Translational diffusion and fluid domain connectivity in a two-component, two-phase phospholipid bilayer

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ABSTRACT The two-dimensional connectivity is examined for mixed bilayers of dimyristoyl phosphatidylcholine (DMPC) and distearoyl phosphatidylcholine (DSPC) as a function of composition and temperature at constant pressure using the fluorescence recovery after photobleaching (FRAP) method. These phospholipid mixtures exhibit peritectic behavior with a large region in which both gel and liquid crystalline phases coexist. Dilauroyl phosphatidylethanolamine covalently linked through the amino function in its head group to the fluorescent nitrobenzodiazolyl group (NBD-DLPE) was used as the fluorescent probe in this study, because it was found to partition almost exclusively in the liquid crystalline phase. The results of these studies show the line of connectivity to be close to the liquidus line on the phase diagram over a rather broad range of concentrations. In this range, a gel phase comprising ~20% of the system disconnects a liquid crystalline phase comprising 80% of the system. The implications of this result are discussed for domain shape and the organization of biological membrane components

INTRODUCTION

The idea that the basic permeability barrier in all biological membranes is a phospholipid bilayer is now widely accepted (Singer and Nicholson, 1972). This acceptance has spawned a multitude of studies on the physical properties of phospholipid bilayer systems of many types. For the most part these studies have focused on one-component systems. Understanding of one-component lipid bilayers has reached a state of considerable sophistication (e.g., Cevc and Marsh, 1987). Such is not the case with bilayers formed from two or more lipid components. It is, however, just such multicomponent bilayers that constitute the permeability barrier in biological membranes (McMurray, 1973). In recent years studies on bilayers composed of two lipid components suggest that phospholipids may in general exhibit deviations from ideal mixing (Silvius, 1982; Sugar and Monticelli, 1985; Cevc and Marsh, 1987). These characteristics strongly imply that the bilayers in biological membranes formed from complex mixtures of lipids can be expected to form a mosaic of stable domains of different composition which may be either liquid crystalline or gel phase in character. In the membranes of a biosynthetically competent cell at constant temperature, the complexity of this domain structure will be governed by the instantaneous balance of the biosynthetic and catabolic activities of the cell. If a bilayer is poised near a transition boundary on the phase diagram describing the compositional domain structure, a very small change in composition will effect a large change in domain structure. This point can be appreciated by considering the connectivity property of domain systems. In a two-component, two-phase bilayer the domain rich in the major component has dispersed in it a discontinuous phase rich in the minor component. As the mole fraction of the minor component is gradually increased at the expense of the major component, a narrow concentration zone is reached in which the initially dispersed phase becomes continuous and the initially continuous phase becomes disperse. At constant pressure, this situation occurs as the temperature is changed in a system of constant composition, or as the composition is changed in a system at constant temperature (Essam, 1972; Snyder and Freire, 1980).

In this paper we examine the two-dimensional connectivity of mixed bilayers of dimyristoyl phosphatidylcholine (DMPC)[1] and distearoyl phosphatidylcholine (DSPC) as a function of composition and temperature at constant pressure using the fluorescence recovery after photobleaching (FRAP) method (Axelrod et al., 1976). These phospholipid mixtures exhibit peritectic behavior with a large region in which both gel and liquid crystalline phases coexist (Knoll et al., 1981). Dilauroyl phosphatidylethanolamine covalently linked through the amino function in its polar group to the fluorescent nitrobenzodiazolyl group (NBD-DLPE) was used as the fluorescent probe in this study. Partitioning studies using fluores-

[1] *Abbreviations used in this paper*: diI, dialkylindocarbocyanine iodide; DLPC, dilauroyl phosphatidylcholine; DMPC, dimyristoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; DSPC, distearoyl phosphatidylcholine; FRAP, fluorescence recovery after photobleaching; NBD-DLPE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-dilauroyl phosphatidylethanolamine.

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cence polarization of probes of this type in DMPC/DSPC mixed bilayers allowed us to select NBD-DLPE as the probe of choice because it was found to partition almost exclusively in the liquid crystalline phase. The results of these studies show the line of connectivity to be close to the liquidus line on the phase diagram over a rather broad range of concentrations. In this range a gel phase comprising ~20% of the system disconnects a liquid crystalline phase which is 80% of the system. A preliminary report of portions of this work has appeared previously (Vaz et al., 1989).

MATERIALS AND METHODS

Materials

Dimyristoyl- and distearoyl phosphatidylcholine were purchased from Avanti Polar Lipids, Inc., Pelham, AL. All lipids were checked for purity by thin-layer chromatography and stored at −20°C as the dry material. NBD-DLPE was prepared as described earlier (Vaz and Hallmann, 1983).

Methods

Preparation of multibilayers

About 2 mg of lipid mixture containing a molar ratio of 1/1,000 of NBD-DLPE was dissolved in 0.4 ml chloroform and allowed to stand for 30 min at room temperature. The solution was then evaporated to a volume of 0.05–0.1 ml by blowing a stream of nitrogen over it. This concentrated solution was then deposited on a siliconized glass microscope slide warmed to 90°C on a hot plate. Care was then taken that the lipid residue did not spread over an area >1 cm^2. The slide with the residue was placed in a vacuum desiccator over anhydrous calcium chloride granules for 4 h and then warmed in an oven to 70°C for an additional 10 min. The dried slide was hydrated by placing it in an atmosphere with a relative humidity close to 100% at a temperature of 70°C for 6 h and then brought to excess water conditions by dropping over the residue a cover slip with a hanging drop of 70 μl 0.01 M sodium phosphate buffer, pH 7.5, containing 0.05 M potassium chloride and 0.02% sodium azide. The slide was left in the humid atmosphere at 70°C for a further 2 h and then cooled gradually down to 20°C over a 4-h period. It was then stored in a chamber over distilled water at 20°C in the dark for at least 3 d before measurements were performed. An occasional sample was stored for periods as long as 30 d before measurement. No differences were observed between samples stored for 3 and 30 d. Before measurement the edge of the cover slip was sealed with a silicone paste (Bayer AG, Leverkusen, FRG) to prevent water evaporation. All preparations were examined by polarized microscopy to be certain that optically uniaxial multilayers of large area were studied. Multibilayers prepared in this way are comprised of several hundred bilayers.

FRAP experiments and recovery curve analysis

FRAP experiments were performed using a uniform circular beam profile with a radius of 3 μm. Details of the instrumentation are shown in Fig. 1. Measurements were either begun at 20°C (heating curves) or at a temperature above the liquidus line for the composition being examined (cooling curves). The sample slide was equilibrated at the temperature desired for at least 30 min before measurements were made. Typical

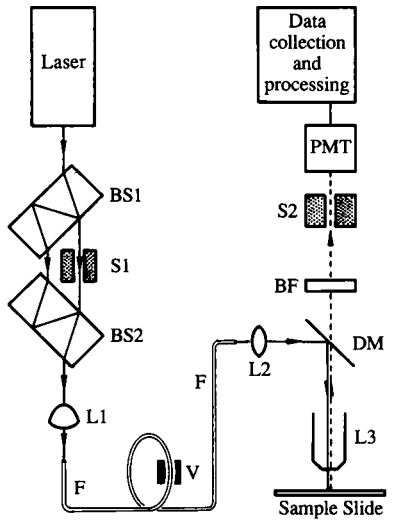

FIGURE 1 Schematic of the FRAP apparatus. The 488-nm line of an argon ion laser (operating at 200 mW power output) is split into two beams with an intensity ratio of 10^{-4} by means of plates *BS1* and *BS2*. The two beams are coaxial after passing *BS2*. The weaker (monitoring) beam illuminates continuously while the intense (bleaching) beam is interrupted, except during the bleaching pulse, by the shutter *S1*. The beams are then focused by lens, *L1*, onto the polished end of an optical fiber, *F*. The emission from the other polished end of the fiber is projected by lens, *L2*, and reflected by a dichroic mirror, *DM*, on the entrance pupil of a microscope objective, *L3*. In the experiments reported here, a Zeiss Ultrafluar 10/0.20 objective was used. A neutral density filter can be placed between *DM* and *L3* to cut down the intensity of the illuminating beam if photobleaching during monitoring is observed. *DM* reflects the illuminating wavelength and transmits the wavelength of light emitted by the fluorophore (NBD) in the sample. A cut-off filter, *BF*, is placed between *DM* and a photomultiplier (type C31034A02, RCA Electro Optics and Devices, Lancaster, PA), *PMT*, which is further protected by a shutter, *S2*. *S2* is closed when *S1* is open to avoid damage to the *PMT* by reflections of the intense bleaching beam. Signal recording is done by photon counting and the entire instrument is controlled by a microcomputer. To smooth the speckle pattern caused by transmission of the coherent laser light through *F*, the fiber is vibrated by an electromagnet, *V*, at a frequency of 100 Hz (Gaub et al., 1984). The use of an optical fiber gives a uniform circular profile, which has the advantage that the shape of the recovery curve is independent of the degree of bleaching. Sample temperature is controlled by use of a Peltier temperature control unit (Cambridge Thermionic Corp., Cambridge, MA) built into the microscope stage. The temperature stability is ±0.1°C.

bleaching times were ~10–30 ms. The FRAP curves were collected in a total of 1,000 channels with a minimum period of 30 ms per channel. The monitoring beam intensity was chosen so that no measurable photobleaching could be observed during a monitoring scan (with the photobleaching beam blocked) for a period equivalent to the time of a typical measurement. The recovery curves were fitted using the Simplex algorithm (Quantum Chemistry Exchange Program, Chandler, J. P., Oklahoma State University, Stillwater, OK) to the equations given by Soumpasis (1983). In cases where the recovery could not be well described as being due to a single diffusing component, an excellent fit was obtained as a summed recovery due to a single diffusing component superimposed upon a linear ramp. This linear ramp reflects one or more

diffusing components with very much slower recovery times. In the data-fitting procedure, the recovery time, τ, the slope of the linear ramp, the fluorescence intensity immediately after photobleaching, $F(0)$, and the fluorescence intensity at infinite time after photobleaching, $F(\infty)$, were adjustable. The fluorescence intensity before photobleaching, $F(i)$, was taken as the mean value of 100 channels measured before the photobleaching pulse.

The equations given by Soumpasis (1983) describe the recovery of fluorescence after photobleaching a uniform circular spot. The assumption is that diffusion of fluorescent unbleached molecules is free to occur across the entire perimeter of the bleached spot. In case of diffusion in bilayers in the gel phase where the recovery observed, if any, is primarily due to one-dimensional diffusion along defects in the gel phase (Derzko and Jacobson, 1980; Schneider et al., 1983) this condition may not obtain. Because an analysis for diffusional recovery in these cases does not exist in the literature, we have used Soumpasis' description even for gel phase recoveries. The very good fits obtained may be fortuitous.

RESULTS

Typical recovery data are shown in Fig. 2. Panels *A*, *B*, and *C* show recovery curves for NBD-DLPE in DMPC/DSPC multilayers of 0.50 mole fraction DMPC at 49.6, 24.9, and 34.9°C, respectively. At the high temperature the bilayers are liquid crystalline, at 24.9°C all gel, and at 34.9°C both liquid crystalline and gel phases coexist with ~61% of the system in the gel phase. A plot of the weighted residuals and the autocorrelation function are shown at the bottom of each panel. In the all-liquid crystalline system at 49.6°C (Fig. 2 *A*) the characteristic recovery time, τ, obtained as a best fit is 0.22 s with no linear ramp. This recovery time and the 99.5% recovery are typical of liquid crystalline phospholipid bilayers at this temperature (Vaz et al., 1985). In the all-gel system at 24.9°C (Fig. 2 *B*) recovery is characterized by more than one recovery time. This type of behavior has been noted earlier in gel phase single component bilayers and has been attributed to diffusion along structural defects in packing in the gel phase in addition to diffusion in defect-free gel phase (Derzko and Jacobson, 1980; Schneider et al., 1983). The value of $\tau = 6.97$ s associated with 46.8% recovery is ~30 times the value expected for a liquid crystalline phase at this temperature (Vaz et al., 1985). The slope of the linear ramp (17 counts/s) reflects the existence of one or more additional τ values at least one of which may represent diffusion in a defect-free gel phase bilayer. It is not possible to estimate this value from the data reported here. The mixed phase system at 34.9°C (Fig. 2 *C*) is also characterized by more than one recovery time. The value of $\tau = 1.17$ s associated with 60.4% recovery is about five times the value expected for diffusion in a liquid crystalline phase, whereas the ramp (13 counts/s) again represents one or more τ values corresponding to diffusivities that are immeasurably slow for the conditions used here.

Fig. 3 is a summary of percent recovery and recovery time constant data for three compositions DMPC/DSPC (molar ratios of 20/80 *A*, *B*; 50/50 *C*, *D*; and 80/20 *E*, *F*) as a function of temperature. Data are presented in Fig. 3 for both heating (*A*, *C*, *E*) and cooling (*B*, *D*, *F*) scans following the protocol outlined in Materials and Methods. The values at each temperature are averages of at least three FRAP determinations. It is apparent that for each system composition there is a characteristic temperature at which the fluorescence recovery becomes 100% and the recovery time constant falls to a value typical of liquid crystalline phosphatidylcholine bilayers in the temperature interval of 20 to 60°C. This characteristic temperature is very similar in both heating and cooling scans for the same composition, which indicates that the 30 min temperature equilibration time between measurements assured that the systems were at equilibrium. Characteristic temperatures for these three systems as well as values obtained in heating scans for DMPC/DSPC molar ratios 35/65 and 65/35 are plotted as filled triangles (▲) on the phase diagram for this system shown in Fig. 4. We suggest that the characteristic temperatures define a locus on the phase diagram along which liquid crystalline phase connectivity changes occur.

DISCUSSION

To our knowledge there have been only two previous reports of FRAP studies on two-component phospholipid bilayers. Klausner and Wolf (1980) used this method to examine the partitioning of dialkylindocarbocyanine dyes (diI) between gel and liquid crystalline phases in very large, single bilayer vesicles formed from either DMPC, dilauroyl phosphatidylcholine (DLPC) or dipalmitoyl phosphatidylcholine (DPPC) as a function of temperature. Several experiments using two diI analogues of different acyl chain length were reported in mixed DLPC/DPPC (50/50) bilayers. The results obtained in this system at 10°C, where gel and liquid crystalline phases coexist, conformed to expectations based on the partition coefficients determined in single component bilayers. Rubenstein et al. (1979) used an NBD-PE probe, derivatized from egg phosphatidylethanolamine, in FRAP studies on multibilayers formed from mixtures of cholesterol and either DMPC or DPPC over the temperature range 14–38°C. These authors reported a region on the phase diagram for DMPC/cholesterol in which the translational diffusion coefficient was that expected for a liquid crystalline phase and a second adjacent region at lower temperature and cholesterol concentration in which the translational diffusion coefficient was about one order of magnitude smaller. The line dividing these two regions, along which the diffusion coefficient changed abruptly,

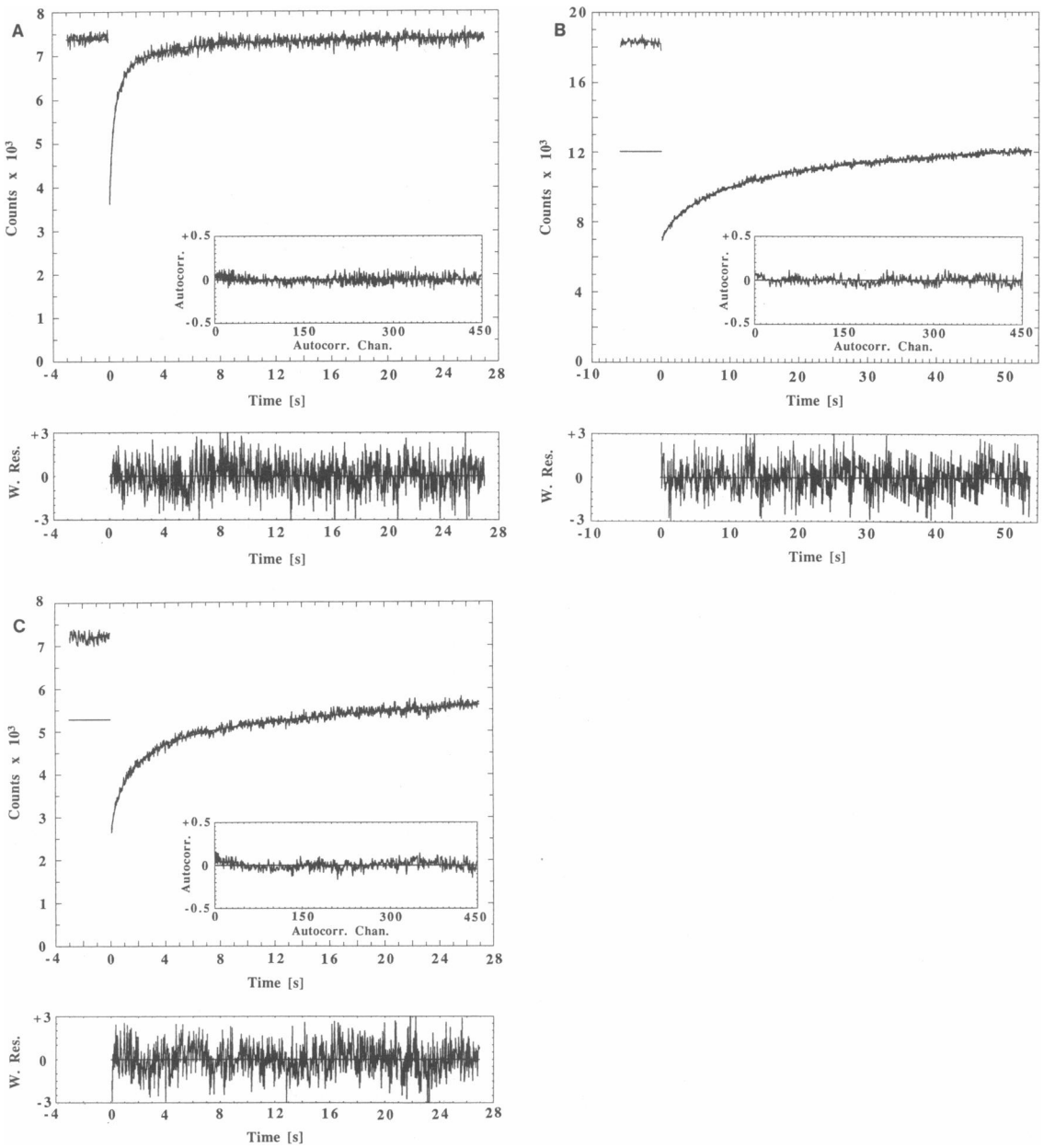

FIGURE 2 Typical fluorescence intensity recovery curves of NBD-DLPE in DMPC/DSPC (50/50, mol/mol). Plots of the autocorrelation function and the weighted residuals in standard deviation units are shown at the bottom of each panel. (*A*) 49.6°C. System is all-liquid crystalline. The best fit was obtained with τ = 0.22 s, $F(0)$ = 2,147 counts and $F(\infty)$ = 7,372 counts. The prebleach fluorescence intensity, $F(i)$, was 7,411 counts. The counting period was 30 ms/channel. (*B*) 24.9°C. System is all-gel. The best fit was obtained with τ = 6.97 s, $F(0)$ = 6,532 counts, $F(\infty)$ = 12,022 counts, and slope of the linear ramp = 13 counts/s. The prebleach fluorescence intensity, $F(i)$, was 18,271 counts. The counting period was 60 ms/channel. The level of $F(\infty)$ is shown as a line in the left margin of the panel. (*C*) 34.9°C. Gel and liquid crystalline phases coexist. The best fit was obtained with τ = 1.17 s, $F(0)$ = 2,353 counts, $F(\infty)$ = 5,282 counts, and slope of the linear ramp = 17 counts/s. The prebleach fluorescence intensity, $F(i)$, was 7,200 counts. The counting period was 30 ms/channel. The level of $F(\infty)$ is shown as a line in the left margin of the panel.

was identified as a phase boundary between liquid crystalline and gel-like phases. Similar but less extensive results were obtained for DPPC/cholesterol mixtures. In a subsequent paper Snyder and Freire (1980) suggested that the abrupt changes in the magnitude of the diffusion coefficient reported by Rubenstein et al. (1979) were the result of connectivity changes in the phase structure of the phosphatidylcholine/cholesterol bilayers. This interpretation was based on the results of Monte Carlo calculations using interaction energies derived from calorimetric

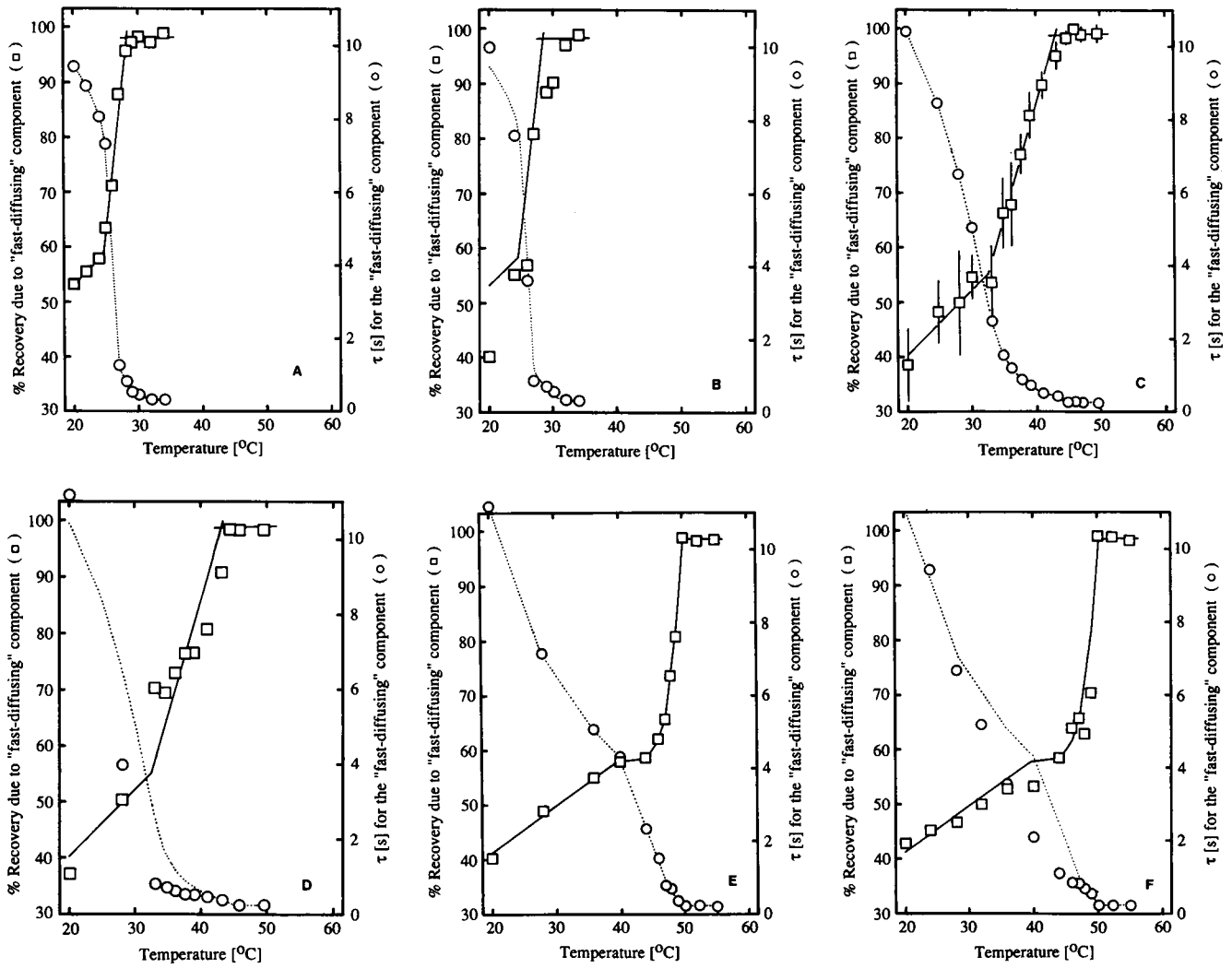

FIGURE 3. Panels *A*, *C*, and *E* are percent recovery and characteristic recovery times for heating scans for 80/20, 50/50 and 20/80, mole/mole, DMPC/DSPC, respectively. The solid and dotted lines are best fit curves for percent recovery and recovery time results, respectively. Panels *B*, *D*, and *F* are the same parameters for cooling scans for the same three compositions, 80/20, 50/50, and 20/80, respectively. For comparison, the solid and dotted lines on the cooling scans are taken from the corresponding heating plots. In the cooling scans, the solid and dashed lines are not fits to the cooling data.

data obtained on phospholipid/cholesterol liposome dispersions by Estep and co-workers (1978, 1979).

Our suggestion that the characteristic temperatures presented in Fig. 4 define a locus on the phase diagram of DMPC/DSPC mixtures along which phase connectivity changes occur is in accord with the view of Snyder and Freire (1980). The rationale for this interpretation of our FRAP data is as follows. The fluorescent lipid derivative used in our work, NBD-DLPE, partitions almost exclusively into the liquid crystalline phase (data not shown). When no liquid crystalline phase is available it probably partitions into structural faults (dislocation or point defects) in the gel phase. Thus at low temperature when the system is totally gel phase, there is $\leq$60% recovery with a recovery time ~30 times the value of the recovery time in the liquid crystalline phase. This recovery and recovery time probably reflect diffusion of probe that is excluded from the gel phase and located in faults in the system (Derzko and Jacobson, 1980; Schneider et al., 1983). The probability that a system of faults exists in the all-gel phase DMPC/DSPC is enhanced by the fact that two immiscible gel phases coexist in this system below 27°C in the concentration interval 0.3–0.65 mole fraction DSPC (Knoll et al., 1981). The extent of recovery is a function of the partition coefficient of the probe between gel phase and faults as well as of the degree of connected-

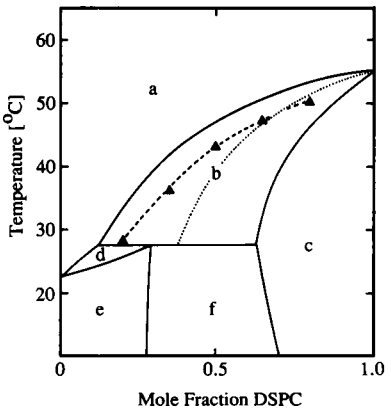

FIGURE 4 Phase diagram for DMP/DSPC mixtures adapted from Knoll et al. (1981). The dashed line connecting the triangles (▲) is the connectivity line. The dotted line connects the midpoints of all tie lines. (*a*) Liquid crystalline; (*b*) gel 1 and liquid crystalline phases coexist; (*c*) gel 1; (*c*) liquid crystalline phase and gel 2 phases coexist; (*c*) gel 2; (*f*) gel 1 and gel 2 coexist.

ness of the faults relative to the dimensions of the bleached spot. The nonrecovering fraction can then be attributed to unconnected faults and/or very slow diffusion of the probe in the ordered gel phase. For the latter case diffusion coefficients of $\leq 10^{-11}$ cm^2/s (Derzko and Jacobson, 1980) and as low as 10^{-16} cm^2/s (Schneider et al., 1983) have been reported.

As the temperature is raised to bring the system into the region of the equilibrium coexistence of both liquid crystalline and gel phases, the probe concentrates in the disconnected liquid crystalline regions. However, after a photobleach, 100% recovery with a time characteristic of the liquid crystalline state is not observed. This is due to the fact that the liquid crystalline phase containing the probe is disconnected, thus recovery of fluorescence by translational diffusion of probe molecules into the bleached area from the unbleached periphery is severely limited. In fact recovery is limited only to those disconnected liquid crystalline domains straddling the circumference of the bleached area. When the system reaches the temperature at which the liquid crystalline phase achieves connectivity, then 100% recovery becomes possible with a time constant characteristic of the liquid crystalline state. This is the case because all liquid crystalline areas within the bleached spot are now connected to each other and to the reservoir of fluorophores in the unbleached periphery. The reverse of this series of conditions obtains as the temperature is lowered to bring the system from all-liquid crystalline to all-gel. Thus as the temperature is lowered into the region of coexistence of both phases, the probe exhibits behavior typical of a liquid crystalline phase until this phase is disconnected by the gel phase. At this temperature probe behavior more typical of the gel phase is observed. Under this interpretation the probe reports the connection or disconnection of the liquid crystalline phase; the condition of the gel phase is not directly assessed.

Heating and cooling data at each of the three compositions shown in Fig. 3 are in remarkably good agreement. This result indicates that the previous thermal history of the samples has no effect on the recovery process. Thus, at each temperature, the 30-min equilibration period appears to have been sufficient to bring the system into phase as well as temperature equilibrium.

Partial recovery of fluorescence intensity in the mixed phase system in which the liquid crystalline phase is disconnected may be due to liquid crystalline domains that straddle the circumference of the bleached spot. This can have only a limited effect on the overall recovery, however. A rough estimate of the maximum contribution to recovery by these straddling domains can be made as follows. The average size of liquid crystalline domains in the DMPC/DSPC system is not known. However, Hui (1981), using diffraction contrast electron microscopy on DLPC/DPPC (50/50) and phosphatidylserine/DPPC (1/2) bilayers, has estimated that a characteristic size of gel domains may be ~0.3–0.5 μm. Experiments using ^{13}C NMR by Blume and co-workers (1982) produced exchange rate data that can be interpreted to give a substantially smaller value for the size of gel domains in dipalmitoyl phosphatidylethanolamine/DPPC bilayers. Let us assume that in the DMPC/DSPC system the liquid crystalline domains are circular and have a maximum diameter of 0.6 μm. It can easily be shown that a domain of any arbitrary shape straddling the circumference of the bleached spot will have the maximum effect on recovery if one half the area of the domain is bleached and the other half is not. Under this condition the maximum number of circular domains of radius r that could straddle the circumference of the bleached spot of radius R will be $2\pi R/2r$. The total area of these straddling domains that is bleached is given by $\pi^2 Rr/2$ and the total area of the bleached spot by πR^2. Thus the fraction of the total area that can recover due to the liquid crystalline domains that straddle the bleached circumference of the spot is $\pi r/2R$. With the values of $r = 0.3$ μm and $R = 3$ μm, this is equal to 0.16. The calculation is independent of the fraction of the bilayer area that is liquid crystalline. It thus appears, based on the assumptions made, that at most a recovery of 16% can be due to liquid crystalline domains straddling the circumference of the bleached spot in two phase bilayers in which the liquid crystalline phases are disconnected.

Examination of Fig. 4 shows that, at compositions above ~0.65 mole fraction DSPC, the points of connectivity lie close to the tie line centers. However, in the composition range of 0.2 to ~0.6 mole fraction DSPC the

gel phase is continuous until the liquid crystalline phase becomes ~80% of the mass of the system. In other words as little as 20% gel phase can disconnect 80% of the other coexisting phase. A similar situation appears to be the case in DLPC/DSPC bilayers over much of the region in which gel and liquid crystalline phases coexist (Vaz and Thompson, preliminary observations). For this to be the case, it seems clear that the geometry of the two coexisting phases must be quite different. If the disconnected liquid crystalline phase domains are taken to be roughly circular in shape, a reasonable assumption for a quasiliquid, then the continuous gel phase must have the shape of a reticulum or network. This situation is illustrated in Fig. 5 in which the hatched areas represent the dispersed liquid crystalline phase and the clear areas the gel phase reticulum. It is interesting to note that, if the liquid crystalline domains were circular and all of the same diameter, the area between the circles when close packed would be ~20% of the total surface and would be independent of the liquid crystalline domain size. For the gel phase to be a reticulum, it is necessary for this phase, when growing as a dispersed phase above the line of connectivity, to be dendritic in form, a condition which obtains when the phase growth rates along two orthogonal directions in the surface are not the same. In one-component system, the arrangement of phosphatidylcholine molecules in the gel phase (L_β or $L_{\beta'}$) is a disordered orthorhombic subcell (Cevc and Marsh, 1987). Whether this is the case in the two-component gel phases in the DMPC/DSPC mixture is not known. However, if the structure is orthorhombic, then asymmetric growth is possible. It is interesting to note that dendritic structures have been observed in multiphasic monolayers at an air/water interface using epifluorescence microscopy (McConnell et al., 1984). Considerable progress has been made in understanding the causes of domain growth in these monolayer systems (Fogedby et al., 1987; McConnell and Moy, 1988; Helm and Möhwald, 1988). In monolayer systems the domains are quite large and easily viewed by fluorescence microscopy. As noted above, however, it is likely that the dimensions of the domains in bilayers such as the DMPC/DSPC system are much smaller. In fact preliminary attempts to visualize them using CCD and laser scanning fluorescence microscopy have been unsuccessful.

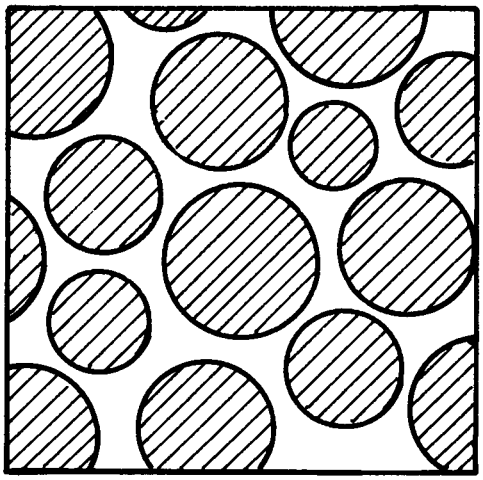

FIGURE 5 Diagram illustrating a centro-symmetric liquid crystalline phase (*hatched areas*) disconnected by a continuous gel phase network (*clear areas*).

For the gel phase reticulum to disconnect the liquid crystalline domains, the barrier integrity of the gel phase network to lateral diffusion of the fluorescent probe must effectively persist over the time frame of the FRAP experiment. This is of the order of 1 min. In the all-gel or the mixed gel/liquid crystalline temperature regions, with increasing temperature all systems exhibit increasing values of percent recovery and decreasing values of τ. The rate of change of these two parameters increases as the mole fraction of DMPC is increased. The effect of this dependence is to cause the characteristic temperature to be more closely defined as the concentration of DMPC is increased. As discussed earlier, it may be that the detailed shape of the percent recovery and τ vs. temperature curves below the characteristic temperature reflect the presence of faults in the gel phase. If this is the case, then the condition of connectivity seen in the FRAP experiment is actually the extensive breaching of the gel phase barrier by faults as the fraction of gel phase is decreased. This then results in free lateral diffusion of the probe and 100% recovery. This view incorporates into the operational FRAP definition of connectivity both dynamic and static aspects of the gel phase fault structure. If this is the case, then the thermodynamic line of connectivity must lie even closer to the liquidus line on the phase diagram.

The fact that the line of connectivity lies close to the liquidus line in the system examined may not be a general phenomenon. It does, however, suggest a possible explanation for an interesting observation made in certain microorganisms and their fatty acid auxotrophs. Numerous studies have demonstrated that some organisms adjust the lipid composition of their membranes so that at the temperature of growth a small fraction of their membrane lipids are in the gel phase (Rottem, et al., 1973; Sinensky, 1974; Melchior and Steim, 1979). It seems possible, that microorganisms may use the coexistence of gel and liquid crystalline phases to provide a domain structure in their membranes to organize protein components. Furthermore because the content of membrane gel phase is low, it may be that the membrane composition is poised near the point of phase connectivity at the temperature of growth so that the membrane

composition can be biosynthetically altered to connect or disconnect the fluid phase regions. This idea can be extended to more complex eucaryotic cell membranes. Thus the domain structure of the membrane bilayer may organize and compartmentalize the two-dimensional surface of the membrane in analogous manner to the way in which the membranes themselves organize and compartmentalize the three-dimensional volume of the cell.

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