Fluid phase connectivity in an isomorphous, two-component, two-phase phosphatidylcholine bilayer

Winchil L. C. Vaz,* Eurico C. C. Melo,* and Thomas E. Thompson‡
*Max-Planck-Institut für Biophysikalische Chemie, D-3400 Göttingen, Federal Republic of Germany; and ‡Department of Biochemistry and Biophysics Program, University of Virginia, Charlottesville, Virginia 22908 USA

ABSTRACT Two-dimensional fluid phase connectivity is examined in mixed bilayers of dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine as a function of composition and temperature at constant pressure using fluorescence recovery after photobleaching (FRAP). These isomorphous phospholipid mixtures exhibit nearly ideal mixing behavior. Dilauroyl phosphatidylethanolamine covalently linked through its amino function to NBD is used as the fluorescent probe in this study. These studies show the line of connectivity to be coincident with the line connecting the midpoints of all tie lines in the two-phase region of the phase diagram.

INTRODUCTION

We have recently reported a study of two-dimensional fluid phase connectivity in bilayers formed from mixtures of dimyristoyl and distearoyl phosphatidylcholines as a function of composition and temperature using the fluorescence recovery after photobleaching method (FRAP). In this peritectic system the line of connectivity was found to be located close to the liquidus over a broad range of concentrations (Vaz et al., 1989).

As a continuation of our study of fluid phase connectivity in bilayer systems, we have examined this property in the nearly ideal, isomorphous binary system of dimyristoyl phosphatidylcholine (DMPC)[1] and dipalmitoyl phosphatidylcholine (DPPC). The phase diagram for bilayers in multilamellar liposomes formed from mixtures of these two lipids has been determined by differential scanning calorimetry (Van Dijck et al., 1977; Chapman et al., 1974; Mabrey and Sturtevant, 1976), by ESR (Shimshick and McConnell, 1973) and by fluorescence polarization (Lentz et al., 1976). This system exhibits relatively small deviations from ideal mixing (von Dreele, 1978; Freire and Snyder, 1980) in contrast to the highly nonideal, peritectic system of dimyristoyl phosphatidycholine/distearoyl phosphatidylcholine which we have examined previously (Vaz et al., 1989). As in the earlier study we use as the fluorescent lipid probe the nitro benzodiazolyl group linked to the amino function of dilauroyl phosphatidylethanolamine (NBD-DLPE). The derivatized phospholipid has been shown by us to partition exclusively into liquid crystalline phosphatidylcholine phases. (Vaz et al., 1989). In sharp contrast to our results in the highly nonideal DMPC/DSPC system, we find in DMPC/DPPC multibilayers that the line of connectivity coincides with the line connecting the midpoints of the tie lines in the two-phase region of the phase diagram.

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

MATERIALS AND METHODS

Materials

Dimyristoyl and distearoyl phosphatidylcholines were purchased from Avanti Polar Lipids, Inc., Birmingham AL. All lipids were critically examined 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

The method of preparation of multibilayers on supporting glass slides and the FRAP apparatus were as previously described (Vaz et al., 1989). Measurements were begun either at 20°C (heating curves) or at a temperature above the liquidus for the compositions under examination (cooling curves). Each sample was equilibrated at the desired temperature for at least 30 min before measurement. At each temperature four bleaches on different areas of each of three different samples were carried out. Temperature was controlled to ± 0.1°C with a Peltier temperature control unit (Cambridge Thermionic Corp., Cambridge, MA). Recovery curve analysis was carried out as previously described (Vaz et al., 1989).

RESULTS AND DISCUSSION

Fig. 1 is a summary of percent recovery and recovery time constants as a function of temperature for the composition 0.50 mole fraction DMPC obtained in the heating mode

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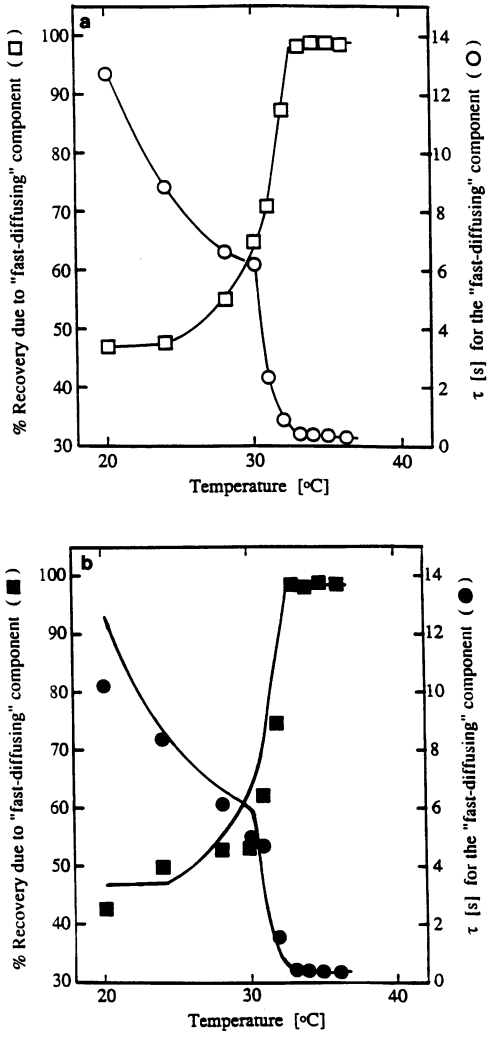

FIGURE 1 (*a*) Percent recovery and recovery time for heating scans for 0.50 mole fraction DMPC. Solid lines are best fit curves. (*b*) Percent recovery and recovery time for cooling scans for 0.50 mole fraction DMPC. For comparison the solid lines on the cooling scans are taken from the heating plots in *a*. Characteristic temperature for connectivity is 32.5°C.

(Fig.1 *a*) and in the cooling mode (Fig. 1 *b*). It is apparent from examination of Fig. 1 that at a characteristic temperature of 32.5°C, the fluorescence recovery becomes 100% and the recovery time constant falls to a value typical of liquid crystalline phosphatidylcholine bilayers at this temperature. This characteristic temperature is very nearly the same in both heating and cooling scans. Characteristic temperatures were found for mixtures of 1.0, 0.75, 0.50, 0.25, and 0 mole fractions of DMPC to be 23.2, 27.7, 32.5, 37.0, and 40.9°C, respectively. These characteristic temperatures are plotted as filled triangles (▲) on the phase diagram at constant pressure shown in

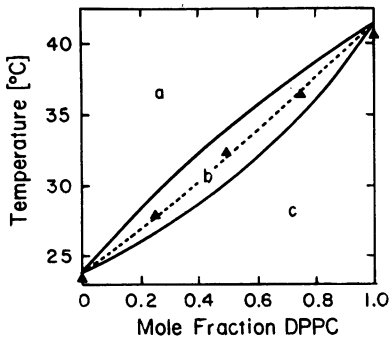

FIGURE 2 Phase diagram for DMPC/DPPC mixtures taken from Mabrey and Sturtevant (1976). Dashed line connects the midpoints of all tie lines. (*a*) Isomorphous liquid crystalline phase region; (*b*) coexistence region of gel and liquid crystalline phases; (*c*) isomorphous gel phase region. (▲) Characteristic temperatures at which connectivity of the fluid phase occurs.

Fig. 2. The phase diagram in this figure, which is based on high-precision differential scanning calorimetric data, is taken directly from Mabrey and Sturtevant (1973). Following our analysis outlined in an earlier paper, we believe the characteristic temperatures define a locus in the plane of the phase diagram along which fluid phase connectivity changes occur (Vaz et al., 1989).

The dashed line in Fig. 2 connects the midpoint of the tie lines in the two-phase region of the phase diagram. With the exception of the characteristic temperatures of the two pure components, the data fall close to this dashed line.

Although no theoretical treatment for phospholipid bilayers is available, it seems reasonable in a two-phase system in which the two components mix ideally that the percolation threshold will occur when the masses of the two coexisting phases are equal. Saxton (1989) has shown that this is the case if percolation is modeled on a triangular lattice in which the lattice points are immobilized and chosen at random. However, if the point obstacles are replaced by hexagonal obstacles, the fraction of blocked sites at the percolation threshold is increased. Xia and Thorpe (1988) using a continuum percolation approach have demonstrated that if the gel phase obstacles are ellipses with random centers and orientations, the fraction of fluid area at the percolation threshold varies from 0.3 for cirlces to 1.0 for needle-like shapes. A percolation threshold of 0.5 using this model is found when the ellipses have an axial ratio of 0.25.

As noted above the characteristic temperatures for the two pure components shown in Fig. 2 do not fall on the dashed line. In this regard it is important to note that the liquidus and solidus lines in Fig. 2 have been corrected for the finite widths of the transitions of the pure components (Mabrey and Sturtevant, 1976). The characteristic tem-

peratures for pure DMPC and DPPC lie within the two-phase region of the main transitions of these lipids but very close to the calorimetrically observed onset of gel phase melting. This behavior has now been observed in all single-component phosphatidylcholines that we have examined. Although we are not certain about the basis of this behavior, it may be that the dynamics of cluster formation and dissolution in the pure system is so rapid on the time scale of the FRAP experiment that the integrity of the gel phase barrier to probe migration is destroyed and the probe is free to move throughout the system in spite of the large proportion of gel phase. That such a condition does not occur in the two-component system may be due to a slower rate of cluster formation. This suggestion is supported by studies on the relaxation of the $P_{\beta'}$ ripple in a DPPC bilayer when the temperature is lowered into the $L_{\beta'}$ region, which indicate that the presence of a second component markedly slows this process (Rock et al., 1989).

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