficulties. Finally, it was noted that fluorescein-labeled ApoC-III changed character over prolonged storage at 4°C as evidenced by less protein incorporation into the multibilayer and slower diffusion rates.

FRAP experiments were done as described (3), with a beam diameter of ≈8 μm. Photobleaching intensities were ≈10 mW for E-PtdCho and $Pam_2PtdCho$ systems and ≈50 mW for E-PtdCho/Chol systems. Measuring beam intensities were attenuated by a factor of 10^5. During the measurement of the initial or prebleach fluorescence (F_i) in the FRAP experiments (19), rapid fading of about 10% of the fluorescence often occurred and then a stable level was achieved. The reason for this behavior is not understood. However, the maximal value of the prebleach fluorescence was selected as F_i because fluorescence recoveries back to this level were routinely obtained. Diffusion coefficients were calculated as described by Axelrod *et al.* (22). Experimental fluorescence recovery curves showed good fits to theoretical diffusion-limited recovery curves for one diffusing component as described by equation 12 of Axelrod *et al.* (22).

Cholate dialysis was done as described by Kagawa and Racker (31) at 4°C for 1 week. The resulting liposomes with or without protein are essentially cholate-free after this process (lipid/cholate, greater than 150:1). Unbound protein was separated from protein-containing liposomes by centrifugation and washing of the pellet. Cholate-dialyzed liposomes with or without protein were labeled with 16-anthroyl palmitate, and fluorescence polarization measurements were done as described by Cadenhead *et al.* (32).

RESULTS

Fig. 1 is an Arrhenius plot of the diffusion of ApoC-III in E-PtdCho and E-PtdCho/Chol bilayers. In the temperature range examined (10–40°C), D for E-PtdCho/Chol was no more than 1/10th that for E-PtdCho. Also, the activation energies for the diffusion of the protein in the two systems were different (Table 1). Fig. 2 is an Arrhenius plot of the diffusion of ApoC-III in $Pam_2PtdCho$ bilayers. A clear transition is seen in this curve between 30 and 35°C. The activation energies for the diffusion process at temperatures above and below this transition also were markedly different (Table 1).

For better understanding of the transition in the plot of D versus $1/T$ in $Pam_2PtdCho$ bilayers between 30 and 35°C (Fig. 2) we examined the influence of ApoC-III on the phase transition of $Pam_2PtdCho$ vesicles. ApoC-III/$Pam_2PtdCho$ vesicles were prepared by cholate dialysis (31) at a lipid/protein molar ratio of about 60:1 and the phase transition in the resulting proteoliposomes was studied by polarization of fluorescence with 16-anthroyl palmitate as the probe (32) as well as by measuring the optical density of the preparation as a function of temperature. The proteoliposomes were compared with protein-free $Pam_2PtdCho$ liposomes prepared in the same manner. The major difference between the protein-containing and the protein-free liposomes was that the pretransition at about 35°C in the protein-free preparation was missing from

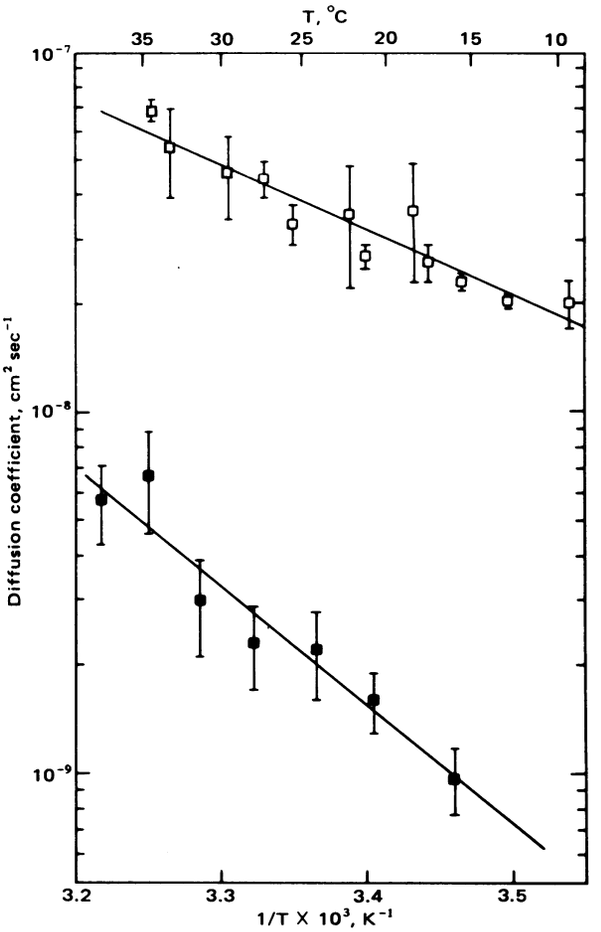

FIG. 1. Arrhenius plots for the diffusion of ApoC-III in E-PtdCho (□) and E-PtdCho/Chol (■) bilayers. Each experimental point is the mean ± SD of five FRAP experiments. The lines are a linear regression least squares analysis of the experimental points. Coefficient of correlation is 0.8976 for the E-PtdCho data and 0.9269 for the E-PtdCho/Chol data. Photobleaching was done at 10 mW for E-PtdCho and 50 mW for E-PtdCho/Chol.

Table 1. Arrhenius activation energies for diffusion of ApoC-III in PtdCho bilayers

Lipid	Temp. range, °C	Activation energy, kcal mol^{-1}
E-PtdCho	15–35	8.1
E-PtdCho/Chol (1:1)	15–35	15.1
$Pam_2PtdCho$	15–30	28.5
$Pam_2PtdCho$	35–45	7.0

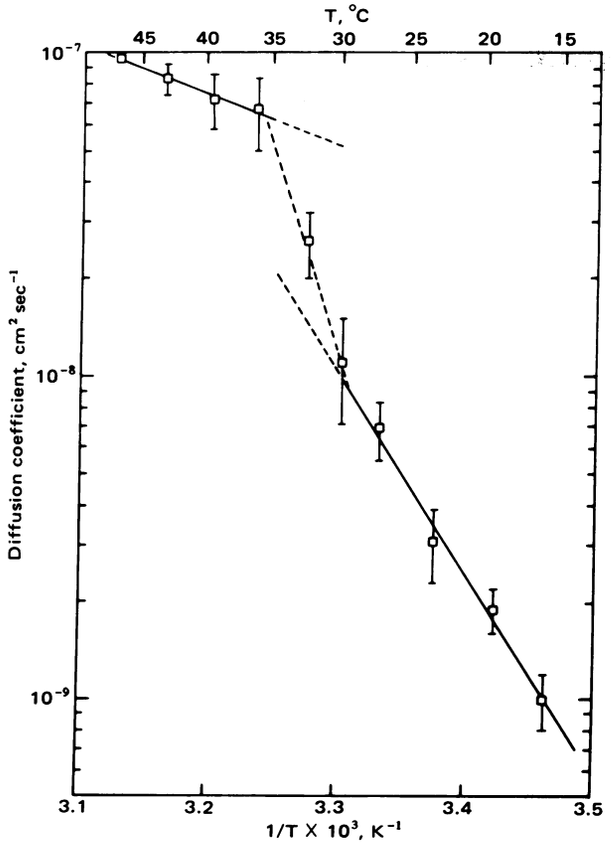

FIG. 2. Arrhenius plot for the diffusion of ApoC-III in $Pam_2PtdCho$ bilayers. Each experimental point is the mean ± SD of five FRAP experiments. The points between 35 and 45°C and between 15 and 30°C were fitted by a linear regression least squares analysis. Coefficient of correlation is 0.9784 between 35 and 45°C and 0.9908 between 15 and 30°C. Photobleaching intensity was 10 mW.

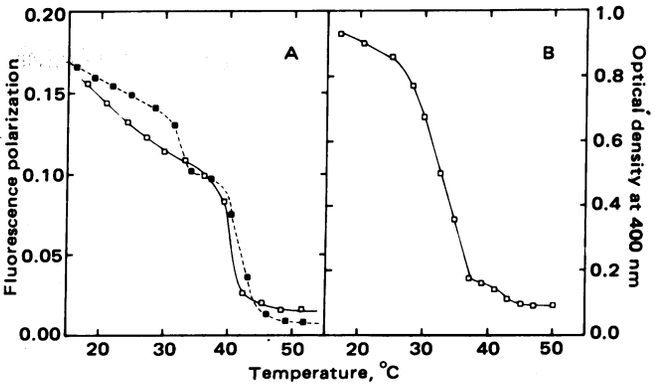

FIG. 3. (*A*) Fluorescence polarization curve for 16-anthroyl palmitate in pure $Pam_2PtdCho$ cholate-dialyzed liposomes (■) and ApoC-III/$Pam_2PtdCho$ cholate-dialyzed liposomes with a lipid/protein ratio of 60:1 (□). The fluorescence probe was added as a methanolic solution to the liposomes just before polarization measurements. The final volume of methanol was less than 1% of the total volume. The probe/lipid ratio was 1:500 in both cases. (*B*) Optical density scan (at 400 nm) of an ApoC-III/$Pam_2PtdCho$ liposome suspension with a lipid/protein ratio of 60:1. A Cary 15 spectrophotometer with a thermostatted cuvette chamber was used; thermal equilibration time was 15 min at each temperature; optical pathlength was 1 cm.

the protein-containing preparation (Fig. 3). Similar results were obtained with the 2-anthroyl palmitate and 1,6-diphenylhexatriene. There was a large, reversible change in optical density in the range 27–35°C in the protein-containing liposomes and a small, reversible change at about 42°C, which is characteristic of the main transition in $Pam_2PtdCho$ liposomes (33). Thus, both polarization and turbidity results indicate that the main transition of the $Pam_2PtdCho$ bilayer liposomes is not appreciably altered by the presence of bound ApoC-III; however, the pretransition, as reflected by fluorescence polarization, is abolished when the protein is bound to the bilayer.

The large change in optical density seen for the protein-containing liposomes was attributed to aggregation of the liposomes induced by the bound protein at temperatures <35°C. This was confirmed to be the case by the technique of dynamic light scattering (34) which indicated that, in the presence of the protein, particles of ≈700 Å in diameter markedly aggregated to give particles of ≈1400 Å in diameter when the temperature was reduced slowly from 35 to 32°C; further cooling to 15°C was accompanied by gradual particle growth to a mean diameter of 1900 Å. (C. C. Hsang, J. T. Ho, and E. P. Day, personal communication).

DISCUSSION

The serum apolipoproteins can be considered as models for some types of membrane-attached proteins, and some useful information on lipid–protein interactions has been obtained through the study of lipid–apolipoprotein interactions (24). In recent models (24, 27), the apoproteins of the C type are depicted as binding to the surface of the lipoprotein particles, being simply adsorbed in the lipid head-group region. ApoC-III adsorption to bilayers is strong (26), the protein–lipid binding constant is not altered by the phase transition in $Pam_2PtdCho$ bilayers (unpublished data), and the peptide does not grossly alter the bilayer structure (24, 26). Furthermore, release of the apolipoprotein from its phospholipid complexes is not accomplished by simply increasing the salt concentration in the system but requires the use of chaotropic agents such as urea, organic solvents, or detergents which significantly perturb the phospholipid structure (ref. 28; unpublished data). Thus, it is clear that the binding of this protein to PtdCho bilayer membranes (in those cases in which the lipid bilayer structure is preserved in the apolipoprotein/PtdCho complex) involves a substantial hydrophobic component. In particular, the model building study of Segrest *et al.* (27) suggests that the bilayer attachment point for ApoC-III may be the apolar face of the amphipathic helix which can be constructed from residues 40 to 68. Taken together, this information suggests that we are measuring the diffusion of ApoC-III in the bilayer head-group region, as depicted in Fig. 4. Our data, as discussed below, are consistent with this picture.

The lateral mobility results presented in this paper show some interesting aspects. First, ApoC-III diffuses rapidly when bound to fluid lipid bilayers. Its lateral diffusion coefficient is comparable to that for lipid analogs (3, 4) and low molecular weight peptides (7) in fluid lipid bilayers (Table 2). In addition, the activation energies for diffusion for ApoC-III are similar for E-PtdCho and $Pam_2PtdCho$ when both are fluid (Table 1); and these energy values for ApoC-III mobility are similar to the values for lipid analog diffusion in E-PtdCho multibilayers (3).

Another noteworthy feature of this study is that Chol slows the diffusion of the protein in E-PtdCho/Chol bilayers by a factor of at least 10. This effect is somewhat larger than the slowing down of lipid or peptide diffusion in E-PtdCho/Chol bilayers, the transport being retarded by a factor of 2–3 (3, 7). Chol makes the diffusion of the protein in E-PtdCho/Chol bilayers similar to that observed in gel-state bilayers (diffusion in $Pam_2PtdCho$ below 30°C).

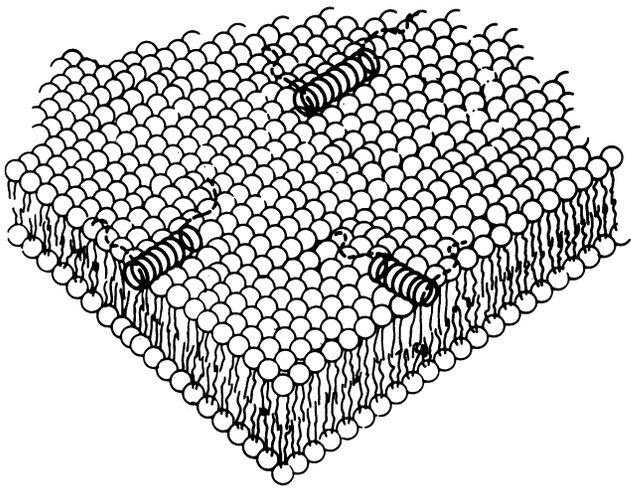

FIG. 4. Proposed membrane binding conformation showing only the amphipathic helical segments of ApoC-III based on recent models (24, 27) [drawing adapted from Singer and Nicolson (35)]. Extent of penetration of ApoC-III into the head-group region is not known.

With regard to the effect of a lipid phase transition, the change in the slope of D versus temperature (Fig. 2) for ApoC-III bound to $Pam_2PtdCho$ bilayers occurs between 30 and 35°C, which does not coincide with the main transition at 42°C for pure $Pam_2PtdCho$ bilayers. Furthermore, the diffusion of the protein is considerably faster than the diffusion of lipid analogs and small peptides in gel phase bilayers. Lipid analogs as well as gramidicin S show large discontinuities in lateral diffusion coefficients at temperatures that nearly coincide with the main phase transition temperature of the bilayer (3, 4, 7) (Table 2). It is difficult to understand why the break in the plot of D versus $1/T$ for diffusion of ApoC-III in $Pam_2PtdCho$ bilayers occurs between 30 and 35°C (Fig. 2), a temperature that does not correspond to the gel–liquid crystalline phase transition temperature of ApoC-III-bearing $Pam_2PtdCho$ bilayers (see Fig. 3). Also, the pretransition seen in protein-free $Pam_2PtdCho$ bilayers by fluorescence polarization is absent when protein is bound, making it unlikely that the break in Fig. 2 is due to the occurrence of the pretransition in the bilayer. It is interesing to note that the ApoC-III/E-PtdCho diffusion data (Fig. 1) can be smoothly extrapolated to fit the ApoC-III/$Pam_2PtdCho$ data (Fig. 2) for $T > 35$°C, suggesting that in $Pam_2PtdCho$ bilayers above 35°C the protein behaves as though it were diffusing in fluid lipid. At any rate, the failure of ApoC-III diffusion to be affected by the main transition suggests a surface location for this protein (Fig. 4).

Table 2. Diffusion of membrane components in bilayers

Diffusing species (reference)	D, cm²/sec: In fluid membranes	D, cm²/sec: In gel phase membranes
Lipid analog:		
NBD-PE (3, 5)	4×10^{-8} *	10^{-10} †
Peptide:		
NBD-gramicidin S (7)	3.5×10^{-8} *	$<10^{-10}$ †
Proteins:		
ApoC-III (this work)	4×10^{-8} *	4×10^{-9} ‡
Lipophilin (36)	$\approx 10^{-8}$ §	$<10^{-10}$ ¶
M-13 coat protein (37)	3.5×10^{-8} §	—

* E-PtdCho at 25°C.
† Dimyristoyl phosphatidylcholine at 20°C.
‡ $Pam_2PtdCho$ at 25°C.
§ Dimyristoyl phosphatidylcholine at 26°C.
¶ Dimyristoyl phosphatidylcholine at 15°C.

The possible lateral transport mechanisms for ApoC-III are intriguing. Simple aqueous diffusion of trapped protein is unlikely because $\tau_{1/2}$ for a diffusion coefficient of 10^{-6} cm² sec^{-1} is about 40 msec for an 8-μm spot. For our instrument, in practice this means that aqueous ApoC-III would appear difficult to bleach at all because recovery takes place so rapidly after the end of the bleach. On the other hand, a complex of ApoC-III irreversibly bound to a small "domain" of bilayer lipid is unlikely also, because in this case one would expect the transport rate to be dramatically retarded as the bilayer is cooled through the phase transition temperature. For the sake of discussion, one might picture the protein as "wiggling" along through the head-group region, generally maintaining the postulated amphipathic helical binding site in close aposition to the membrane surface. The similarity of ApoC-III and lipid analog diffusion above the transition temperature suggests that both motions suffer a rate limitation associated with the head-group region at temperatures above the transition temperature. Indeed, Evans and Hochmuth (38) pointed out that kinetic interactions in the head-group and glycerol backbone region appear to determine the surface viscosity of monolayers; hence, these interactions can be supposed to play the dominant role in determining the magnitude of lipid lateral diffusion coefficient. These same dynamic interactions may define the "fluidity" of the head-group region which controls the magnitude of the ApoC-III diffusion constant. Below the transition temperature, however, lipid diffusion is controlled by the large increase in viscosity in the gel state, but the protein mobility continues to be controlled by head-group interactions which are not altered until a temperature that corresponds to the pretransition is reached (for review, see ref. 39).

Another mobility mechanism could consist of a dissociation of the apoprotein from the bilayer followed by a brief aqueous "hop" and finally a reassociation with the bilayer. Our current studies do not distinguish between these two diffusion mechanisms. Regardless of mechanism, the data presented here probably represent an upper limit for the diffusion of integral membrane proteins and, furthermore, suggests that the C peptides could possess considerable mobility on the surface of the very low density lipoproteins.

More general methods of "reconstitution" of protein-containing lipid bilayers are required for lateral diffusion studies using the FRAP techniques (36, 37). The method used in this work cannot be applied to all proteins that bind to lipid membranes, particularly those that traverse the entire width of the bilayer. Ideally, results from single, noninteracting bilayers bearing proteins are required for comparison with the cell membrane studies. In model systems, it would also be desirable to evaluate the dependence of the protein self-diffusion coefficient on its membrane concentration. Lateral and rotational diffusion measurements on the same model systems may also allow the Saffman–Delbrück theory (40) to be tested.

We thank Dr. Demetrious Papahadjopoulos for his helpful comments on the manuscript and Dr. Jake Bello for use of his Cary 15 temperature-controlled spectrophotometer. We also thank Dr. W. Virgil Brown for supplying part of the protein used in this study. This work was supported by Grant CA-16743 from the National Cancer Institute. K.J. is an Established Investigator of the American Heart Association.

1. Edidin, M. (1974) *Annu. Rev. Biophys. Bioeng.* 3, 179–201.
2. Cherry, R. J. (1976) in *Biological Membranes*, eds. Chapman, D. & Wallach, D. F. H. (Academic, London), Vol. 3, pp. 47–102.
3. Wu, E. S., Jacobson, K. & Papahadjopoulos, D. (1977) *Biochemistry* 16, 3936–3941.

4. Fahey, P. F. & Webb, W. W. (1978) *Biochemistry* **17,** 3046–3053.
5. Smith, B. A. & McConnell, H. M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2759–2763.
6. Wolf, D. E., Schlessinger, J., Elson, E. L., Webb, W. W., Blumenthal, R. & Henkart, P. (1977) *Biochemistry* **16,** 3476–3483.
7. Wu, E. S., Jacobson, K., Szoka, F. & Portis, A. (1978) *Biochemistry* **17,** 5543–5550.
8. Cherry, R. J., Mueller, U., Henderson, R. & Heyn, M. P. (1978) *J. Mol. Biol.* **121,** 283–298.
9. Vaz, W. L. C., Austin, R. H. & Vogel, H. (1979) *Biophys. J.* **26,** 415–426.
10. Vaz, W. L. C., Wu, E. S. & Jacobson, K. (1979) *Biophys. J.* **25,** 178a.
11. Peters, R., Peters, J., Tews, K. H. & Bahr, W. (1974) *Biochim. Biophys. Acta* **367,** 282–294.
12. Edidin, M., Zagyansky, Y. & Lardner, T. J. (1976) *Science* **191,** 466–468.
13. Jacobson, K., Wu, E. S. & Poste, G. (1976) *Biochim. Biophys. Acta* **433,** 215–222.
14. Zagyansky, Y. & Edidin, M. (1976) *Biochim. Biophys. Acta* **433,** 209–214.
15. Schlessinger, J., Koppel, D. E., Axelrod, D., Jacobson, K., Webb, W. W. & Elson, E. L. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2409–2413.
16. Schlessinger, J., Webb, W. W., Elson, E. L. & Metzger, H. (1976) *Nature (London)* **264,** 550–552.
17. Axelrod, D., Ravdin, P., Koppel, D. E., Schlessinger, J., Webb, W. W., Elson, E. L. & Podleski, T. R. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4594–4598.
18. Schlessinger, J., Axelrod, D., Koppel, D. E., Webb, W. W. & Elson, E. L. (1977) *Science* **195,** 307–309.
19. Jacobson, K., Derzko, Z., Wu, E. S., Hou, Y. & Poste, G. (1977) *J. Supramol. Struct.* **5,** 565–576.
20. Axelrod, D., Ravdin, P. M. & Podleski, T. R. (1978) *Biochim. Biophys. Acta* **511,** 23–38.
21. Axelrod, D., Wight, A., Webb, W. W. & Horwitz, A. (1978) *Biochemistry* **17,** 3604–3609.
22. Axelrod, D., Koppel, D. E., Schlessinger, J., Elson, E. L. & Webb, W. W. (1976) *Biophys. J.* **16,** 1055–1069.
23. Brewer, H. B., Schulman, R., Herbert, P., Ronan, R. & Wehrly, K. (1972) *Adv. Exp. Med. Biol.* **26,** 280.
24. Morrisett, J. D., Jackson, R. L. & Gotto, A. M. (1977) *Biochim. Biophys. Acta* **472,** 93–133.
25. Morrisett, J. D., David, J. S. K., Pownall, H. J. & Gotto, A. M. (1973) *Biochemistry* **12,** 1290–1299.
26. Träuble, H., Meddelhoff, G. & Brown, W. V. (1974) *FEBS Lett.* **49,** 269–275.
27. Segrest, J. P., Jackson, R. L., Morrisett, J. D. & Gotto, A. M. (1974) *FEBS Lett.* **38,** 247–253.
28. Brown, W. V., Levy, R. I. & Fredrickson, D. S. (1969) *J. Biol. Chem.* **244,** 5687–5694.
29. Robles, C. & Van Den Berg, D. (1969) *Biochim. Biophys. Acta* **187,** 520–526.
30. Papahadjopoulos, D. & Miller, N. (1967) *Biochim. Biophys. Acta* **135,** 624–638.
31. Kagawa, Y. & Racker, E. (1971) *J. Biol. Chem.* **246,** 5477–5487.
32. Cadenhead, D. A., Kellner, B. M. J., Jacobson, K. & Papahadjopoulos, D. (1977) *Biochemistry* **16,** 5386–5392.
33. Yi, P. & MacDonald, R. (1973) *Chem. Phys. Lipids* **11,** 114–134.
34. Day, E. P., Ho, J. T., Kunz, R. K. & Sun, S. T. (1977) *Biochim. Biophys. Acta* **470,** 503–508.
35. Singer, S. J. & Nicolson, G. (1972) *Science* **175,** 920–931.
36. Derzko, Z. & Jacobson, K. (1978) *Biophys. J.* **21,** 204a.
37. Smith, L. M., Smith, B. A. & McConnell, H. M. (1979) *Biochemistry* **18,** 2256–2259.
38. Evans, E. & Hochmuth, R. (1978) *Curr. Top. Membr. Transp.* **10,** 1–64.
39. Seelig, J. (1978) *Biochim. Biophys. Acta* **515,** 105–140.
40. Saffman, P. G. & Delbruck, M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3111–3113.