Protein-Catalyzed Phospholipid Exchange between Gel and Liquid-Crystalline Phospholipid Vesicles†

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ABSTRACT: Bovine liver phospholipid exchange protein catalyzes the transfer of phosphatidylcholine between two populations of single bilayer phospholipid vesicles. Donor vesicles are prepared from egg phosphatidylcholine–phosphatidic acid–lactosylceramide (90:2:8 mol %); acceptor vesicles are prepared from phosphatidylcholine–phosphatidic acid (98:2 mol %). Activity is determined from the rate of transfer of ^{3}H-labeled egg phosphatidylcholine from donor to acceptor vesicles in the presence of phospholipid exchange protein. Donor vesicles are quantitatively precipitated by *Ricinus communis* agglutinin, while acceptor vesicles remain in the supernate. When egg phosphatidylcholine acceptor vesicles over the temperature range 11–45 °C are used, a linear Arrhenius plot is obtained, in keeping with the observation that these membranes exist only in the liquid-cyrstalline state. When dimyristoylphosphatidylcholine acceptor vesicles under the same conditions are used, however, a biphasic plot is seen with decreasing transfer activity at lower temperatures. The discontinuity occurs at 31 °C and corresponds with the onset of the liquid-crystalline to gel phase transition. The incorporation of cholesterol into dimyristoylphosphatidylcholine vesicles at a concentration sufficient to abolish the thermotropic phase transition yields a monophasic Arrhenius plot of transfer activity. The results indicate that bovine liver phospholipid exchange protein interacts catalytically with phospholipid bilayer vesicles composed of saturated or unsaturated phosphatidylcholines but preferentially with liquid-crystalline membranes.

Interactions between lipids and proteins have been extensively studied in recent years. In much of the accumulated work (Sandermann, 1978; Chapman et al., 1979), the principal focus has been the detailed description at the molecular level of structural and functional phenomena in biological membranes, plasma lipoproteins, and artificially reconstituted systems. It has become generally accepted that for many lipid–protein interactions the chemical properties and the physical organization of the lipids are important parameters. Thus, factors such as the polar head groups of phospholipids and the chain length and degree of unsaturation of the constituent fatty acids may profoundly influence certain lipid–protein interactions. Also contributing to these interactions when membranes are involved is the phase behavior of the membrane, defined by the gel–liquid-crystalline transition and lateral phase separations.

Phospholipid exchange proteins, isolated primarily from bovine liver, heart, and brain, have served as useful investigative tools in membrane research. These cytosolic proteins bind stoichiometrically specific classes of phospholipid molecules and catalyze the transfer of these phospholipids among a variety of natural and artificial membranes (Kamp et al., 1973; Ehnholm & Zilversmit, 1973; Helmkamp et al., 1974). In these systems, lipid–protein interactions actually occur at two distinct levels: (1) *phospholipid–phospholipid exchange protein* for the membrane-dissociated, water-soluble complex and (2) *membrane–phospholipid exchange protein* for the binding complex during which phospholipid exchange may proceed. With respect to the former level of interaction, the major bovine liver phospholipid exchange protein exhibits an absolute specificity for PtdCho[1] molecules, but a broad specificity toward the fatty acyl moieties (Kamp et al., 1977). Concerning the latter level, the major bovine brain phospholipid exchange protein, which transfers both PtdIns and PtdCho, binds preferentially to mixed phospholipid membranes containing low proportions of PtdIns or PtdEtn and to PtdCho membranes composed of unsaturated fatty acyl residues (Harvey et al., 1974; Helmkamp, 1980a,b).

In this communication, we describe a new assay system which permits the rapid measurement of phospholipid transfers between two populations of single bilayer lipid vesicles. This is accomplished by the incorporation of the glycolipid LacCer into one membrane population and its subsequent precipitation by *Ricinus communis* agglutinin (Curatolo et al., 1978). We report on the activity of bovine liver phospholipid exchange protein toward phospholipid vesicles of different fatty acid composition. The influence of the gel–liquid-crystalline phase transition on phospholipid exchange and the addition of cholesterol to the bilayer membrane are also examined.

Experimental Procedures

Lipids. PtdCho was isolated from fresh egg yolks, and synthetic PtdCho's were prepared from *sn*-glycerol-3-phosphocholine and the appropriate acyl anhydride, as previously described (Helmkamp, 1980b). Fatty acids, >99% purity, were purchased from Nu Check Prep, Elysian, MN. Phosphatidic acid was obtained from egg PtdCho by using partially purified phospholipase D from Savoy cabbage (Helmkamp, 1980a). LacCer and GlcCer were purchased from Miles Laboratories, Inc., Elkhart, IN, and cholesteryl [1-^{14}C]oleate was purchased from New England Nuclear, Boston, MA; they were used without further purification. Egg PtdCho was hydrolyzed with phospholipase A_2 purified from *Crotalus adamanteus* venom (Wells, 1975) and reacylated with [9,10-^{3}H]oleic acid by using rat liver microsomes (Robertson & Lands, 1962); [^{3}H]PtdCho was purified by chromatography on Unisil silicic acid (Clarkson Chemical, Williamsport, PA). All lipids were chromatographically pure when analyzed on thin layers of silica gel HR developed in chloroform–methanol–acetic acid–water (50:25:7:3 v/v)

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[1] Abbreviations used: PtdCho, phosphatidylcholine; Myr_2PtdCho, dimyristoylphosphatidylcholine; Pal_2PtdCho, dipalmitoylphosphatidylcholine; PtdIns, phosphatidylinositol; PtdEtn, phosphatidylethanolamine; LacCer, lactosylceramide; GlcCer, glucosylceramide; RCA, *Ricinus communis* agglutinin; DPH, 1,6-diphenyl-1,3,5-hexatriene.

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(Skipski et al., 1963). They were stored in chloroform–methanol (2:1 v/v), under N_2 at −20 °C.

Membrane Preparation and Characterization. Details of the preparation of sonicated, single bilayer vesicles have been reported (Helmkamp, 1980a,b). Donor vesicles contained ^{3}H-labeled egg PtdCho–phosphatidic acid–LacCer (90:2:8 mol %), while acceptor vesicles contained PtdCho–phosphatidic acid–cholesteryl [^{14}C]oleate (98:2:0.3 mol %). Cholesteryl oleate served as a nonexchangeable lipid to monitor vesicle recovery. The buffer used throughout these experiments was 10 mM Hepes and 50 mM NaCl (pH 7.4). Phosphorus analyses of membrane preparatins were done according to published procedures (Rouser et al., 1970). Vesicle concentrations were generally in the range 2–5 mM lipid phosphorus. Techniques to monitor the fluorescence polarization of DPH in donor and acceptor vesicles have likewise been described (Helmkamp, 1980b).

Phospholipid Exchange Protein. Previously published procedures were followed to purify phosphatidylcholine exchange protein from fresh bovine liver (Kamp et al., 1973). In order to obtain an electrophoretically pure protein preparation, the material was further chromatographed on a 2.5 × 10 cm column of DEAE-Sephacel (Pharmacia, Piscataway, NJ) and eluted with a linear gradient of 0–100 mM NaCl in 5 mM sodium phosphate and 0.1 mM dithiothreitol (pH 7.4). Exchange protein activity, monitored by the microsome–vesicle assay system (Kamp et al., 1973), was centered at 12 mM NaCl. The protein was stored in 50 mM sodium phosphate (pH 7.2) containing 50% (v/v) glycerol at −20 °C. Protein concentrations were based upon an extinction coefficient of 3.7×10^4 M^{-1} cm^{-1} at 280 nm (Wirtz & Moonen, 1977).

Vesicle–Vesicle Assay of Phospholipid Exchange Activity. Phospholipid exchange activity was determined by measuring the rate of transfer of [^{3}H]PtdCho from donor to acceptor vesicles. Unless otherwise specified for certain experiments, 200 nmol (based on lipid phosphorus) each of donor and acceptor vesicles were incubated together in the presence or absence of phospholipid exchange protein at 37 °C for 30 min. The buffer was 10 mM Hepes and 50 mM NaCl (pH 7.4). The total volume of 0.5 mL also included 1 mg of fatty poor bovine plasma albumin (Sigma Chemical Co., St. Louis, MO). The reaction was terminated by the addition of 0.2 mL of ice-cold RCA in the above buffer (2.2 mg mL^{-1}), which had been either donated generously by Dr. L. L. Houston, The University of Kansas, Lawrence, KS, or purchased from Miles Laboratories, Inc., Elkhart, IN. After 15 min at 0 °C, the reaction mixtures were spun in 1.5-mL polypropylene tubes in an Eppendorf Micro centrifuge (Model 5412) at 15600*g* for 2 min. An aliquot of the supernate was transferred to scintillation vials containing 10 mL of 3a70 counting cocktail (Research Products International Corp., Elk Grove Village, IL) and analyzed for ^{3}H and ^{14}C radioactivity. In some experiments, the vesicle preparations and reaction mixtures were extracted with chloroform–methanol (1:2 v/v) (Bligh & Dyer, 1959) and analyzed for radioactivity. For expression of transfer activity in terms of nanomoles of PtdCho per hour, it was assumed that 70% of the donor vesicle PtdCHo was available for binding to phospholipid exchange protein (Koter et al., 1978).

Results

Properties of the Vesicle–Vesicle Assay System. The potential usefulness of the vesicle–vesicle assay system was established by demonstrating that RCA was capable of selectively precipitating those vesicles into which 8 mol % LacCer had been incorporated. As can be seen in Figure 1, when donor and acceptor vesicles were treated with increasing quantities of agglutinin and then centrifuged, donor vesicles were precipitated and acceptor vesicles remained in the supernatant. Under the conditions of the normal assay procedure in which the final agglutinin concentration was 0.63 mg mL^{-1}, the recovery of acceptor vesicles was 92–98%, while the contamination of the supernatant by donor vesicles was 2–3%. Furthermore, this optimal separation was unaffected by increases in the amount of bovine plasma albumin added to the reaction mixture or by increases in times allocated for agglutination at 0 °C and centrifugation.

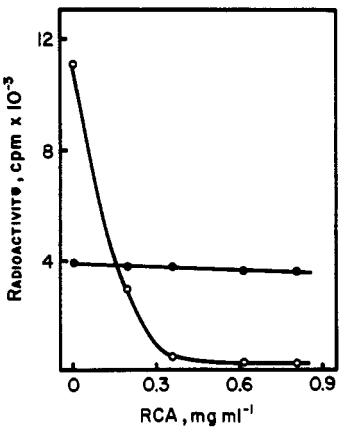

FIGURE 1: Precipitation of LacCer-containing vesicles by *Ricinus communis* agglutinin. Details of the preparation of donor and acceptor vesicles and their incubation at 37 °C for 30 min are found under Experimental Procedures. Vesicles were not mixed, and no exchange protein was present. Following agglutination and centrifugation, the supernatants were analyzed for donor vesicle ^{3}H radioactivity (○) and acceptor vesicle ^{14}C radioactivity (●).

When phosphatidylcholine exchange protein was added to the assay mixture, transfer of [^{3}H]PtdCho was observed from donor to acceptor vesicles. The magnitude of transfer was proportional to added exchange protein in the range 30–120 ng and was linear with time up to 45 min. Transfers in the range of 20 nmol of PtdCho represent 15% of the donor pool and therefore reflect initial reaction kinetics. In the absence of exchange protein, transfer was independent of vesicle composition at 37 °C; typical values were 3.4 ± 0.5 nmol h^{-1} for egg PtdCho, 4.1 ± 0.5 for Myr_2PtdCho, and 3.6 ± 0.4 for Myr_2PtdCho–cholesterol vesicles. Fusion among single bilayer vesicles may be a contributing factor to the observed protein-independent transfers, but this process is minimized by the inclusion of phospatidic acid in all vesicle preparations and the use of short incubation conditions. In other experiments, the LacCer content of donor membranes could be increased from 8 to 15 mol % without change in the rate of phospholipid transfer. Also, the incorporation of 10 mol % GlcCer in acceptor membanes was without vesicle recovery and the rate of phospholipid transfer. Thus, the presence of these glycolipids in the phospholipid vesicle bilayer has no significant effect on phospholipid exchange protein–membrane interaction.

It should be pointed out that the present assay system has several important advantages. Reactions are rapidly carried out with readily available materials. Less exchange protein is required to obtain meaningful rates of transfer. Near complete recovery of each membrane population is possible, and the lipid composition of each membrane can be manipulated. Several other vesicle–vesicle systems have been employed for phospholipid exchange measurements, including the incorporation into vesicles of sufficient phosphatidic acid to allow those vesicles to bind to DEAE-cellulose (Hellings et al., 1974), the incorporation of Forssman antigen into phos-

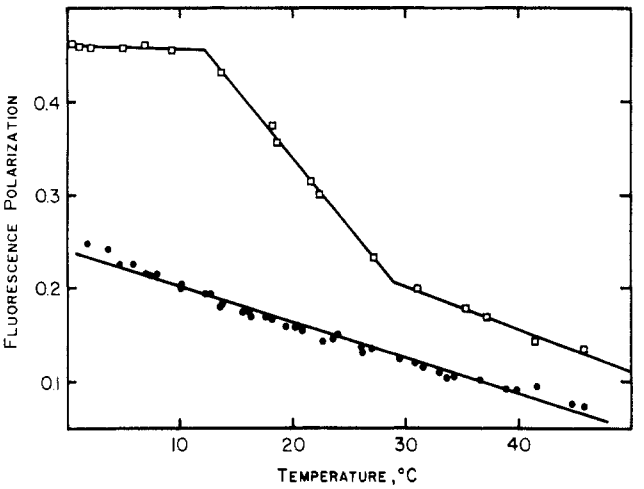

FIGURE 2: Polarization of DPH fluorescence in phospholipid vesicles. Phospholipid concentrations were 0.4 mM; DPH concentration, added as a solution in tetrahydrofuran, was 0.5 μM. Samples were heated to new temperatures and allowed to equilibrate completely before subsequent polarization measurements. Appropriate corrections for light scattering were made. Acceptor vesicles were prepared from egg PtdCho (●) or Myr_2PtdCho (□) and also contained 2 mol % egg phosphatidic acid.

pholipid bilayers and the subsequent immunoprecipitation of those membranes (Ehnholm & Zilversmit, 1973), and the use of dimannosyldiacylglycerol in vesicle preparations and the formation of precipitable complexes with concanavalin A (Sasaki & Sakagami, 1978).

Characterization of Phospholipid Vesicles by Fluorescence Polarization. Because of differences in the fatty acid composition of PtdCho used to prepare membrane vesicles, it was necessary to define the thermotropic phase behavior of the phospholipid bilayers. To this end, the rotational mobility of DPH in the bilayer was investigated. DPH partitions to the hydrophobic interior of a lipid bilayer but prefers neither the gel phase nor the liquid-crystalline phase (Lentz et al., 1976). The rotational mobility and, in turn, the fluorescence polarization of DPH are sensitive not only to temperature but also to the transition between gel and liquid-crystalline phases (Shinitzky & Barenholz, 1978).

The data in Figure 2 summarize the temperature dependence of fluorescence polarization of DPH incorporated into acceptor phospholipid vesicles. For egg PtdCho vesicles, the liquid-crystalline phase was observed over the entire temperature range, to be expected from a reported phase transition temperature of −15 to −5 °C (Ladbrooke et al., 1968). For Myr_2PtdCho vesicles, on the other hand, a transition between gel and liquid-crystalline phase was measured between 12 and 30 °C with a midpoint of 21 °C, in comparison to a transition temperature of 23 °C for pure Myr_2PtdCho liposomes (Phillips et al., 1969). Because these vesicle membranes are highly sonicated, single bilayer structures and contain 2 mol % egg phosphatidic acid, a broader transition range and somewhat lower transition temperature may be anticipated (Jacobson & Papahadjopoulos, 1975; Tsong & Kanehisa, 1977). The fluorescence polarization of DPH incorporated into donor phospholipid vesicles was also examined and found to be identical with that described above for egg PtdCho acceptor vesicles, indicating that the inclusion of 8 mol % LacCer in the egg PtdCho phospholipid bilayer did not alter the liquid-crystalline properties of that structure.

Variation in Fatty Acid Composition of Acceptor Vesicle Phosphatidylcholine. Vesicles were prepared with egg PtdCho or Myr_2PtdCho and compared over the temperature range 11–45 °C for their ability to function as acceptors of [^{3}H]-

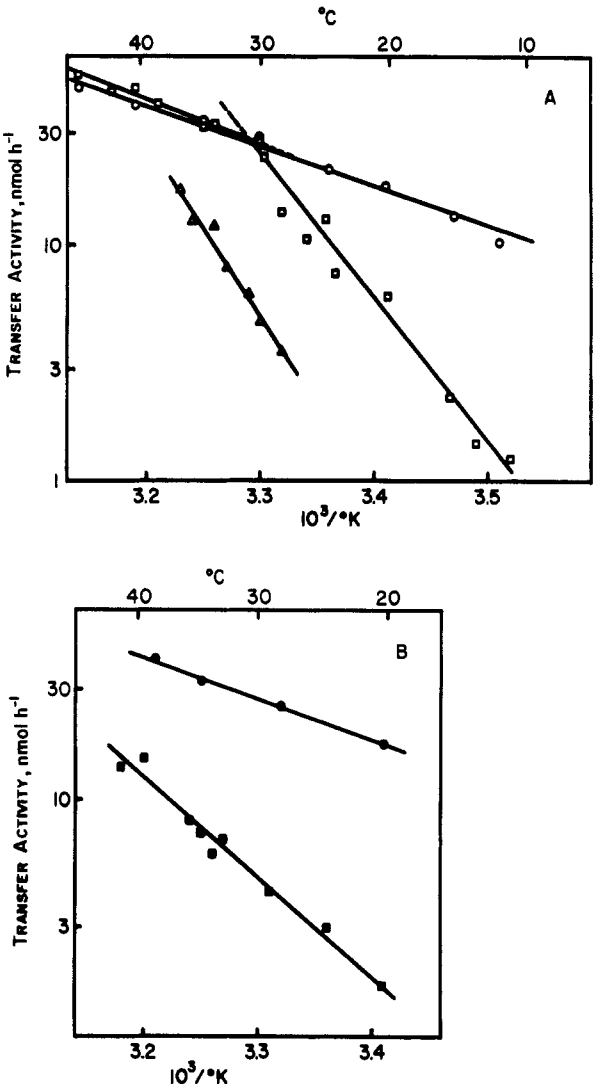

FIGURE 3: Arrhenius plots of rates of PtdCho transfer. Incubations were performed in duplicate and contained egg PtdCho donor vesicles and the indicated acceptor vesicle and quantity of exchange protein. (A) Egg PtdCho vesicles and 0.12 μg of exchange protein (○); Myr_2PtdCho vesicles and 0.30 μg of exchange protein (□); Pal_2PtdCho vesicles and 0.30 μg of exchange protein (△). (B) Egg PtdCho–cholesterol (33 mol %) vesicles and 0.12 μg of exchange protein (●); Myr_2PtdCho–cholesterol (33 mol %) vesicles and 0.50 μg of exchange protein (■).

PtdCho in exchange protein catalyzed reactions. The results were expressed as Arrhenius plots of transfer activity (Figure 3A). Egg PtdCho acceptor vesicles exhibited a continuous activity–temperature relationship over the entire temperature range, characterized by an apparent activation energy of 33 kJ mol^{-1}. Significantly, in this temperature range, egg PtdCho vesicles did not show any change in phase behavior (Figure 2). In contrast, Myr_2PtdCho vesicles displayed a discontinuity at 31 °C. Above this temperature, the temperature dependence of PtdCho transfer was essentially identical with observed egg PtdCho, and the apparent activation energy was 35 kJ mol^{-1}. Below 31 °C, transfer rates decreased much more sharply than with the egg PtdCho acceptor vesicles; for this region of the temperature range, the apparent activation energy was 115 kJ mol^{-1}. The temperature of this discontinuity corresponded with onset temperature of the liquid-crystalline to gel transition established for these vesicles (Figure 2). It is clear that all lipid bilayers, whether saturated or unsaturated, gel or liquid crystalline, can support reasonable rates of phospholipid transfer. Thus, gel-state phospholipid bilayers still participate in protein-catalyzed phospholipid transfer but

Table I: Effect of Cholesterol on Bovine Liver Phospholipid Exchange Protein Activity at 37 °C

composition of acceptor vesicle[a]		exchange protein assayed (μg)	PtdCho transfer activity (nmol h^{-1})
phospholipid species	cholesterol content (mol %)		
egg PtdCho	0	0.12	36.4
	33	0.12	31.6
Myr_2PtdCho	0	0.30	35.8
	15	0.30	17.8
	33	0.30	7.4

[a] Vesicles also contained 2 mol % egg phosphatidic acid.

with markedly increased apparent activation energies and decreased rates.

It is interesting to compare the magnitude of the thermodynamic parameter for protein-catalyzed phospholipid transfer (33–35 kJ mol^{-1}) with that determined for intermembrane transfers in the absence of phospholipid exchange protein. The apparent activation energies of the transfer of spin-labeled PtdCho between phospholipid vesicles was 82 kJ mol^{-1} (Maeda & Ohnishi, 1974) and from Sendai virus to human erythrocyte ghosts was 43 kJ mol^{-1} (Kuroda et al., 1980). For the transfer of [^{3}H]PtdCho from phospholipid vesicles to high density lipoprotein, the value was 52 kJ mol^{-1} (Jonas & Maine, 1979). It is obvious that part of the rate enhancement by phospholipid exchange protein involves a lowering of the apparent activation energy of the transfer process. Also depicted in Figure 3A is the temperature dependence of phospholipid exchange protein activity with acceptor vesicles prepared with Pal_2PtdCho, another saturated phospholipid whose transition temperature is 42 °C (Phillips et al., 1969). For the very limited temperature range 28–39 °C, the Arrhenius plot was monophasic and the apparent activation energy was 141 kJ mol^{-1}.

Effect of Cholesterol on Phospholipid Exchange. The ability of cholesterol to alter the phase behavior of phospholipid bilayers has been well documented (Hinz & Sturtevant, 1972; Oldfield & Chapman, 1972). It was, therefore, of interest to incorporate cholesterol into acceptor membranes and measure the rates of protein-catalyzed phosphatidylcholine transfer into these vesicles. The results of such experiments are presented in Table I. When egg PtdCho was the major phospholipid in the acceptor vesicle, cholesterol caused a modest decrease in transfer activity. At 33 mol % cholesterol, the activity was 88% of that observed with vesicles containing no cholesterol. For Myr_2PtdCho vesicles, the changes were more striking: 15 mol % cholesterol gave vesicles which were only half as active as pure phospholipid vesicles; 33 mol % cholesterol reduced the activity even further to 26% of control. It should be noted that the vesicle recovery and level of background transfer were no different as a consequence of adding cholesterol to the membranes.

Acceptor membranes containing 33 mol % cholesterol were also used to construct Arrhenius plots for rates of phospholipid transfer (Figure 3B). It is seen that transfer activity with egg PtdCho–cholesterol vesicles still exhibited a continuous temperature dependence. The apparent activation energy, 33 kJ mol^{-1}, was essentially no different from that found for the cholesterol-free vesicles. Moreover, the Myr_2PtdCho–cholesterol vesicles also displayed a continuous activity–temperature relationship in the temperature range 20–41 °C. The monotonic slope of the Arrhenius plot was in sharp contrast to that depicted in Figure 3 in the absence of cholesterol and yielded an activation energy, 81 kJ mol^{-1}, which was intermediate between the extremes reported above. Clearly, choesterol was able to abolish the effect of the gel–liquid-crystalline phase transition on the activity of bovine liver phospholipid exchange protein.

Discussion

Hydrated phospholipids may assume a variety of highly ordered physical orientations, many of which are dictated by the chemical properties of the phospholipid molecules and the temperature, pH, and ionic composition of the system (Luzzati et al., 1968). In general, changes in bilayer arrangements of phospholipid molecules involve a readily reversible transition between gel and liquid-cyrstalline phases (Lee, 1977). Upon increasing the temperature of single bilayer vesicles, the thickness of the bilayer is decreased, the vesicle radius is increased, and the membrane surface undergoes lateral expansion (Watts et al., 1978). These dimensional changes are accompanied by an increased amount of bound water, presumably to the polar head groups, and by the reorganization of the fatty acyl hydrocarbon chains from a rigid, all-trans configuration to the more relaxed gauche configuration. The influence of the gel–liquid-crystalline phase transition on membrane-associated phenomena include permeation to water, glucose, and alkali cations (Papahadjopoulos et al., 1973; Wu & McConnell, 1973; Blok et al., 1976), binding of organic dyes (Tsong, 1975; Jacobson & Papahadjopoulos, 1976), incorporation of proteins (Pownall et al., 1977, 1978), kinetic activity of enzymes (Kimelberg and Papahadjopoulos, 1974), and transbilayer mobility of phospholipids (de Kruijff & van Zoelen, 1978). While the physiochemical details of these events have yet to be elucidated fully, it is apparent that several mechanisms may be significant.

On the one hand, the transition from the liquid-crystalline phase to the gel phase involves considerable ordering of the fatty acyl moieties and a dramatic loss of molecular motion in the hydrocarbon region of the bilayer. The resulting changes in membrane fluidity, however defined, have given rise to the concepts of viscotropic regulation of certain membrane functions (Kimelberg & Papahadjopoulos, 1974; Sandermann, 1978) and viscotropic adaptation by cells and cellular membranes to various environmental conditions (Sinensky, 1974; Thilo et al., 1977; Silvius & McElhaney, 1980). Documentation of such viscotropic effects has usually relied upon the shape of Arrhenius plots whose discontinuities, when present, should correspond to the phase transition temperature or temperature range of the membrane being investigated. A clear preference for one phase or the other is often indictated, as in the enhanced interaction of M13 virus coat protein with gel-phase Myr_2PtdCho vesicles (Kimelman et al., 1979).

On the other hand, the phase transition also implies the coexistence of gel and liquid-crystalline phases over a discreet temperature range, the magnitude of which is strongly dependent upon the structural orientation of the phospholipid molecules. The coexistence and, hence, the separation of these two radically different phases generate boundaries or lattice defects which in turn promote enhanced permeation by small molecules or increased penetration by proteins. The signifiance of this mechanism is recognized by maximal activation at or near the phase transition and diminished activities as the membrane system moves toward a pure gel or pure liquid-crystalline phase. A particularly striking example of this phenomenon is the selective hydrolysis of vesicle PtdCho by a number of phospholipases A_2 at the substrate phase transition (Op den Kamp et at., 1975; Wilschut et al., 1978; Kensil & Dennis, 1979).

In describing the interaction of bovine liver phospholipid exchange protein with single bilayer vesicles in the region of

gel–liquid-crystalline phase transition, we may readily distinguish between the two mechanisms. The data clearly indicated significant transfer activity with liquid-crystalline bilayer membranes. At the onset of the phase transition with Myr_2PtdCho acceptor vesicles, i.e., for those temperatures at which gel and liquid-crystalline phases coexist, transfer activity decreased. This decrease was accompanied by pronounced increases in the apparent activation energy for protein-catalyzed phospholipid transfers to the acceptor vesicles.

Phospholipid transfer was facilitated by the use of fluid, liquid-crystalline phospholipid bilayer membranes. In fact, the difference in relative membrane fluidity of the egg PtdCho and Myr_2PtdCho vesicles, expressed by the fluorescence polarization of DPH, may account for the differences in the rates of PtdCho transfer with these two vesicle populations. Such a correlation between transfer activity and membrane fluidity has been established for bovine brain phospholipid exchange protein (Helmkamp, 1980b). We may also interpret the effect of cholesterol on phospholipid transfer in terms of decreased membrane fluidity, in keeping with the influence of cholesterol on molecular motion within a phospholipid bilayer (Kawato et al., 1978; Rubenstein et al., 1979). Under the present conditions where the Myr_2PtdCho–cholesterol bilayers assumed an organizational and fluidity state intermediate between the pure gel and pure liquid-crystalline phaaes, reduced rates of transfer were recorded. A smaller decrease in transfer activity was noted for egg PtdCho–cholesterol acceptor vesicles, again reflecting the expected change in fluidity in these membranes.

The incorporation of cholesterol in certain membrane populations could have several important consequences on the vesicle–vesicle assay system. It is well established that cholesterol spontaneously redistributes among single bilayer phospholipid vesicles (Haran & Shporer, 1977; Backer & Dawidowicz, 1979). The extent to which this occurs in our system is estimated to be no more than 20% of the initial acceptor membrane cholesterol concentration, based on transfer half-life of 1.5 h at 37 °C for egg PtdCho vesicles (Backer & Dawidowicz, 1979). We feel that this extent of cholesterol transfer is, nevertheless, still too large since we maintain equivalent concentrations of donor and acceptor vesicles rather than the large excesss of cholesterol-free vesicles employed to achieve pseudo-first-order kinetics in the above report. Furthermore the half-life of cholesterol exchange is longer for vesicles prepared from saturated PtdCho's (Bloj & Zilversmit, 1977). Another consequence of cholesterol inclusion is in the alteration of physical parameters of single bilayer vesicles. The vesicle radius increases from 10.2 to 13.3 nm upon addition of 33 mol % cholesterol to egg PtdCho vesicles containing 4 mol % phosphatidic acid (Johnson, 1973), while the transbilayer distribution (outside/inside ratio) of PtdCho decreases from 2.7 to 2.1 for Myr_2PtdCho vesicles containing 0 and 33 mol % cholesterol, respectively, and is virtually unchanged (2.0) for egg PtdCho vesicles containing these two cholesterol concentrations (de Kruijff et al., 1976). This means that the pool of transferable Myr_2PtdCho in our system decreases from 143 to 133 nmol in the absence and presence of 33 mol % cholesterol. We feel that this small change cannot account for the more dramatic cholesterol-induced decrease in transfer activity at 37 °C for Myr_2PtdCho acceptor vesicles.

We conclude from the present data that a critical aspect of phospholipid exchange protein catalysis involves an intimate association between protein and membrane, even perhaps penetration of the protein into the network of organized phospholipid molecules. A direct demonstration of complex formation between bovine liver phospholipid exchange protein and phospholipid vesicles has recently been achieved (Wirtz et al., 1979). While there is yet no physical evidence that penetration does occur, such an event could greatly facilitate the exchange of protein-bound and membrane-bound PtdCho that accompanies each cycle of intermembrane phospholipid transfer (Demel et al., 1973). A rather substantial energy barrier must exist for the transfer of a PtdCho molecule between an accommodating lipid environment and even the most hydrophobic protein environment (Tanford, 1973); this barrier would cerainly be greater if water were not excluded from the interfacial regions to which exchange protein binds. The fluid environment of a liquid-cyrstalline lipid bilayer, the increased fluidity of unsaturated phospholipid membranes, and the general hydrophobic character of the phospholipid exchange protein (Kamp et al., 1973) all promote effective interaction between membrane and protein.

One experimental system, namely the use of phospholipid monolayers, has been adapted for the study of lipid–protein interactions (London et al., 1973). With particular reference to phospholipid exchange protein, the addition of the bovine liver protein to the aqueous subphases of pure and mixed phospholipid monolayers resulted in no detectable change in the surface pressures of the lipid films (Demel et al., 1973, 1977). One interpretation of these results would be to argue against membrane penetration of exchange protein. Alternatively, if penetration does take place, it may involve changes in lipid organization in the vicinity of the protein (Pink & Chapman, 1979) or in protein conformation within the membrane (Bloom, 1979), neither of which would necessarily perturb gross membrane structure. Additional experimental approaches, including the use of sensitive spectroscopic probes and techniques, are needed to provide further insight into the precise nature of phospholipid exchange protein–membrane interactions.

Acknowledgments

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