Fluorescence Studies of Dipalmitoylphosphatidylcholine Vesicles Reconstituted with the Glycoprotein of Vesicular Stomatitis Virus†

William A. Petri, Jr., Ranajit Pal, Yechezkel Barenholz, and Robert R. Wagner*

ABSTRACT: The vesicular stomatitis virus glycoprotein (G) was reconstituted into dipalmitoylphosphatidylcholine (DPPC) vesicles by detergent dialysis. The DPPC gel to liquid-crystalline phase transition of the DPPC–G protein vesicles was monitored by the fluorescence anisotropy of *trans*-paranaric acid, 16-(9-anthroyloxy)palmitoylglucocerebroside, 1,6-diphenyl-1,3,5-hexatriene, and 4-heptadecyl-7-hydroxycoumarin. The DPPC transition temperature measured by all four fluorescent probes was lowered in the presence of the G protein and the DPPC gel state was disordered by the G protein as evidenced by a decreased fluorescence anisotropy for all four probes below the phase-transition temperature. A possible ordering of the DPPC liquid-crystalline state by the G protein was indicated by an increased anisotropy of *trans*-paranaric acid and 16-(9-anthroyloxy)palmitoylglucocerebroside in the liquid-crystalline state of DPPC–G protein vesicles. The G protein in addition affected the ionization of the 4-heptadecyl-7-hydroxycoumarin in lipid vesicles, increasing the apparent pK of the probe from 9.05 to 9.45.

Vesicular stomatitis (VS)[1] virus contains a single virally coded glycoprotein (G) in its membrane. The G protein has an estimated molecular weight of 69 000 (Wagner, 1975), of which ~6000 daltons is a protease-resistant hydrophobic tail fragment embedded in the virus membrane (Mudd, 1974; Schloemer & Wagner, 1975). Sequencing of a cDNA clone of the 3′ end of G mRNA has provided evidence that the G protein has 20 consecutive hydrophobic amino acids traverse the virion membtane and that 29 amino acids from the carboxy-terminus protrude from the inner membrane surface (Rose et al., 1980). Further evidence that the G protein is transmembrane has come from studies of in vitro translation of G mRNA in the presence of microsomal vesicles: during translation the G protein is inserted by its amino terminus into the lumen of endoplasmic reticulum vesicles, leaving a 3000-dalton fragment at the carboxy terminus accessible to protease on the outside of the vesicles (Katz et al., 1977; Toneguzzo & Ghosh, 1978).

Pure G protein has been isolated from VS virus and reconstituted into liposomes by detergent dialysis (Petri & Wagner, 1979; Miller et al., 1980); the micellar form of G protein also partitions into preformed sonicated vesicles (Petri & Wagner, 1980). Phosphatidylcholine–G protein vesicles formed by octyl glucoside dialysis contain glycoprotein spikes protruding in the same external orientation as in the VS virus membrane. Proteolytic digestion of these vesicles with thermolysin leaves a hydrophobic glycoprotein tail fragment embedded in the membrane that migrates identically on polyacrylamide gels with the tail fragment from VS virus treated with thermolysin (Petri & Wagner, 1979).

The G protein reconstituted into dipalmitoylphosphatidylcholine (DPPC) vesicles by detergent dialysis was found to exert a profound effect upon the DPPC gel to liquid-crystalline phase transition monitored by differential scanning calorimetry (Petri et al., 1980). Increasing the G protein concentration from 0 to 0.15 mol % decreased the transition temperature from 41 to 39 °C, broadened the temperature range of the transition, and decreased the transition enthalpy change from 8.0 to 4.6 kcal/mol (Petri et al., 1980). The present studies report investigations of the mechanism by which the G protein affects the DPPC phase transition as measured by fluorescence depolarization of four probes inserted in G protein–DPPC vesicles.

Experimental Procedures

Isolation of VS Viral Glycoprotein. VS virus of the Indiana serotype was purified by differential, rate zonal, and equilibrium centrifugation in sucrose and tartrate gradients 21 h after infection of baby hamster kidney-21 cells with 0.1 plaque-forming unit/cell of plaque-purified virus (McSharry & Wagner, 1971). The VS virus glycoprotein and phospholipids were released from purified virus with 30 mM octyl glucoside (octyl β-D-glucopyranoside) (Calbiochem, La Jolla, CA) as previously described (Petri & Wagner, 1979). The glycoprotein was purified from the phospholipids by sedimentation into a 15–30% sucrose gradient containing 60 mM octyl glucoside, 0.5 M NaCl, and 50 mM Tris (pH 7.6). The pooled protein-containing fractions from the gradient were 97% pure as determined by polyacrylamide gel electrophoresis and contained no detectable cholesterol and approximately one molecule of residual phospholipid per molecule of glycoprotein (Petri & Wagner, 1979).

Reconstitution of VS Viral Glycoprotein into Vesicles. 1,2-Dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) was obtained from Avanti Biochemicals (Birmingham, AL). The glycoprotein was reconstituted with the phospholipid by the detergent dialysis method (Petri & Wagner, 1979). The glycoprotein purified free of cholesterol and phospholipid was added to 2 mL of 0.25 mM DPPC in 60 mM octyl glucoside and 50 mM KCl. The detergent solution of glycoprotein and phospholipid was then extensively dialyzed against 50 mM KCl for 48 h at 41 °C.

Fluorescence. The fluorescence depolarization of 1,6-diphenyl-1,3,5-hexatriene (diphenylhexatriene; Aldrich, Milwaukee, WI) as a function of temperature was determined as previously described (Suurkuusk et al., 1976). A modified Perkin-Elmer MPF3 spectrofluorimeter with polarizers in the excitation and emission beams was used to measure fluores-

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[1] Abbreviations used: VS virus, vesicular stomatitis virus; G protein, viral glycoprotein; DPPC, dipalmitoylphosphatidylcholine.

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cence depolarization, and the sample temperature was measured with a Yellow Springs Instrument thermistor probe connected to a digital ohmmeter. Diphenylhexatriene (1 mM) dissolved in tetrahydrofuran was added to the reconstituted vesicles at a ratio of 1 molecule for every 200 DPPC molecules and after an incubation for 1 h at 50 °C was excited at 360 nm and fluorescence recorded at 430 nm, using the MPF3 390-nm cutoff filter to reduce scattered light. The fluorescence measurements were corrected for scattered light, which was never more than 5% of the fluorescence intensity perpendicular to the plane of the excitation beam. The fluorescence of *N*-methylacridinium perchlorate was used to correct for the differential sensitivity of the photomultiplier for horizontally and vertically polarized light.

The use of diphenylhexatriene fluorescence depolarization as a specific probe for the hydrophobic region of the lipid bilayer has been described by Shinitzky & Barenholz (1974). The anisotropy of DPH fluorescence, r, is defined by

$$r = \frac{(I_\parallel/I_\perp) - 1}{(I_\parallel/I_\perp) + 2}$$

where $I_\parallel$ is the fluorescence intensity parallel to and $I_\perp$ is the fluorescence intensity perpendicular to the plane of polarization of the excitation beam. A more complete discussion of the assumptions used in the treatment of diphenylhexatriene fluorescence depolarization has been given (Shinitzky & Barenholz, 1978).

The 16-(9-anthroyloxy)palmitoylglucocerebroside (anthroyloxycerebroside) probe was a kind gift from Dr. Maria Correa-Freire (University of Virginia, Charlottesville, VA). Fluorescence depolarization of the anthroyloxycerebroside as a function of temperature was measured in the same manner as for diphenylhexatriene, except that anthroyloxycerebroside was excited at 389 nm and fluorescence recorded at 455 nm, using the MPF3 340 cutoff filter to reduce scattered light. The fluorescence anisotropy for anthroyloxycerebroside was calculated identically with that for diphenylhexatriene anisotropy.

The anthroyloxycerebroside probe was added at a level of 1 molecule for every 200 molecules of DPPC in chloroform. The chloroform was removed by a stream of N_2 and the DPPC–anthroyloxycerebroside mixture resuspended in 60 mM octyl glucoside and 50 mM KCl containing the appropriate amount of glycoprotein. Reconstitution of the G protein vesicles occurred over 48 h of extensive dialysis in the dark at 41 °C against 50 mM KCl.

4-Heptadecyl-7-hydroxycoumarin (heptadecylcoumarin; Molecular Probes, Plano, TX) or *trans*-paranaric acid (Molecular Probes) was added as a 1 mM solution in tetrahydrofuran to the reconstituted vesicles at a ratio of 1 molecule for every 150 DPPC molecules. The cuvettes containing the *trans*-paranaric acid were sealed under N_2 before fluorescence measurements. After incubation for 1 h at 50 °C, the vesicles containing heptadecylcoumarin were excited at 325 nm and the fluorescence was measured at 450 nm, using the 430-nm cutoff filter; the vesicles containing *trans*-paranaric acid were excited at 323 nm and the fluorescence was measured at 410 nm, using the 390-nm cutoff filter. The fluorescence anisotropies for heptadecylcoumarin and *trans*-paranaric acid were calculated as for diphenylhexatriene. The vesicles for the heptadecylcoumarin fluorescence anisotropy measurements were reconstituted in 10 mM Tris, pH 8.5.

The vesicles for the pH titration of the heptadecylcoumarin were made from total viral lipids extracted from purified VS virions by the procedure of Folch et al. (1957) and were reconstituted with or without 1 mol % G protein by octyl glucoside dialysis against 50 mM KCl. The excitation spectrum was recorded by fixing the emission maximum at 450 nm. The degree of ionization of the probe was calculated from the peak area at 325 and 380 nm for un-ionized and ionized molecules, respectively. The pH of the solution was varied by adding aliquots of 0.01 N NaOH. In a separate experiment, the pH of one solution was measured by pH meter after adding aliquots of 0.01 N NaOH to the vesicle preparation.

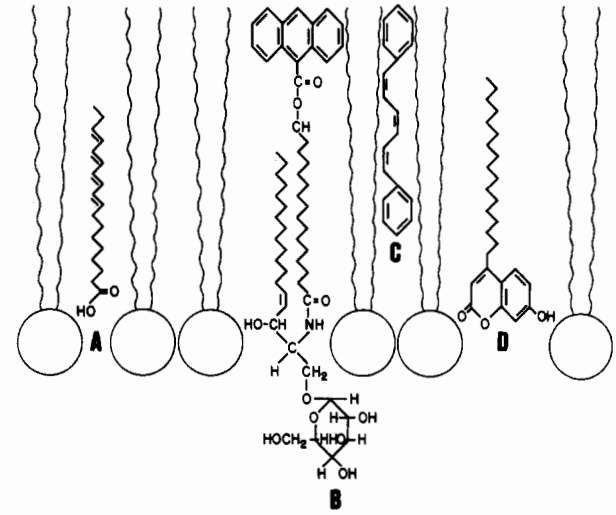

FIGURE 1: Diagrammatic representation of putative membrane location and chemical structures of (A) *trans*-paranaric acid, (B) 16-(9-anthroyloxy)palmitoylglucocerebroside, (C) 1,6-diphenyl-1,3,5-hexatriene, and (D) 4-heptadecyl-7-hydroxycoumarin.

Lipid and Protein Analysis. The phosphorus content of the vesicles was measured by a modification of the Bartlett procedure (Marinetti, 1962). The protein was determined by the method of Lowry et al. (1951). The mole percent of glycoprotein was calculated by assuming a molecular weight of 69 000 for the glycoprotein.

Results

The steady-state fluorescence depolarization of four fluorescent probes which are sensitive to the gel to liquid-crystalline transition of DPPC have been used to monitor the structural changes in the DPPC bilayer caused by the G protein. The chemical structures and putative membrane locations of the four fluorescent probes are depicted in Figure 1. *trans*-Paranaric acid (Figure 1A) is a naturally occurring polyene fatty acid which has an enhanced quantum yield in and preferentially partitions into gel state phospholipids (Sklar et al., 1979). The location in the bilayer of the fluorescent group of 16-(9-anthroyloxy)palmitoylglucocerebroside is unambiguous, with the fluorescent anthracene-9-carboxylic acid attached to the terminal methyl group of the cerebroside (Figure 1B). In contrast to *trans*-paranaric acid, 1,6-diphenyl-1,3,5-hexatriene (Figure 1C) partitions equally between gel and liquid-crystalline state phospholipids and is thought to be located in the hydrophobic core of the bilayer (Shinitzky & Barenholz, 1978). The fluorescent hydroxycoumarin group of 4-heptadecyl-7-hydroxycoumarin is hydrophilic and presumably superficially located in the bilayer (Figure 1D). Heptadecylcoumarin, in addition to being sensitive to the gel to liquid-crystalline phase transition of DPPC (R. Pal, W. A. Petri, Jr., Y. Barenholz, and R. R. Wagner, unpublished experiments), can also be used to monitor the ionic environment near the phospholipid head groups by measuring the probe's apparent pK by its fluorescence spectrum (Fromherz, 1973). It was of interest to compare the effect of the reconstituted G protein on the anisotropic motion of these four fluorescent

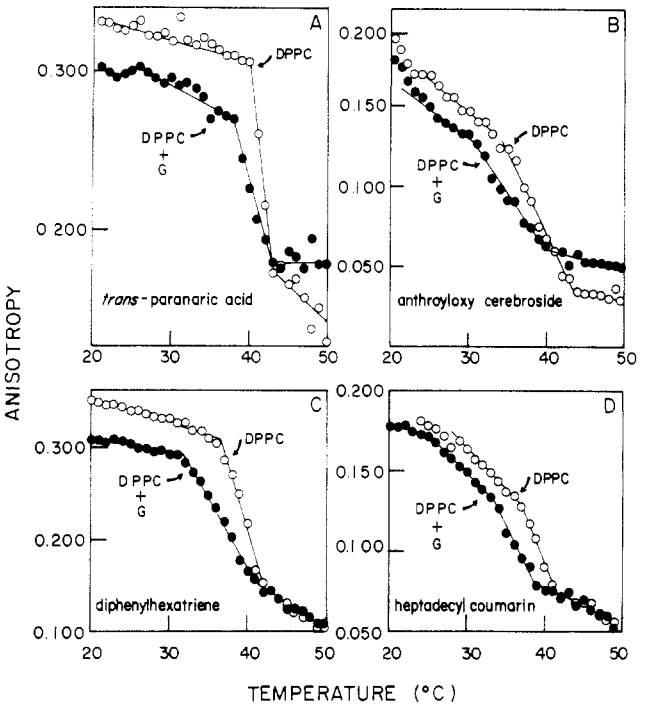

FIGURE 2: Fluorescence anisotropy of (A) *trans*-paranaric acid, (B) 16-(9-anthroyloxy)palmitoylglucocerebroside, (C) 1,6-diphenyl-1,3,5-hexatriene, and (D) 4-heptadecyl-7-hydroxycoumarin in G protein–DPPC vesicles as a function of temperature. The fluorescent probes were incorporated into the reconstituted vesicles and the fluorescence anisotropy measured as described under Experimental Procedures. The DPPC vesicles contained either no glycoprotein (○) or (A) 0.45 mol %, (B) 0.34 mol %, (C) 0.56 mol %, (D) 0.40 mol % glycoprotein (●).

probes which presumably are located in different regions of the lipid bilayer.

Fluorescence Depolarization of trans-Paranaric Acid. The fluorescence polarization of the fatty acid *trans*-paranaric acid (Figure 1A) has been successfully used to monitor the phase transition of DPPC dispersions (Sklar et al., 1979). The preferential partitioning and enhanced quantum yield of *trans*-paranaric acid in the DPPC gel state make this probe uniquely sensitive to small amounts of gel state phospholipids (Sklar et al., 1979).

Figure 2A depicts the fluorescence depolarization of *trans*-paranaric acid in DPPC vesicles reconstituted with or without the VS virus glycoprotein by detergent dialysis. The DPPC vesicles without the glycoprotein showed a sharp transition at 41.4 °C, with the fluorescence anisotropy increasing from 0.174 at 43 °C to 0.306 at 40 °C, in close agreement to the data by Sklar et al. (1979) for DPPC dispersions (where the data were presented as the ratio of $I_{\parallel}/I_{\perp}$). The phase transition of the DPPC–glycoprotein vesicles was decreased to 40.5 °C and occurred over a wider temperature range than that of the pure DPPC vesicles. The anisotropy difference was also smaller, with the anisotropy above the phase transition higher than that for pure DPPC and the anisotropy below the phase transition lower than for pure DPPC (Figure 2A).

Fluorescence Depolarization of Anthroyloxycerebroside. Anthroyloxy fatty acid derivatives have been used as probes of DPPC liposomes (Cadenhead et al., 1977; Tilley et al., 1979). Of the anthroyloxy fatty acids, 16-(9-anthroyloxy)-palmitic acid has been shown to have the least perturbing effect on the DPPC bilayer structure as judged by transition temperature lowering (Cadenhead et al., 1977). Linkage of 16-(9-anthroyloxy)palmitate to glucocerebroside (Figure 1B) permits a more exact knowledge of the location of the fluorescent probe within the bilayer (M. Wong, M. Correa-Freire, Y. Barenholz, and T. Thompson, unpublished results). The fluorescence depolarization of anthroyloxycerebroside reconstituted with DPPC by detergent dialysis at a probe to DPPC ratio of 1:200 is shown in Figure 2B. The DPPC phase transition monitored by anthroyloxycerebroside fluorescence depolarization was centered at 39 °C, with the anisotropy of 0.036 at 44 °C increasing to 0.124 at 34 °C. Previous work with 16-(9-anthroyloxy)palmitate in DPPC multilamellar dispersions at a lower probe to DPPC ratio of 1:500 showed similar anisotropy changes but a higher transition temperature of 41 °C (Cadenhead et al., 1977). The fluorescence anisotropy of anthroyloxycerebroside reconstituted into DPPC vesicles containing 0.34 mol % G protein demonstrated a lowering of the phase transition temperature to 35.5 °C (Figure 2B). As was the case for *trans*-paranaric acid, the anthroyloxycerebroside fluorescence anisotropy in DPPC–G protein vesicles was increased in the liquid-crystalline state and decreased in the gel state (Figure 2B).

Fluorescence Depolarization of Diphenylhexatriene. The use of diphenylhexatriene (Figure 1C) to monitor the phase transition of DPPC multilamellar dispersions has been well defined (Suurkuusk et al., 1976; Lentz et al., 1978). The phase-transition temperature monitored by diphenylhexatriene fluorescence anisotropy for DPPC detergent dialysis vesicles was 39.5 ° (Figure 2C), somewhat lower than the transition temperature of 40.6 °C reported by Lentz et al. (1978) for DPPC multilamellar dispersions. The fluorescence anisotropies in the gel and liquid-crystalline state of the DPPC vesicles were almost identical with those reported by Suurkuusk et al. (1976) for DPPC multilamellar dispersions. G protein reconstituted with DPPC at a level of 0.56 mol % lowered the transition temperature to 36.5 °C, as monitored by diphenylhexatriene fluorescence anisotropy (Figure 2C). The anisotropy of diphenylhexatriene in the DPPC gel state was decreased in the presence of the G protein, but in contrast to the results from *trans*-paranaric acid and anthroyloxycerebroside, there was no change in the fluorescence anisotropy above the phase transition in the liquid-crystalline state.

Fluorescence Depolarization of Heptadecylcoumarin. Heptadecylcoumarin (Figure 1D) has been used as a pH indicator at the phospholipid–water interface of monomolecular lipid films (Fromherz, 1973). The location of the fluorescent hydroxycoumarin group at the membrane surface provided an interesting probe of the DPPC phase transition. The fluorescence lifetime of heptadecylcoumarin in DPPC multilamellar vesicles is not significantly different between the DPPC gel and liquid-crystalline states; the fluorescence lifetimes determined by both phase and modulation methods on an SLM fluorimeter (Urbana, IL) were 4.6 ns at 50 °C and 4.8 ns at 20 °C (R. Pal, W. A. Petri, Jr., Y. Barenholz, and R. R. Wagner, unpublished results). The fluorescence anisotropy of heptadecylcoumarin for the DPPC vesicles showed a sharp transition at 39.5 °C (Figure 2D); DPPC–G protein vesicles had a phase transition of 36 °C. The heptadecylcoumarin fluorescence anisotropy was decreased for the DPPC–G protein vesicles in the gel state but, similarly to diphenylhexatriene, was unchanged in the liquid-crystalline state from the anisotropy of pure DPPC vesicles (Figure 2D).

Titration of the Heptadecylcoumarin Probe in Lipid Vesicles with or without G Protein. The heptadecylcoumarin probe was titrated with NaOH in detergent dialysis vesicles composed of total VS viral lipids reconstituted with or without 1 mol % G protein (Figure 3). Viral lipids were substituted for DPPC as the DPPC vesicles were not stable to titration

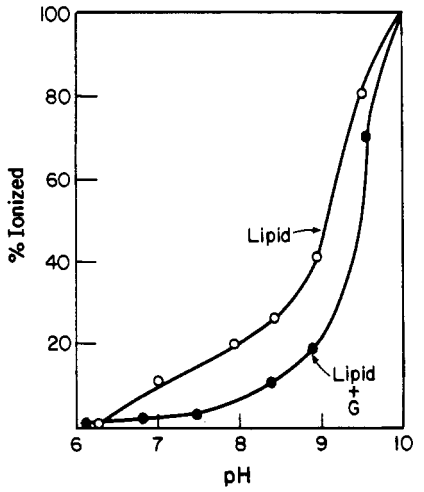

FIGURE 3: Ionization of heptadecyl-7-hydroxycoumarin in G protein–viral lipid vesicles as a function of pH. Viral lipid vesicles (○) and viral lipid vesicles containing 1 mol % G protein (●) were prepared by detergent dialysis against 50 mM KCl. The probe (1 mM) in tetrahydrofuran was added to the vesicles at a probe to phospholipid ratio of 1:200; the vesicles were incubated at 37 °C for 3 h and the excitation spectra were recorded by fixing the emission wavelength to 450 nm. The degree of ionization of heptadecyl-7-hydroxycoumarin was calculated by measuring the area of the peaks at 325 and 380 nm which represent the excitation maxima for the un-ionized and ionized molecule, respectively. The pH of the solution was varied by adding aliquots of 0.01 N NaOH, and the pH was measured in separate experiments with a pH meter.

with NaOH. The presence of G protein in the vesicles affected the probe ionization, as reflected in the apparent pK of the molecule. The apparent pK of the probe in lipid vesicles was 9.05, which was increased to 9.45 in the G protein–viral lipid vesicles (Figure 3).

Discussion

The primary findings of these studies are that the VS viral G protein reconstituted in DPPC vesicles causes a decrease in the phase-transition temperature, a disordering of the gel state, and a possible ordering of the liquid-crystalline state of DPPC. All of the fluorescent probes used demonstrated a decrease in the phase-transition temperature in the presence of G protein. The decrease was the smallest for *trans*-parinaric acid, with a 1 °C drop in the transition temperature, which was likely due to the preferential partition of the *trans*-parinaric acid into the undisturbed gel state phospholipids. The other three probes showed decreases in the transition temperature of 3–4.5 °C with 0.34–0.56 mol % G protein; these results agree with previous studies from this laboratory in which differential scanning calorimetry data showed a 2 °C drop in the DPPC transition temperature with 0.15 mol % G protein (Petri et al., 1980).

The number of molecules of DPPC removed from the phase transition by a G protein molecule was estimated by differential scanning calorimetry to be 270 ± 150, corresponding to a mol % G protein of 0.19 to 0.83. Nevertheless, it was possible to demonstrate the presence of a phase transition by fluorescence anisotropy at protein-to-lipid ratios at which the scanning calorimetry data predicted there would be no observable phase transition. There are two possible explanations for this disagreement between the fluorescence and scanning calorimetry data. First, at high protein concentrations, the G protein may self-aggregate so that it interacts with a fewer number of DPPC molecules per molecule of G protein. The transition temperature lowering caused by the G protein became progressively smaller as the G protein concentration increased, which may be a reflection of G protein aggregation (Petri et al., 1980). If the G protein did aggregate at the high protein-to-lipid ratios studied by fluorescence anisotropy, the scanning calorimetry data of 270 DPPC molecules removed from the transition per G protein would still be valid as a measure of the effect of the G protein monomer on the DPPC phase transition.

As a second explanation for this discrepancy, the fluorescence probes may not be accurately reporting the thermotropic behavior of all the DPPC in the reconstituted vesicles but may be preferentially located in relatively undisturbed DPPC domains containing little G protein. The use of four different fluorescent probes located in different areas of the membrane in this study should minimize such a possibility.

The decrease in the fluorescence anisotropy of all four probes in the DPPC gel state in the presence of G protein is evidence that the G protein disrupts the ordering of the fatty acyl chains in the gel state. This disordering of the gel phase is at least partly responsible for the decreased DPPC transition enthalpy change caused by the G protein (Petri et al., 1980). A similar disordering of the gel phase of dimyristoylphosphatidylcholine by the M13 coat protein has been seen by using *cis*-parinaric acid fluorescence intensity (Kimelman et al., 1979). The increase in anisotropy of the liquid-crystalline state of G protein–DPPC vesicles seen with *trans*-parinaric acid and anthroyloxycerebroside suggests that the G protein may additionally depress the transition enthalpy change by ordering the fatty acyl chains in the liquid-crystalline state. Diphenylhexatriene may not have detected the ordering effect of the G protein in the liquid-crystalline state because of the likely location of this probe near the highly mobile terminal methyl groups of the fatty acyl chains (Shinitzky & Barenholz, 1978). The heptadecylcoumarin which also did not detect the G protein mediated ordering of DPPC in the liquid-crystalline state is also located in a very mobile environment, as judged by its anisotropy in the gel state of only 0.15–0.18, compared to an anisotropy of 0.28 in glycerol at 5 °C (R. Pal, W. A. Petri, Jr., Y. Barenholz, and R. R. Wagner unpublished results).

The final explanation for the increase in the apparent pK of the heptadecylcoumarin in the presence of G protein cannot be resolved at this time. While it is clear from Figure 3 that the presence of the G protein results in an increase in the apparent pK, the contribution to the change in the surface potential of the membrane by the presence of the G protein and by the G protein induced change in the phospholipid packing seen by fluorescence anisotropy cannot presently be separated.

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Fluorescence Lifetime and Time-Resolved Polarization Anisotropy Studies of Acyl Chain Order and Dynamics in Lipid Bilayers†

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ABSTRACT: The time-resolved fluorescence intensity and anisotropy decays of *cis*- and *trans*-parinaric acids and phosphatidylcholines labeled with *trans*-parinaric acid have been characterized in bilayers formed by several phosphatidylcholines and by dipalmitoylphosphatidylcholine–cholesterol mixtures, at several temperatures. Both a conventional free-running nitrogen flashlamp and the novel synchrotron source at the Stanford Linear Accelerator Center (SLAC) were used as excitation sources for a modified single photon counting fluorescence lifetime apparatus. The measured emission decay kinetics of both isomers of parinaric acid were biexponential in all but one of the lipid systems examined. The fluorescence anisotropy of parinaric acid was large and constant in gel phase lipids, but showed a very rapid (~2 GHz) decay of large amplitude in fluid lipids. In all lipid systems studied, the fluorescence anisotropy decayed to a nonzero asymptote, in striking contrast to the behavior observed in viscous solvent solutions. The asymptotic anisotropy was used to calculate an "order parameter" of the emission transition dipole. The value of the order parameter is quite close to that obtained by deuterium NMR. Cholesterol increased the order parameter measured in fluid dipalmitoylphosphatidylcholine but did not substantially affect the rate of angular relaxation. Experiments conducted with *trans*-parinaroylphosphatidylcholines yielded results virtually identical with those obtained with *trans*-parinaric acid.

Investigations of the dynamic and order properties of the angular degrees of freedom of acyl chains in phospholipid bilayers are important for several reasons. The phospholipid bilayer is the foundation of biological membranes, and the phospholipid acyl chains form the environment of many functionally important membrane proteins. Phospholipid bilayers are also intrinsically interesting structures. They can be modeled theoretically only after extending or rethinking techniques for dealing with isotropic three-dimensional systems. The existence of phospholipid phase transitions, involving collective disordering of the acyl chains, expands the scope and content of such modeling by introducing phase equilibria and large changes in order and dynamics. Finally, most of our knowledge of the effect of other membrane lipids (e.g., cholesterol) and membrane proteins on the structure of lipid bilayers comes from studies of their effect on the acyl chain dynamics and order.

The properties of acyl chains in bilayers have been investigated with a broad spectrum of techniques, including X-ray scattering (Rand et al., 1975; Janiak et al., 1976; Brady & Fein, 1977), ^{2}H NMR (Seelig & Seelig, 1974a,b; Brown & Seelig, 1979; Stockton & Smith, 1976; Oldfield et al., 1978; Davis, 1979; Brown, 1979), ^{1}H NMR (Hemminga & Berendson, 1972; Seiter & Chan, 1973), nitroxide ESR (Hubbell & McConnell, 1971; Jost et al., 1971), polarized, time-resolved fluorometry (Chen et al., 1977; Kawato et al., 1977), and phase fluorimetry (Lakowicz et al., 1979). The overall trends seen by magnetic resonance techniques have been recently reviewed by Bocian & Chan (1978), and the results of ESR and deuterium NMR have been compared by Gaffney & McConnell (1974).

Polarized, time-resolved fluorometric techniques have several advantages over other methods. First, both dynamic and order information can be extracted from the decay data in a straightforward way. Since experiments are performed in the time rather than frequency domain, the dynamic information

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