Lateral Diffusion of Cytochrome P-450 in Phospholipid Bilayers†

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ABSTRACT: The lateral diffusion coefficient (D) of cytochrome P-450 (P-450) has been measured in lipid multibilayers with the method of fluorescence recovery after photobleaching. In the liquid-crystal phase of egg phosphatidylcholine (EPC) and dimyristoylphosphatidylcholine (DMPC), the diffusion of P-450 is fast with D about 2×10^{-8} cm^2/s. In DMPC multibilayers, P-450 diffusion dropped by a factor of 20 near the liquid crystal to gel phase transition region, and D is about 5×10^{-10} cm^2/s in the gel phase. A value of 50 mol % of cholesterol reduced the diffusion of P-450 in the liquid-crystal phase only slightly but enhanced the diffusion of P-450 in the gel phase significantly. In EPC membranes, P-450 diffusion underwent a stepwise drop as the cholesterol contents increased from 20 to 30 mol %. With the assumption of a lateral diffusion mediated electron transfer between P-450 and NADPH-P-450 reductase and with $D = 2.5 \times 10^{-8}$ cm^2/s for both enzymes, the reduction rate for P-450 in liposomes was calculated and compared with the reported experimental value.

It is now well established that integral membrane components, such as lipids and proteins, can diffuse laterally. Subjects concerning lateral diffusion have been extensively reviewed recently (Edidin, 1974; Cherry, 1979; Peters, 1981). Most measurements were made on cell surfaces where the complexity of cellular structure makes the data interpretation difficult. As an alternative, lateral diffusion can be studied in well-defined lipid model membranes where parameters affecting the lateral diffusion can be controlled and individually examined. Several laboratories have measured the lateral diffusion of proteins in model membranes, among which are peptides gramicidin S (Wu et al., 1978) and gramicidin C (Tank et al., 1982), protein apoC-III (Vaz et al., 1979), M-13 coat protein (Smith et al., 1979), lipophilin (Derzko & Jacobson, 1978), and glycophorins (Vaz et al., 1981; Wu et al., 1981). In this paper, we report our recent measurement of the lateral diffusion of cytochrome P-450 (P-450)[1] in lipid multibilayers.

Cytochrome P-450 is a component enzyme of the hepatic microsomal monooxygenase system. This membrane-bound enzyme system catalyzes the biotransformation of a wide array of exogenous and endogenous substrates (Conney, 1967; Gillette et al., 1972; Sato & Omura, 1978). In this system, the reducing equivalent from NADPH is transferred through NADPH-P-450 reductase to P-450; the reduced form of P-450 then catalyzes the oxygenation of various substrates by molecular oxygen (White & Coon, 1980). In rat liver microsomes, the number of P-450 molecules is about 20–30 times greater than that of the reductase (Estabrook et al., 1976). The organization and interaction of these enzymes molecules in biomembranes have been extensively studied (Estabrook et al., 1976; Peterson et al., 1976; Yang, 1975, 1977; DePierre & Ernster, 1977). Existing data strongly suggest that monooxygenase enzymes can move laterally in the membrane (Yang, 1975, 1977; Yang & Strickhart, 1975; Miwa et al., 1979; Taniguchi et al., 1979), but direct evidence of lateral diffusion has not been demonstrated. In this study, fluorescently labeled cytochrome P-450 was reconstituted in multibilayers formed of dimyristoylphosphatidylcholine (DMPC), egg phosphatidylcholine (EPC), and their cholesterol mixtures. The lateral diffusion of P-450 was then studied by the method of fluorescence recovery after photobleaching (FRAP) (Axelrod et al., 1976; Jacobson et al., 1976a; Zagyansky & Edidin, 1976; Edidin et al., 1976).

Materials and Methods

Materials. DMPC and EPC were obtained from Sigma, and cholesterol was supplied by Applied Science. These lipids were used without further purification. Fluorescently labeled lipid, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE), was purchased from Avanti Biochemicals. Lipids and lipid/cholesterol mixtures were stored at −20 °C under N_2 in a methanol/chloroform mixture (1/1 v/v). All other chemicals were reagent grade, and water was double distilled.

Purification and Fluorescence Labeling of P-450. P-450 was purified by the procedure of West et al. (1979) from liver microsomes of male Long-Evans rats (body weight 100–125 g) that had received daily injections of phenobarbital (75 mg/kg of body weight) for 4 days. The enzyme preparation contained 13–15 nmol of P-450/mg of protein and moved as a single band with an estimated molecular weight of 52 000 in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Baskin & Yang, 1980). The sulfhydryl group directed fluorescein maleimide (Molecular Probes) was used to label P-450. Usually, 0.3 mg of this reagent was allowed to react with 130 nmol of P-450 at room temperature for 90 min in 2 mL of 0.05 M phosphate buffer, pH 7.4, containing 20% glycerol. The modified protein was separated from the labeling reagent by Sephadex G-25 gel chromatography in a 1.5 × 40 cm column. The protein fractions were pooled and dialyzed overnight against the same buffer containing 10% glycerol. The amounts of fluorescein and P-450 in the labeled protein were quantitated by A_{496nm} and A_{417nm}, respectively. The ratio between A_{496nm} and A_{417nm} did not change after a second gel chromatography but decreased slightly upon dialyzing for 2 or 3 days, probably due to the degradation of the fluorescein

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[1] Abbreviations: P-450, cytochrome P-450; DMPC, dimyristoylphosphatidylcholine; EPC, egg phosphatidylcholine; FRAP, fluorescence recovery after photobleaching; NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine; T_m, liquid crystal to gel phase transition temperature; D, lateral diffusion coefficient; MBL, multibilayer; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

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chromophore. The number of fluorescein molecules bound to P-450 was dependent on the concentration of the reagent used. Under the aforementioned conditions, the labeled protein contained one molecule of fluorescein per molecule of P-450. The samples were stored frozen prior to use.

P-450 Reconstitution and Formation of Lipid Multibilayers. Lipids and lipid/cholesterol were brought to room temperature prior to use. To prepare multibilayers, 1.5 μmol of lipids (or lipid/cholesterol) was deposited on a clean microscope slide in an area of diameter 0.8–1 cm. Organic solvent was removed by evaporating the sample under dry N_2 and then in a vacuum for 2 h. Reconstitution of P-450 was achieved by gently spreading 12 μL of stock protein solution (26.5 nmol/mL in 10% glycerol solution) over the solvent-free lipid film. The sample was then incubated for 30 min in a water-saturated chamber at 30 °C for DMPC and at room temperature for EPC. At the end of incubation, excess water on the sample was removed with tissue paper. To form oriented multibilayers, a 20 × 30 mm glass cover slip was pressed over the hydrated lipid for 5 min. The edges of the cover slip were immediately sealed with paraffin to prevent dehydration. Sealed DMPC samples were cycled through the phase-transition temperature (T_m) several times. The samples were used within 48 h.

Fluorescent lipid NBD-PE was introduced in the multibilayers by premixing with lipids prior to sample preparation. A constant NBD-PE to lipid ratio of 1/4000 was maintained in all the samples. Glycerol was incorporated in the sample during the hydration process by incubating the lipid film with water containing various amounts of glycerol.

Fluorescence Recovery after Photobleaching. Details of FRAP methods have been described and reviewed extensively (Axelrod et al., 1976; Jacobson et al., 1976b; Cherry, 1979). A brief description of the apparatus and experimental parameters used in this work is presented below.

The 488-nm emission of a Spectra Physics argon laser (Model 165) was used as the fluorescence excitation source. The laser beam was focused through an Olympus Vanox microscope with a vertical illuminator on the multibilayer samples. For a 40× objective, the radius of the laser beam at the specimen plane is about 1.1 μm with an uncertainty of 20%. The fluorescence was measured with a cooled photomultiplier (ITT, FW-130). The amplified and photon-discriminated signal was either stored in a multichannel analyzer (Northern Traco, 702N) or displayed on a photon counter (Ortec, 6903), depending on whether it was a fast ($D > 10^{-9}$ cm^2/s) or a slow ($D < 10^{-9}$ cm^2/s) diffusional process. In the former case, the stored recovery curve was subsequently least-squares fitted to the recovery equation of Axelrod et al. (1976) to obtain the diffusion coefficient. In the latter case, the output of the photon counter was plotted with a strip chart recorder, and the recorded recovery curve was visually analyzed for the diffusion coefficient.

In a typical experiment, three to four flat areas in a given sample were randomly selected for FRAP measurements. In the case of fast recovery, photobleaching was repeated 6–10 times at the same spot of a selected area, and the recovery curves were electronically averaged and then stored for further analysis. In the case of slow diffusion, photobleaching was repeated twice at the same spot, and both recovery curves were stored and analyzed independently. Diffusion coefficients determined from these stored recovery curves were then combined with coefficients determined similarly for other flat areas to yield a single diffusion coefficient for the sample at the given temperature. All measurements were repeated on at least three independently prepared samples. Error bars shown in the figures represent the standard deviations; they do not include the error resulting from the 20% uncertainty in the beam radius.

Results

Morphology of Multibilayers. P-450-containing multibilayers prepared as described above, when examined with cross-polarized microscopy, exhibited familiar flat blocks (Wu et al., 1977; Asher & Pershan, 1979) surrounded by strong birefringent boundaries. Most of these flat blocks were about 15–20 μm in size, which is small as compared to the multibilayers prepared without P-450, but such size was sufficient for FRAP measurements. Fluorescence in flat blocks was very weak but appeared to be uniform. Protein aggregation was noticed in birefringent boundaries for most samples. As a result, the exact amount of protein that had been incorporated in multibilayers was not known. In the gel phase of DMPC, two different morphologies were noticed in the membranes. Visualized under the cross-polarized microscope, one type appeared to be smooth, and the other type showed a pebbling appearance (Derzko & Jacobson, 1980). FRAP measurements reported here were made on smooth membranes only. Multibilayer morphology was not affected by the incorporation of cholesterol. No exclusion of proteins from membrane by high cholesterol concentration was noticed (Wu et al., 1978; Tank et al., 1982).

Lateral Diffusion of P-450 in DMPC Multibilayers. In the liquid-crystal phase of DMPC, the fluorescence recovery after photobleaching was fast and almost complete. Occasionally, partial recovery was noticed in some randomly chosen flat areas, indicating the existence of immobile proteins. In these areas, repeated photobleaching at the same spot increased the extent of recovery progressively, but the recovery rate, which determines the diffusion coefficient, remained unchanged. In the gel phase of DMPC, fluorescence photobleaching resulted in partial recovery only. Frequently, we also observed a component of extremely fast fluorescence recovery superimposed on the otherwise normal recovery curve. The diffusion coefficient for this fast component was greater than 2×10^{-7} cm^2/s. We attributed this to the free diffusion of P-450 in the aqueous phase. As a precaution against this artifact, the first 15 data channels (out of 1024 channels) of the recovery curve were ignored in the data processing.

The lateral diffusion coefficient (D) of P-450 in DMPC membrane is shown in Figure 1 as a function of temperature. The D value at 25 °C was 2.5×10^{-8} cm^2/s and increased slightly at higher temperature. The diffusion coefficient of P-450 dropped by a factor of 20 as the temperature was lowered from 24 to 22 °C. Below 22 °C, further temperature decrease reduced D only slightly.

Lateral Diffusion of P-450 in DMPC Multibilayers Containing Cholesterol. The effect of cholesterol on P-450 diffusion is shown in Figure 2. In DMPC membranes with 50 mol % cholesterol, the sharp change in D near the phase-transition temperature was abolished (Figure 2, curve A). Above the phase-transition temperature, 50 mol % cholesterol lowered P-450 diffusion slightly, whereas below the phase-transition temperature cholesterol enhanced P-450 diffusion substantially. However, the activation energy for the diffusion of P-450, 8.3 kcal/mol, was comparable to those for lipid probes (Wu et al., 1977) and small peptides (Wu et al., 1978; Tank et al., 1982) in membranes of similar compositions. In DMPC multibilayers containing 25 mol % cholesterol, the lateral diffusion of P-450 increased continuously between 5 and 35 °C (Figure 2, curve B). Within this temperature range,

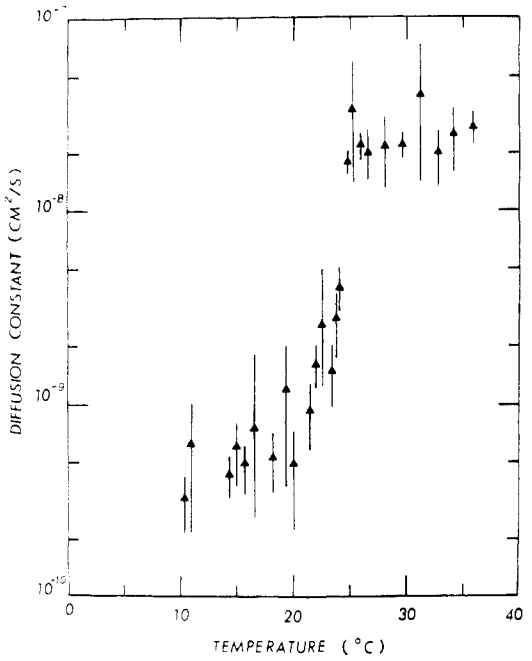

FIGURE 1: Temperature dependence of lateral diffusion coefficient of cytochrome P-450 in DMPC multibilayers. Temperature is accurate to ±0.5 °C. Error bars represent the standard deviations. The gel to liquid crystal phase transition temperature for DMPC is 23.6 °C.

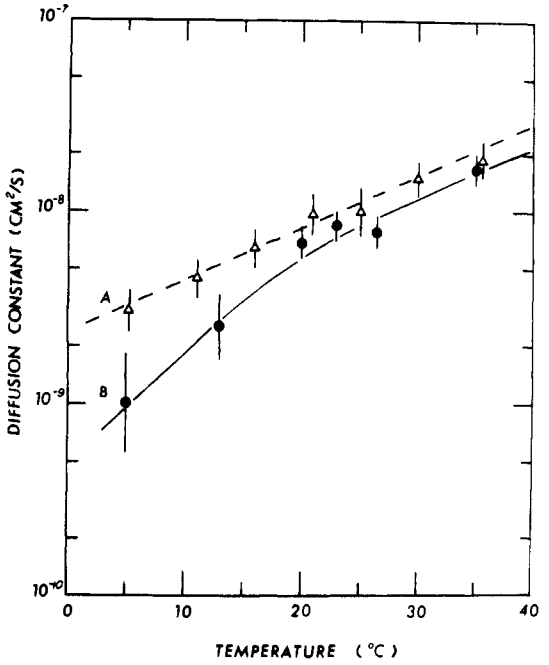

FIGURE 2: Temperature dependence of lateral diffusion coefficient of cytochrome P-450 in DMPC multibilayers containing 50 (Δ, curve A) and 25 mol % (●, curve B) cholesterol.

P-450 diffusion was slower than that in DMPC containing 50 mol % cholesterol.

Lateral Diffusion of P-450 in EPC Multibilayers. Lateral diffusion of P-450 in EPC membranes was measured between 5 and 35 °C, a temperature range in which EPC is in the liquid-crystal phase. The fluorescence recovery after photobleaching exhibited the same qualitative features as found in the liquid phase of DMPC membranes. For instance, incomplete fluorescence recovery was also found in some flat membrane blocks during the first photobleaching. The *D* value of P-450 in EPC membranes is shown in Figure 3 (curve A) as a function of temperature.

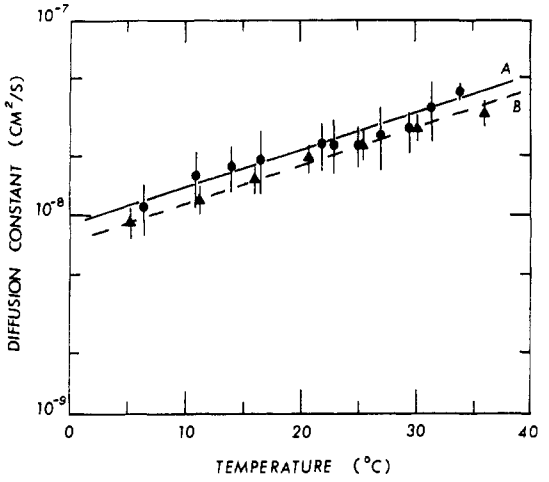

FIGURE 3: Temperature dependence of lateral diffusion coefficient of cytochrome P-450 in EPC multibilayers (●, curve A) and EPC multibilayers containing 50 mol % cholesterol (▲, curve B). In the temperature range covered, EPC is in the liquid-crystal state. The activation energy for both curves is about 8 kcal/(mol·deg).

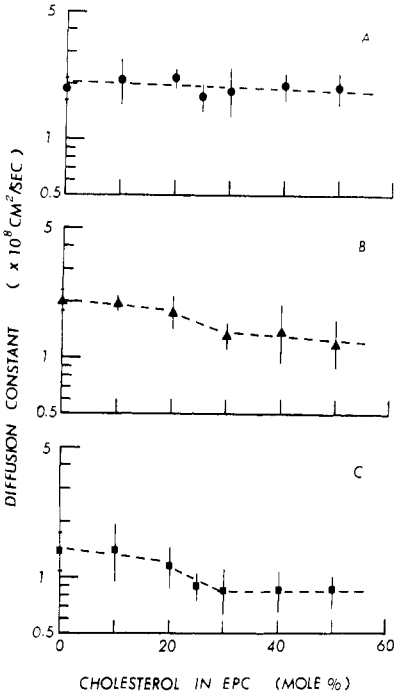

FIGURE 4: Cholesterol dependence of lateral diffusion of cytochrome P-450 in EPC multibilayers at 25 (●, curve A), 15 (▲, curve B), and 5.3 °C (■, curve C). Cholesterol concentration is accurate to ±5 mol %.

Lateral Diffusion of P-450 in EPC Multibilayers Containing Cholesterol. The lateral diffusion of P-450 in EPC membranes containing 50 mol % cholesterol is shown as a function of temperature in Figure 3 (curve B). The diffusion of P-450 was impeded only slightly by cholesterol, and the activation energy of diffusion was almost identical with that in EPC membranes without cholesterol.

The cholesterol dependence of P-450 diffusion in EPC multibilayers was studied at three different temperatures, 25, 15, and 5.3 °C, and the result is shown in Figure 4. At 25 °C, the diffusion of P-450 was essentially constant up to 50 mol % cholesterol (Figure 4A). Although a minor depression in *D* appeared to occur between 20 and 30 mol % cholesterol, the significance of this depression was unclear due to large experimental uncertainties. At 15 and 5.2 °C, the diffusion of P-450 decreased slightly as the cholesterol concentration

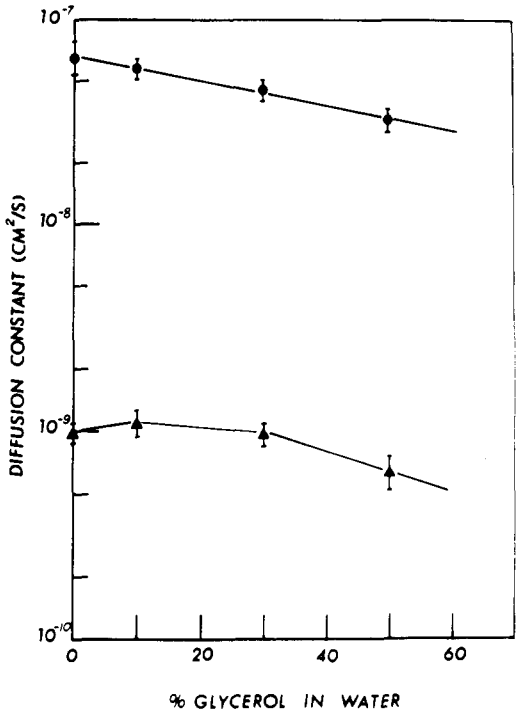

FIGURE 5: Lateral diffusion coefficients of NBD-PE in DMPC multibilayers formed by hydrating dry lipid films with water/glycerol mixtures. Molar ratio of DMPC/NBD-PE = 5000/1. At 26 °C (●), DMPC is in the liquid-crystal phase, and at 15 °C (▲), DMPC is in the gel phase.

increased (Figure 4B,C). Between 20 and 30 mol % cholesterol, a significant drop in D was noticed. The lateral diffusion coefficient remained essentially constant at higher cholesterol concentrations.

Lipid Diffusion in DMPC Multibilayers Containing Glycerol. To study the effect of glycerol on lipid diffusion, the lateral diffusion of a lipid analogue NBD-PE, was measured in multibilayers formed by hydrating DMPC with water and glycerol mixtures. FRAP measurements were made at 26 and 15 °C, which are above and below the phase transition temperature of DMPC, respectively. Figure 5 shows the lateral diffusion coefficient of NBD-PE as a function of glycerol concentration. At 26 °C, the D value of NBD-PE decreased steadily as the glycerol concentration increased; D varied from 7×10^{-8} cm^2/s in glycerol-free samples to 3.5×10^{-8} cm^2/s in multibilayer samples containing 50% glycerol. At 15 °C, the D of NBD-PE at first increased from 10^{-9} cm^2/s in glycerol-free samples to 1.2×10^{-9} cm^2/s in samples with 10% glycerol and then decreased steadily as the glycerol concentration increased further. In samples containing 50% glycerol, the D values of NBD-PE dropped to 7×10^{-10} cm^2/s.

Discussion

Several recent reports indicate that small proteins such as gramicidin S (Wu et al., 1978), gramicidin C (Tank et al., 1982), coat protein M-13 (Smith et al., 1979), and glycophorin (Vaz et al., 1981; Wu et al., 1981) all exhibit quantitatively similar lateral mobility in lipid bilayers. In the liquid-crystal phase of DMPC, the diffusion coefficient of these proteins falls within a range between 10^{-8} and 5×10^{-8} cm^2/s. These results qualitatively agreed with the theory of Saffman & Delbruck (1975) that predicted that in a viscosity-controlled lateral diffusion, D is only weakly dependent on the size of proteins embedded in the membrane. However, the validity of this prediction has never been critically tested. It is useful to perform such a test with the measurements made on a big protein such as P-450. Saffman–Delbruck's (1975) theory predicts that

$$D = \frac{kT}{4\pi\eta h}\left[\ln\left(\frac{\eta h}{\eta_w a}\right) - \gamma\right]$$

where k is the Boltzmann constant, T is the absolute temperature, η and η_w are the viscosity of membrane and water, respectively, h is the membrane thickness, a is the radius of the intramembranous section of the protein molecules, and γ is the Euler constant. To test this equation, one has to know the location and the size of P-450 in the membrane. The exact location of P-450 in the membrane is unknown (Gut et al., 1982; DePirre & Ernster, 1977), but the observation that P-450 diffusion was sharply reduced when DMPC was changed from liquid-crystal to gel phase indicates that P-450 is deeply embedded in the membrane. The molecular size of P-450 used in this study is not known but may be similar to that of bacterial P-450 due to similar molecular weights. The latter was recently determined from X-ray crystallographic studies to have an effective radius $a = 22$ Å (Gunsalus, 1981; Schwartz et al., 1982). With this information we can test the size dependence of D by comparing the diffusion coefficients of P-450 ($D_{P\text{-}450}$) and NBD-PE (D_{PE}); both were measured in this work on the same membranes. If we assume $\eta = 1.5$ P, $a = 4$ Å for NBD-PE, and $h = 45$ Å for a bilayer, the calculated $D_{P\text{-}450}/D_{PE} = 0.42$. The experimentally measured values at 25 °C are $D_{P\text{-}450} = (2.5 \pm 0.6) \times 10^{-8}$ cm^2/s and $D_{PE} = (6 \pm 1) \times 10^{-8}$ cm^2/s. This yields a ratio $D_{P\text{-}450}/D_{PE} = 0.42$, in agreement with the calculated value. Such agreement confirms the validity of Saffman–Delbruck's equation. However, one has to be aware of the limit of the above test, since the dependence of D in molecular size is through a logarithmic factor. If we increase the size of P-450 by a factor of 2 to $a = 44$ Å and maintain all other parameters the same, the calculated $D_{P\text{-}450}/D_{PE} = 0.36$, which, within the experimental uncertainties, still agrees with the measured value. It is clear that a quantitative test of the dependence of D on molecular size requires either more accurate experimental data or measurements performed on larger proteins.

As the temperature decreased from 24 to 22 °C, the lateral diffusion coefficient of P-450 in DMPC dropped by a factor of 20 as a result of lipid liquid crystal to gel phase transition. Similar sharp drops have also been reported for the diffusion of other reconstituted proteins (Wu et al., 1981; Smith et al., 1979). However, there were some differences. In the case of P-450 diffusion, the 20-fold decrease in D occurred gradually within a temperature range of more than 2 °C. In contrast, the diffusion coefficient of other proteins usually drops by more than 200-fold and within a somewhat narrower temperature range. Such quantitative differences cannot be explained by the difference in membrane fluidity alone, because lipid diffusions in all the cases appear to be the same. A possible contributing factor is the difference in protein sizes. In the gel phase, electron microscopy and X-ray diffraction results (Copeland & McConnell, 1980; Janiak et al., 1976, 1979) indicate that DMPC forms corrugated bands with a periodicity of about 200 Å. In addition to these banded structures, crystal defects also prevail over most multibilayers. Recent results (Chan et al., 1981) indicate that diffusions along these defects might have dominated the observed fluorescence recovery processes. The effect of proteins on the ordered lipid structure is not clear. It is possible that a large protein like P-450 is potentially able to induce a higher degree of crystal disorder than smaller peptides such as gramicidins or M-13 coat proteins and thus provide more defect channels for the

observed fast fluorescence recovery. The small amount of glycerol (10%) added to the multibilayer samples during the hydration process may also have some effect on the observed temperature dependence of P-450 diffusion, presumably through the glycerol-induced changes in the microstructures of multibilayers (McDaniel et al., 1983).

Recently, there were several theoretical investigations on the effect of cholesterol–lipid interactions on lateral diffusion (Owicki & McConnell, 1980; Snyder & Freire, 1980; Saxton, 1982). Below the T_m, these theories depicted a DMPC/cholesterol membrane as a continuum of solid lipid interspersed with liquidlike cholesterol-rich domains (Snyder & Freire, 1980) or as an array of alternating parallel domains of fluid and solid lipid (Owicki & McConnell, 1980). At low cholesterol concentrations, the lateral diffusion in macroscopic scale is very slow as it is restricted by the solid lipid. As the cholesterol concentration increases, the cholesterol-rich domains expand. At about 20 mol % cholesterol, these interstitial liquid domains connect with each other forming a network of macroscopic dimension. As a result, the lateral diffusion over a macroscopic scale, as measured by FRAP method, is expected to increase significantly. These theories successfully explained the experimental observations of Rubinstein et al. (1979). At higher cholesterol concentration, the sharp change in D near the T_m, due to gel to liquid-crystal transition, no longer exists. This agrees with our observations that in DMPC membranes containing 25 and 50 mol % cholesterol, the D value of P-450 increased gradually with temperature (Figure 2).

Above the T_m, in the liquid phase of lipid, the dependence of lateral diffusion on cholesterol was not explicitly discussed in those theories. But they are expected to be applicable, provided one treats the membrane as a continuum of liquid lipid interspersed with solidlike cholesterol-rich domains. A change in D near 20 mol % cholesterol can also be predicted due to the change in the lateral connectivity of cholesterol-rich domains (Snyder & Freire, 1980). This prediction was confirmed by the observed cholesterol dependence of P-450 diffusion in EPC membranes (Figure 4). The measurements were made between 25 and 5 °C. Within this temperature range, EPC is in liquid phase. At 5 and 15 °C, there was a noticeable decrease in D near 20 mol % cholesterol, in agreement with the theory. However, the magnitude of this decrease is somewhat small as compared to the predicted large increase in D at 20 mol % cholesterol below the T_m. This is expected; the magnitude of the change in D is strongly dependent on the parameter r, the ratio of the diffusion coefficient in the solid domain to that in the fluid domain (Saxton, 1982). For a DMPC/cholesterol mixture, $r \sim 0.05$ below the T_m, and $r \geq 0.9$ above the T_m. In EPC/cholesterol mixtures, r probably has similar values. At 25 °C (curve A, Figure 4), there was a minor depression in D near 20 mol % cholesterol, the significance of the decrease is not clear due to large experimental uncertainties.

Since cytochrome P-450 was stored in solution containing 10% glycerol, some glycerol was inevitably incorporated in the MBL samples during the hydration processes. Glycerol, in addition to its effect on membrane structure, may affect the lateral diffusion of P-450 by changing the viscosities of both the aqueous phase and the lipid membrane. If one assumes that the glycerol concentration in the aqueous phase of the MBL is the same as that in the protein stock solution (Diamond & Katz, 1974), then 10% glycerol raises the aqueous viscosity from 1 to 1.15 cP and is not expected to affect the lateral diffusion of P-450 because the dependence of D on aqueous phase viscosity is logarithmical (Saffman & Delbruck, 1975). The partitioning of glycerol in the membrane may affect the lateral diffusion significantly since membrane viscosity is inversely proportional to the lateral diffusion coefficient (Saffman & Delbruck, 1975). However, the exact amount of glycerol partitioned in the membrane cannot be determined because of excessive hydration in the MBL samples. To determine the effect of glycerol on lateral diffusion, we therefore measured the lateral diffusion of NBD-PE in multibilayers formed by hydrating DMPC with water containing various amounts of glycerol (Figure 5). Measurements were made at two temperatures, 26 and 15 °C. At 26 °C, DMPC is in the liquid-crystal phase. Glycerol, with a viscosity of about 5 P, lowered the diffusion of NBD-PE (Figure 5, curve A). In membranes hydrated with 10% glycerol, the diffusion of NBD-PE decreased by a factor of 1.2. If this decrease is due to the membrane viscosity increase, then the true lateral diffusion of P-450 in a glycerol-free membrane should be at least 1.2-fold faster than those reported in Figures 1–4. At 15 °C, DMPC is in the gel phase. The addition of glycerol in MBL may reduce the membrane viscosity and, hence, increase the lateral diffusion. For lipid analogue NBD-PE, the lateral diffusion remained almost constant at low glycerol concentrations (Figure 5, curve B) but decreased slightly at higher glycerol concentrations. Such decreases cannot be attributed to a change in membrane viscosity, and the possibility that it resulted from glycerol-induced changes in membrane structure (McDaniel et al., 1983) should be considered.

Taniguchi et al. (1980) have recently measured the reduction rate of P-450 in reconstituted liposomes of DMPC. They observed a sharp drop in reduction rate during liquid crystal to gel phase transition and concluded that the reduction of P-450 by reductase is a diffusion-controlled process. This drop (by a factor of 20) is quantitatively consistent with the presently observed sharp drop (by a factor of 20) in P-450 diffusion in the phase-transition region of DMPC (Figure 1). The reduction rate for P-450 in a diffusion-limited process can be estimated from collision frequency (Hardt, 1979) by assuming that both P-450 and reductase are diffusing at the same rate of $D = 2.5 \times 10^{-8}$ cm^2/s, every collision between P-450 and reductase results in an electron transfer, and the molar ratio of lipid/P-450/reductase is 400/1/1, the same concentration as that used in the experiment of Taniguchi et al. (1980). The calculated reaction rate under such assumptions is about 5×10^3 s^{-1}, almost 3 orders of magnitude faster than the experimentally measured value (Taniguchi et al., 1980). This discrepancy can be partly accounted for if some constraints, such as molecular shape factors, orientations, and rotational diffusions (Schmitz & Shurr, 1972), were imposed on the reaction.

The present work shows that P-450 can diffuse rather rapidly in multibilayers with a $D = (2–3) \times 10^{-8}$ cm^2/s, which is fast enough to sustain a diffusion-limited reaction in liposomes. However, in the microsomal membranes, the mobility of P-450 is also affected by protein–protein interactions and lipid heterogeneity. Recently, several measurements on the rotational diffusion of P-450 have been reported (Kawato et al., 1982; Gut et al., 1982), and the results of these measurements suggest that the rotational motion of P-450 in liposomes of high protein concentration is partially restricted, due to the self-aggregation of P-450. Since lateral diffusion in bilayers is not very sensitive to the size of diffusant, it is not clear whether microaggregates were present in MBL samples studied herein.

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Registry No. P-450, 9035-51-2; DMPC, 13699-48-4; cholesterol, 57-88-5; glycerol, 56-81-5.

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