Calorimetric and Fluorescence Characterization of Interactions between Cytochrome b_5 and Phosphatidylcholine Bilayers†

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ABSTRACT: The interactions of cytochrome b_5 with dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine lipid bilayers have been studied with high-sensitivity differential scanning calorimetry and fluorescence spectroscopy. The incorporation of cytochrome b_5 into large single lamellar vesicles causes a reduction in the enthalpy change associated with the lipid phase transition. Analysis of the dependence of this enthalpy change on the protein/lipid molar ratio indicates that each cytochrome b_5 molecule prevents 14 ± 1 lipid molecules from participating in the gel to liquid-crystalline transition and that this number is independent of the phospholipid acyl chain length. Resonance energy transfer between the intrinsic tryptophan fluorescence of cytochrome b_5 and pyrenedecanoic acid indicates that, in the liquid-crystalline phase, protein and lipid molecules are uniformly distributed within the bilayer plane. In the gel phase, pyrenedecanoic acid partitions into the boundary layer lipid causing a dramatic decrease in the fluorescence intensity of cytochrome b_5. The excimer/monomer ratios of pyrenedecanoic acid decrease upon increasing the protein/lipid molar ratio, indicating that the presence of protein molecules within the bilayer slows down the lateral mobility of the lipid probes. The picture that emerges from this set of experiments is that cytochrome b_5 perturbs one layer of lipid around the hydrophobic segment of the protein and that this layer is unable to undergo the gel–liquid-crystalline transition, remaining instead in a relatively disordered configuration above and below the transition temperature of the bulk lipid.

The arrangement of membrane proteins within the lipid bilayer matrix as well as the interactions between protein and lipid components of biological membranes has been the subject of considerable interest in recent years. From those studies, it became evident that the incorporation of membrane proteins into lipid bilayer membranes had a perturbing effect on the structural state of the surrounding lipid and that this perturbation might have profound effects on the properties and behavior of the membrane. The availability of purified membrane proteins that can be reconstituted into well-characterized lipid vesicles has permitted the use of a variety of physical techniques directed to probe the nature of the protein–lipid interaction (Curatolo et al., 1977; Chapman et al., 1979; Marsh et al., 1982). In this paper, we present the results of a calorimetric and fluorescence investigation of the interactions of cytochrome b_5 with single lamellar vesicles prepared from dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine.

Cytochrome b_5 is an intrinsic membrane protein with a molecular weight of 16 700 (Spatz & Strittmatter, 1971); it consists of two independent domains, a hydrophilic region that bears the heme group and a functionally distinct hydrophobic domain that anchors the molecule to the membrane (Visser et al., 1975; Spatz & Strittmatter, 1971; Strittmatter et al., 1972). Cytochrome b_5 is mainly found in the endoplasmic reticulum of liver cells (Jarasch et al., 1979) and can be isolated by detergent extraction. The isolated lipid-free protein binds rapidly and completely to preformed phosphatidylcholine vesicles and other membranes (Strittmatter et al., 1972; Sullivan & Holloway, 1973; Roseman et al., 1978; Leto & Holloway, 1979). It has also been documented (Roseman et al., 1977; Enoch et al., 1977; Leto et al., 1980) that cytochrome b_5 exchanges rapidly between phosphatidylcholine vesicles and other membrane systems. This intermembrane exchange of an intrinsic membrane protein may prove to be of importance in a variety of biological processes including the assembly of biomembranes within the cell.

The studies presented in this paper describe the effects of cytochrome b_5 on the thermotropic behavior of the lipid bilayer and evaluate the nature and magnitude of the lipid perturbation exerted by the protein. This investigation has been accomplished by combining high-sensitivity scanning calorimetry with fluorescence measurements that measure energy transfer between the intrinsic tryptophan fluorescence of cytochrome b_5 and pyrenedecanoic acid probes embedded within the bilayer and the rate of excimer formation of the pyrene probe.

Materials and Methods

Dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine were obtained from Avanti Biochemicals (Birmingham, AL) and used without further purification. Rabbit liver cytochrome b_5 was prepared by the method originally described by Ozols (1974) and modified by Leto (1980). Pyrenedecanoic acid was obtained from Molecular Probes (Plano, TX) and used without further purification. Stock solutions of pyrenedecanoic acid were prepared with tetrahydrofurane (THF) or acetonitrile as solvent and stored under nitrogen, in the dark at 4 °C.

All the vesicle preparations used for these experiments were fused unilamellar vesicles prepared essentially as described by Schullery et al. (1980). DMPC[1] or DPPC was first dried from a chloroform solution and lyophilized overnight. The dried lipid was suspended in 50 mM KCl containing 0.02% sodium azide to give a concentration of 70 mM. The lipid suspensions

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[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; Pipes, 1,4-piperazinediethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid.

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were sonicated and centrifuged above the lipid phase transition temperature and then incubated at 4 °C for 1 week as described by Wong et al. (1982). This low-temperature incubation triggers a spontaneous fusion process and produces a homogeneous population of single lamellar vesicles of ~700-Å diameter (Wong et al., 1982).

Cytochrome b_5 was incorporated into DMPC or DPPC fused unilamellar vesicles by incubating the desired amounts of protein and lipid as outlined by Leto et al. (1980). These membrane preparations were checked for degree and sidedness of protein incorporation and stability to heme loss. Aliquots containing 1 mg of lipid were layered on a Sephadex G-100 column (0.8 × 25 cm) and eluted with Pipes buffer, pH 8.1–1.0 mM EDTA. The 0.5-mL fractions were collected, and spectral scans were taken from 280 to 450 nm. The cytochrome b_5 concentrations were calculated from an extinction coefficient of 114000 mol cm^{-1} at 412 nm. A total of 50 μL of each fraction was analyzed for lipid phosphorus by the method of Bartlett (1959). The incorporation of cytochrome b_5 was found to be ~100% into the outer monolayer with less than 1% loss of heme absorption over the course of all experiments performed.

The sideness of cytochrome b_5 was checked by placing an aliquot of the stock (final concentration 20 μg of lipid/mL) in a cuvette containing 3.7 μg of purified NADH–cytochrome b_5 reductase protein (water-soluble proteolytic fragment, M_r 40000). The absorbance spectrum was recorded, and the absorbance at 412 and 425 nm was measured. The cuvette solution was made 50 mM in NADH, and the spectrum was remeasured. Additional NADH had no effect, nor did NADH without reductase present. Finally, a few crystals of dithionite (a membrane-permeable reductant) were added to the cuvette, and no further reduction was observed (<2%), indicating that all the cytochrome b_5 was located in the outer layer of the vesicles.

The calorimetric experiments were performed with a scanning calorimeter of the heat-conduction type as described by Suurkuusk et al. (1976) at a scanning rate of 15 °C/h or alternatively with a Microcal MCI differential scanning calorimeter at a scanning rate of 20 °C/h. Lipid concentrations for the calorimetric experiments ranged between 5 and 10 mg/mL.

All temperature scanning fluorescence experiments were performed in a modified Aminco Bowman spectrofluorometer equipped with a photon counter and a thermostated cuvette holder connected to a Neslab RTE-8 refrigerated bath circulator equipped with a temperature programmer (ETP-3). The temperature of the sample preparations was monitored during the experiments with a thermistor immersed inside the sample cell. Fluorescence intensities and temperatures were simultaneously recorded on a four-channel Hewlett-Packard 3467A logging multimeter. Fluorescence spectra were measured with an SLM 4800 spectrofluorometer.

Resonance energy transfer experiments between cytochrome b_5 and pyrenedecanoic acid were performed by measuring the fluorescence emission spectrum of cytochrome b_5 incorporated into phospholipid vesicles (excited at 280 nm) at increasing concentrations of pyrenedecanoic acid. The measured intensities were corrected by subtracting the signal obtained from similar vesicle preparations containing equal amounts of pyrenedecanoic acid but without cytochrome b_5.

All resonance energy transfer experiments were analyzed with the computer using the method of Monte Carlo calculations developed by Freire & Snyder (1982). This method allows generation of protein–lipid distributions for arbitrary protein–lipid compositions, protein dimensions, and intermolecular potentials. Once a particular protein distribution is obtained, the computer is instructed to distribute a preselected number of probe molecules among the lipid molecules; in this way, the entire ensemble of position coordinates for the donor and acceptor groups can be generated. Having the position coordinates of donors and acceptors, it is possible to calculate the spectral properties of the system in a straightforward manner. The ratio of the fluorescence intensity of the donor in the presence of acceptors to that of the donor alone is given by the expression

$$\frac{Q_{DA}}{Q_D} = \frac{1}{N_D}\sum_i \frac{1}{1 + \sum_j (R_0/R_{ij})^6}$$

where R_0 is the distance of half-transfer efficiency and R_{ij} is the distance between the ith donor and jth acceptor (Snyder & Freire, 1982). The advantages of this method over conventional analytical calculations is that no special assumptions or approximations are required in order to generate statistically precise quenching ratios for arbitrary donor–acceptor distributions. In fact, this method can be used to test the accuracy of approximate analytical treatments existing in the literature (Snyder & Freire, 1982). The main source of error in these calculations is the assumption of a random orientation of donors and acceptors that introduces an error of up to ~10% in R_0. Complete details are given in Synder & Freire (1982). This assumption is, however, not unique to this method.

Results

Scanning Calorimetry. Scanning calorimetric scans were performed on DMPC and DPPC large unilamellar vesicles containing different mole fractions of cytochrome b_5. These large unilamellar vesicles give rise to very sharp peaks in the calorimeter, similar to those observed with multilamellar liposomes. The gel–liquid-crystalline phase transition of these vesicles is characterized by a ΔH of 5.4 kcal/mol and a T_m of 24.1 °C for DMPC and a ΔH of 6.8 kcal/mol and a T_m of 41.2 °C for DPPC. The half-height widths ($\Delta T_{1/2}$) are 0.4 and 0.7 °C for DPPC and DMPC, respectively. As shown in Figure 1, the incorporation of cytochrome b_5 into these vesicles causes a monotonic decrease in the enthalpy change (area under the heat capacity curve) for the gel–liquid-crystalline transition but only a negligible change in the phase transition temperature. It should also be mentioned that the half-height width of the transition increases only 0.1 °C up to a protein/lipid molar ratio of 1/100, indicating that cytochrome b_5 does not largely disrupt the cooperative behavior of the lipid bilayer matrix. Only at protein/lipid ratios higher than 1/50 does the heat capacity function become skewed toward the low-temperature end of the transition, apparently due to the existence of two superimposed peaks, a sharp component having the transition characteristics of the pure lipid and a broader component centered at a somewhat lower temperature. As seen in Figure 1, the incorporation of cytochrome b_5 also affects the phospholipid pretransition; it gradually diminishes its amplitude until it is not longer detectable by the calorimeter at protein/lipid mole ratios greater than 1/50. The decrease in ΔH upon increasing the protein/lipid ratio indicates that the protein molecules are preventing some of the lipid molecules from undergoing the lipid phase transition. The dependence of ΔH on the protein/lipid molar ratio can be analyzed in terms of the equation (Correa-Freire et al., 1979)

$$\Delta H/\Delta H_0 = 1 - N_A(P/L)$$

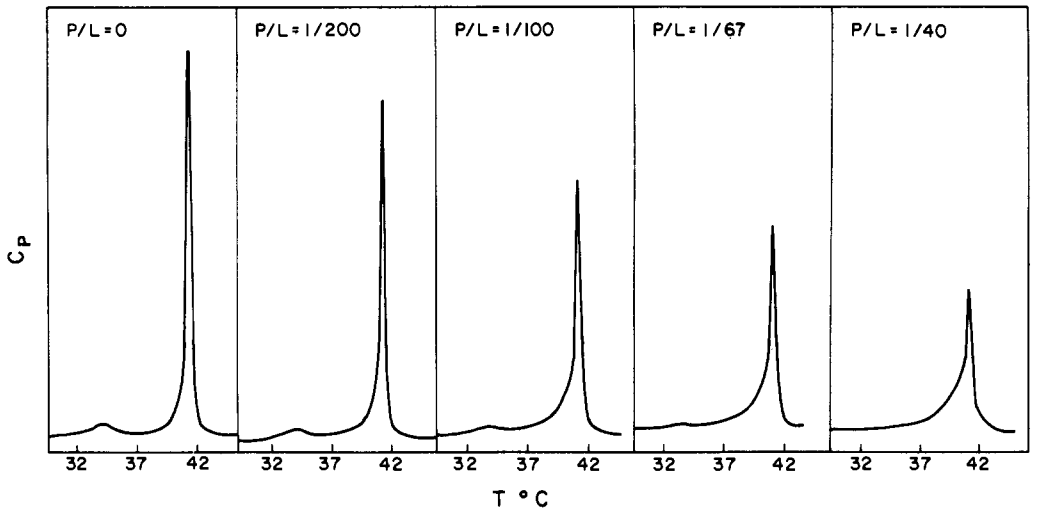

FIGURE 1: Excess heat capacity function vs. temperature for DPPC large single lamellar vesicles containing different concentrations of cytochrome b_5.

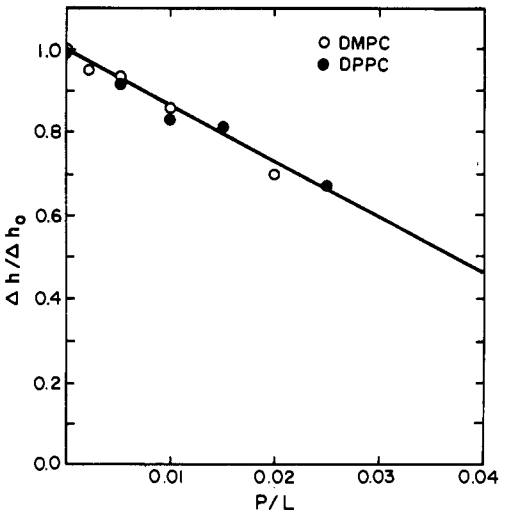

FIGURE 2: Relative decrease in enthalpy change associated with gel–liquid-crystalline transition of DMPC (○) and DPPC (●) as a function of cytochrome b_5/lipid molar ratio.

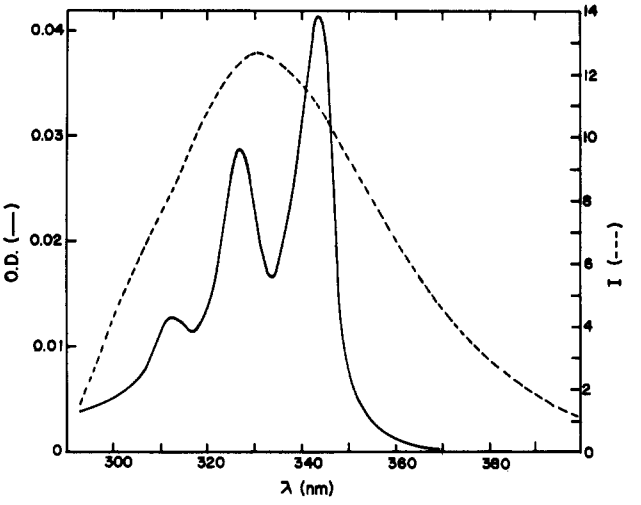

FIGURE 3: Corrected fluorescence emission spectrum of cytochrome b_5 incorporated in large DMPC single lamellar vesicles (---) and absorption spectrum of pyrenedecanoic acid (—). The excitation wavelength for the fluorescence spectrum was 280 nm. Both spectra were taken at 28 °C.

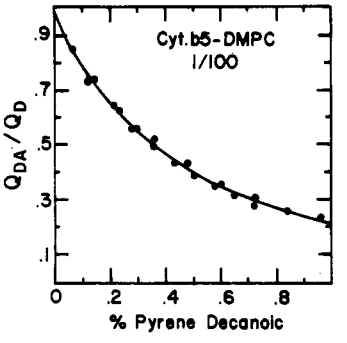

FIGURE 4: Quenching of cytochrome b_5 tryptophan intrinsic fluorescence upon addition of pyrenedecanoic acid. The solid line is the theoretical curve calculated with Monte Carlo methods on the assumption of a uniform distribution of cytochrome b_5 on the bilayer surface. The excitation wavelength was 280 nm, and the emission wavelength was 340 nm. The lipid concentration was 58 μM.

where ΔH_0 is the enthalpy change in the absence of protein and N_A is the mean number of lipid molecules prevented from participating in the gel–liquid-crystalline phase transition per protein molecule. As shown in Figure 2, a linear least-squares analysis of the data for both DMPC and DPPC indicates that each cytochrome b_5 molecule subtracts 14 ± 1 lipid molecules from the phase transition.

Cleavage of the hydrophilic domain of cytochrome b_5 by the addition of trypsin to the vesicle preparations did not affect the heat capacity profiles, indicating that the hydrophobic portion of the protein is responsible for the decrease in ΔH and that the hydrophilic domain interacts very little if at all with the membrane surface. This result is consistent with the proposed three-dimensional structure of cytochrome b_5 in which the hydrophilic domain is separated from the hydrophobic domain by a joining segment of approximately nine amino acid residues (Strittmatter & Dailey, 1982). This result is also consistent with the observation that cytochrome b_5 has only a minor effect on the cooperative behavior of the lipid phase transition, as expected from the interaction with the small hydrophobic domain and not from the relatively bulky hydrophilic region.

Resonance Energy Transfer. As shown in Figure 3 there is a very strong overlap between the fluorescence emission spectrum of cytochrome b_5 and the absorption spectrum of pyrenedecanoic acid, thus allowing energy-transfer experiments directed to assess the planar distribution of these molecules within the bilayer. The low wavelength of the cytochrome b_5 tryptophan emission (330 nm) supports the conclusion that

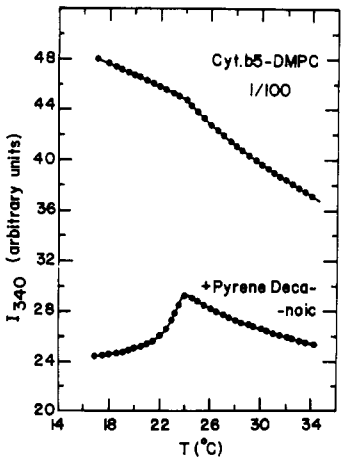

FIGURE 5: Temperature dependence of cytochrome b_5 fluorescence in the absence and in the presence of pyrenedecanoic acid. Note the large change in fluorescence intensity at T_m for the sample containing pyrenedecanoic acid. Below T_m, pyrenedecanoic acid preferentially partitions into the boundary-layer lipid whereas above T_m pyrenedecanoic acid is uniformly distributed within the bilayer. The excitation wavelength was 280 nm and the lipid concentration 58 μM. The amount of pyrenedecanoic acid in the bottom curve is 0.3 mol %.

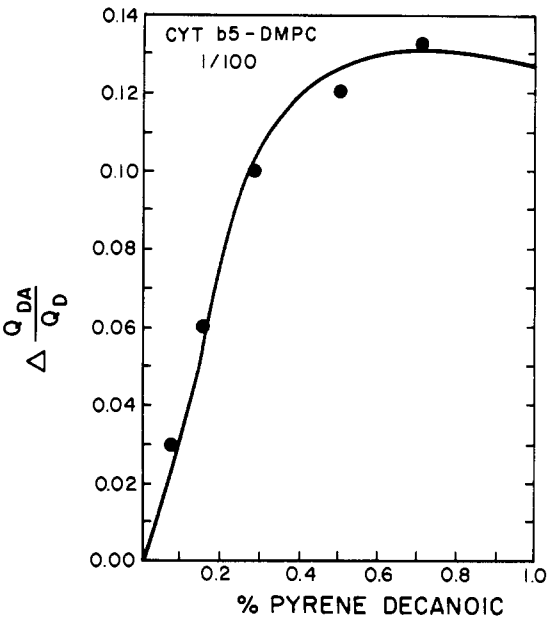

FIGURE 6: Differential change in cytochrome b_5 fluorescence above and below T_m as a function of pyrenedecanoic acid mole fraction. The solid line is the theoretical curve, on the assumption that below T_m pyrenedecanoic acid partitions into the boundary-layer lipid (see text for details). For these experiments the excitation wavelength was 280 nm and the emission wavelength 340 nm. The lipid concentration was 58 μM.

this residue is located in a hydrophobic environment, probably 20–22 Å from the bilayer surface (Strittmatter & Dailey, 1982). Figure 4 shows a typical quenching profile for cytochrome b_5 incorporated into DMPC vesicles at a mole ratio of 1/100 and 30 °C as a function of the mole fraction of pyrenedecanoic acid. As shown in the figure, pyrenedecanoic acid concentrations of less than 1 mol % are required to obtain the entire quenching curve. These low concentrations of lipid probe minimize any possible perturbation of the membrane induced by the probes themselves. The solid line in the figure is the theoretical curve expected for a uniform distribution of proteins and lipid probes within the membrane surface. The theoretical curve was calculated as described by Snyder & Freire (1982) by using Monte Carlo techniques and assuming an R_0 value of 42 Å.

Figure 5 shows the effects of the lipid phase transition on the tryptophan emission spectrum of cytochrome b_5. In the absence of pyrenedecanoic acid or any other fluorescent probe, the lipid phase transition is visible only as a small break in the emission vs. temperature profile. A similar effect has previously been reported for the M13 viral coat protein incorporated into DMPC vesicles by Kimelman et al. (1979). In the presence of pyrenedecanoic acid, however, there is a large decrease in the emission intensity of the protein fluorescence upon cooling the system from the liquid-crystalline to the gel phase. The significantly larger amount of quenching existing below the phase transition temperature indicates that in the gel phase the pyrene lipid probes are closer to the cytochrome b_5 molecules than in the liquid-crystalline phase; i.e., below T_m pyrenedecanoic acid preferentially partitions into the boundary-layer lipid. Since the partition coefficient of pyrene fatty acid derivatives is much larger in the liquid-crystalline phase than in the gel phase (Galla & Sackmann, 1974, 1975; Galla & Hartmann, 1980), this increased concentration of pyrenedecanoic acid around the protein molecules suggests that the boundary-layer lipid remains in a disordered conformation below the phase transition.

Figure 6 shows the dependence of the amplitude of the quenching change associated with the lipid-phase transition as a function of the mole fraction of pyrenedecanoic acid. The quantity plotted, $\Delta Q_{DA}/Q_D$, is defined as the difference in tryptophan quenching above and below the lipid phase transition temperature and is calculated from the equation:

$$\Delta \frac{Q_{DA}}{Q_D} = \left.\frac{Q_{DA}}{Q_D}\right|_{T>T_m} - \left.\frac{Q_{DA}}{Q_D}\right|_{T<T_m}$$

Below T_m, pyrenedecanoic acid partitions into the boundary-layer lipid, causing an increase in the tryptophan quenching. As shown in Figure 6, the magnitude of this increase is proportional to the amount of pyrenedecanoic acid present in the bilayer. The solid line in Figure 6 is the expected concentration dependence obtained by Monte Carlo calculations assuming that, above T_m, cytochrome b_5 and lipid probes are uniformly distributed within the bilayer (see Figure 4) and that, below T_m, pyrenedecanoic acid partitions into a layer one lipid thick around the protein molecules. For these calculations the cross-sectional radius of the hydrophobic demain of cytochrome b_5 was assumed to be 16 Å (Visser et al., 1975; Vaz et al., 1979).

Excimer Formation. The technique of excimer formation using pyrene derivatives embedded within the lipid bilayer matrix is well documented in the literature [see Galla & Hartmann (1980) for a recent review] and has been used to investigate lipid lateral mobilities as well as phase-separation phenomena. Figure 7 shows the temperature dependence of the ratio I'/I of the fluorescence intensities of pyrenedecanoic acid measured at the maxima of the excimer (I') and monomer bands (I) for several cytochrome b_5/DPPC ratios. If the system is cooled from above to below the lipid phase transition temperature, there is an increase in the excimer/monomer ratio at T_m. In the absence of protein this increase is relatively large and is due to the fact that pyrenedecanoic acid is excluded from the gel phase and concentrated in the remaining fluid regions of the bilayer; finally, as the entire bilayer becomes solid, pyrenedecanoic acid aggregates forming static dimers whose properties are not diffusion controlled (Galla & Sackmann, 1974, 1975). As the protein/lipid ratio increases, the amplitude of the change in the excimer/monomer ratio associated with the lipid phase transition gradually decreases.

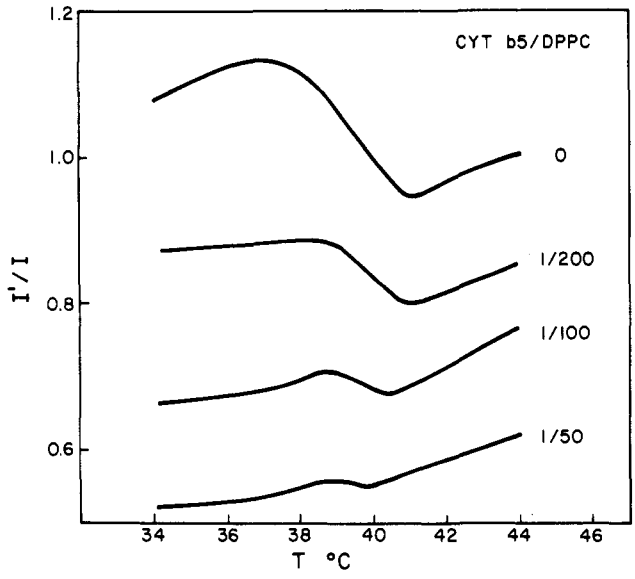

FIGURE 7: Excimer/monomer ratios of pyrenedecanoic acid vs. temperature for DPPC large single lamellar vesicles containing different amounts of cytochrome b_5. In these experiments the samples were excited at 320 nm, and the emissions of monomer and dimer were taken at their maxima at 395 and 480 nm, respectively. The lipid concentration was 50 μM, and the amount of pyrenedecanoic acid was 5 mol %.

This result is consistent with the calorimetric and fluorescence energy transfer data, which indicate that the presence of protein molecules prevents some of the lipid molecules from participating in the gel–liquid-crystalline transition and that this lipid remains in a disordered configuration at all temperatures. As the concentration of protein increases, more lipid remains in this disordered state below T_m, thus preventing the aggregation of pyrenedecanoic acid. This protein effect is very similar to the one observed after addition of cholesterol into the lipid bilayer (Estep et al., 1978; Galla & Hartmann, 1980).

Above the lipid phase transition the formation of excited dimers is a diffusion-controlled process as demonstrated by Galla & Sackmann (1974). Under these conditions the addition of cytochrome b_5 causes a progressive decrease in the excimer/monomer ratios (see Figure 6). Since the ratio (I'/I) is directly proportional to the lateral diffusion coefficient of the lipid probes within the membrane, this result indicates that the presence of cytochrome b_5 slows down the free movement of the probes. Monte Carlo calculations of lipid lateral diffusion (W. Van Osdol and E. Freire, unpublished results) indicate that this effect can be interpreted in terms of the proteins acting as obstacles or barriers to the free diffusion of the lipids without being necessary to assume a major structural perturbation of the lipid bilayer matrix.

Discussion

The calorimetric results presented in this paper indicate that the major effect of cytochrome b_5 on the thermotropic behavior of the lipid bilayer is a progressive reduction in the enthalpy change associated with the gel–liquid-crystalline phase transition. This effect is similar to the one observed with other membrane proteins (Chapman et al., 1979; Curatolo et al., 1977), suggesting the existence of a rather general and nonspecific perturbing effect. The dependence of the enthalpy decrease upon the protein/lipid molar ratio indicates that each cytochrome b_5 molecule prevents 14 ± 1 phospholipid molecules from undergoing the phase transition. This number is equivalent to the number of lipid molecules in the outer monolayer of the vesicle that can be fit in a single layer around a cylinder of 16-Å radius. Vas et al. (1979) have previously estimated a Stokes radius of ~16 Å for the cytochrome b_5 hydrophobic tail; Visser et al. (1975) calculated a Stokes radius of 17.4 Å from hydrodynamic measurements. Thus, the calorimetric results give support to the idea that the protein perturbation of the bilayer is only local and that it extends to only one layer of lipid around the protein. Furthermore, the calorimetric data suggest that the major perturbation induced by cytochrome b_5 is in the outer layer of the membrane and that the inner layer is affected very little if at all. The existence of two superimposed peaks at high protein/lipid ratios might be the result of such molecular arrangement. If such is the case, the sharp component would correspond to the inner layer of the bilayer whereas the broad component would correspond to the largely perturbed outer layer. Since cytochrome b_5 is able to rapidly exchange on this time scale between lipid vesicles (Roseman et al., 1977; Leto et al., 1980), it is very unlikely that the two overlapping peaks observed with the calorimeter arise from an uneven distribution of the protein among the lipid vesicles.

The lipid molecules surrounding the protein molecule are unable to undergo the gel–liquid-crystalline transition, remaining in the same physical state below and above the transition temperature of the bulk lipid. The boundary-layer lipid appears to be in a relatively disordered state, judging from the fact that, below T_m, pyrenedecanoic acid is excluded from the bulk lipid and partitions around the protein molecules. This conclusion is consistent with the observations of Kimelman et al. (1979) for the M13 coat protein using *trans*-parinaric acid as a lipid probe; these authors observed that, below T_m, *trans*-parinaric acid was excluded from the boundary-layer lipid due to its preferential partioning in the gel phase. Thus, the protein molecules appear to be surrounded by a relatively disordered layer of lipid whose structural state is somewhat insensitive to the physical state of the rest of the membrane. These conclusions agree with deuterium nuclear magnetic resonance experiments on cytochrome b_5–DMPC bilayers that indicate that, below T_m, the presence of the protein prevents some of the lipid molecules from undergoing crystallization (Oldfield et al., 1978).

Even though the perturbations exerted by individual protein molecules are only local, the superposition of these local perturbations gives rise to macroscopic effects that affect the properties and behavior of the lipid bilayer as a whole. As discussed above, the incorporation of protein molecules into the lipid bilayer slows down the lateral diffusion of the lipid probes even in the absence of a major structural change in the lipid bilayer. This is due to a reduced collision frequency of the lipid probes induced by the presence of the protein molecules within the bilayer. The presence of protein molecules, acting as obstacles for the free lateral diffusion of the lipid molecules, causes an increase in the mean number of diffusional steps required for the collision of two lipid probes and results in a reduced rate of excimer formation. It must be noted that the incorporation of cytochrome b_5 into the lipid bilayer does not affect the excimer fluorescence lifetime of pyrenedecanoic acid (C. Rigell, S. Georghiou, and E. Freire, unpublished results), in agreement with the interpretation that the reduced rate of excimer formation observed in the presence of the protein is a purely diffusional phenomenon.

This effect of the protein molecules on the collision frequency of other molecules embedded within the bilayer will affect the reaction rates of second-order and higher order reactions within the membrane. Also, since this effect arises from the topological distribution of molecules along the plane of the membrane, changes in the state of aggregation of

membrane proteins as well as the formation of phase-separated domains will undoubtedly affect the efficiency and kinetic parameters of membrane-located biochemical reactions.

Registry No. Cytochrome b_5, 9035-39-6; DMPC, 13699-48-4; DPPC, 2644-64-6.

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Chlortetracycline as a Probe of Membrane-Associated Calcium and Magnesium: Interaction with Red Cell Membranes, Phospholipids, and Proteins Monitored by Fluorescence and Circular Dichroism†

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ABSTRACT: The fluorescence emission and circular dichroism spectra of chlortetracycline (CTC) have been measured, including the effects of multivalent cations (Ca, Mg, La), of medium polarity, and of interaction with human red cell membranes, lipids, and a variety of proteins. An obligatory role of Ca in the association of CTC with membranes was demonstrated. Binding and kinetic constants for the CTC–Ca chelate interaction with membranes and phospholipids were determined. The results suggest that the CTC–Ca chelate fluorescence is greatly enhanced in the vicinity of membrane phospholipid head groups. The circular dichroism spectra indicate a number of distinct CTC conformations corresponding to chelation of specific cations, to interaction with membranes and phospholipids, and to medium polarity. The high quantum yield CTC–Ca conformation associated with membranes or phospholipids was identified by its characteristic circular dichroism spectrum and is different from the CTC–Ca conformation in nonpolar media (80% methanol).

Membrane calcium translocation is involved in many aspects of cell physiology including cellular secretion, muscle contraction, mitochondrial function, and membrane transduction of hormonal messages. The use of tetracyclines as a fluorescent probe of membrane-associated calcium and magnesium was first proposed by Caswell and Hutchison in 1971, and there have been many applications to a variety of functioning membrane systems since then. We have previously used chlortetracycline (CTC)[1] fluorescence to probe membrane

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[1] Abbreviations: CTC, chlortetracycline; RCM, red cell membrane(s); CD, circular dichroism; LL, lysolecithin; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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