chelate. This is noteworthy because dipicolinate and 1,10-phenanthroline have nearly identical first and second affinity constants for zinc ion (Sillen & Martell, 1971) and neither affects the equilibrium constant for the spontaneous dissociation of zinc from the holoenzyme (this work; Lindskog & Malmstrom, 1962).

It is difficult to compare the pseudo-first-order rates of these ligand-assisted dissociations with the so-called "spontaneous dissociation" of zinc from the enzyme because the latter rate, normally assumed to be first order, may in fact be pseudo-first-order due to the participation of water (solvent) in the reaction. A pragmatic comparison of the differences among these processes can be made if the former are defined relative to a 1 M standard state and the latter is assumed to be first order. A $\Delta G^{\ddagger}$ of about 24 kcal can be calculated for the rate of the spontaneous dissociation by using the association rate constant (Henkens & Sturtevant, 1968) and the equilibrium constant. Upon comparison with the $\Delta G^{\ddagger}$ of 18 kcal found for dipicolinate and the $\Delta G^{\ddagger}$ of 23 kcal calculated for 1,10-phenanthroline, it can be seen that dipicolinate is much more efficient.

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Effects of Phospholipid Fatty Acid Composition and Membrane Fluidity on the Activity of Bovine Brain Phospholipid Exchange Protein†

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ABSTRACT: The interaction of bovine brain phospholipid exchange protein with membranes has been investigated as a function of membrane phospholipid fatty acid composition. Single bilayer vesicles were prepared by sonication, centrifugation, and molecular sieve chromatography and were used as acceptor membranes in the exchange protein catalyzed transfer of phosphatidylinositol from rat liver microsome donor membranes. For the series egg phosphatidylcholine and dioleoyl-, and dielaidoyl-, and dimyristoylphosphatidylcholine, initial rates of phosphatidylinositol transfer were highest with the two cis-unsaturated species and lowest with the saturated species, the trans-unsaturated species being intermediate. A progressive decrease in transfer rate was noted with vesicles containing a mixture of egg phosphatidylcholine and dimyristoylphosphatidylcholine as the molar proportion of the latter phospholipid increased. Apparent Michaelis constants for the interaction between exchange protein and different vesicle preparations decreased in the order saturated > trans unsaturated > cis unsaturated. Maximum velocities were independent of fatty acid composition. The fluorescence polarization of diphenylhexatriene in vesicle preparations also decreased in the same order, under conditions well above the thermotropic gel to liquid-crystalline phase transition of all phospholipids studied. These results suggest that the fatty acid composition, the degree of unsaturation, and in particular the hydrocarbon fluidity of the membrane are important determinants in the activity of bovine brain phospholipid exchange protein.

In recent years the physicochemical characterization of biological and artificial membranes has been advanced through the use of phospholipid exchange proteins. Among the applications which have been described are the insertion of spectroscopic probes into membranes, the determination of membrane topography, and the measurement of transbilayer mobility (Rothman & Dawidowicz, 1975; Barsukov et al., 1967; Bloj & Zilversmit, 1976; Shaw et al., 1977; Brophy et al., 1978; Sandra & Pagano, 1978; van den Besselaar et al., 1978; DiCorleto & Zilversmit, 1979). Each of these depends upon selective associations between exchange proteins and transferable phospholipid molecules, such as the specificity of bovine liver exchange protein for PtdCho[1] (Kamp et al., 1977).

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Also of importance are the interactions of exchange proteins with membranes which must occur during protein-catalyzed, intermembrane transfers of phospholipids. These interactions have been shown to be extraordinarily sensitive to the lipid composition of the membranes, particularly the polar head groups of amphiphilic lipids in a variety of membranes. For example, the activity of bovine heart exchange protein is enhanced by the incorporation of diphosphatidylglycerol into multilamellar liposomes (DiCorleto & Zilversmit, 1977); the activity of bovine brain exchange protein is likewise enhanced by the incorporation of PtdEtn or sphingomyelin into single bilayer vesicles (Helmkamp, 1980). While these and other studies (Hellings et al., 1974; Harvey et al., 1974; Johnson & Zilversmit, 1975; Wirtz et al., 1976, 1979) point to specific effects of polar head groups on phospholipid exchange protein activity, contributions from the fatty acyl chains have not been considered in detail. This is of particular importance since the naturally occurring phospholipids employed in such studies have very diverse fatty acid compositions (White, 1973). We have, therefore, prepared a series of PtdCho vesicles of defined fatty acid composition and investigated them as acceptors of phospholipids from rat liver microsomes in the presence of bovine brain phospholipid exchange protein. While both PtdCho and PtdIns can be transferred by bovine brain phospholipid exchange protein, PtdIns is the preferred substrate, even when its molar proportion in participating membranes is significantly less than that of PtdCho (Helmkamp et al., 1974; Harvey et al., 1974; Demel et al., 1977). The bidirectionality of phospholipid exchange, from microsome to vesicle as well as from vesicle to microsome, has also been established (Harvey et al., 1974). In the current study rates of PtdIns transfer are reported as functions of acyl chain unsaturation, temperature, and membrane fluidity.

Experimental Procedures

Lipids. Phosphatidylcholine was isolated from fresh egg yolks (Bangham et al., 1974) and hydrolyzed to phosphatidic acid with partially purified phospholipase D from Savoy cabbage (Davidson & Long, 1958); phosphatidic acid was converted to its sodium salt (Papahadjopoulos & Miller, 1967). Synthetic phosphatidylcholines were prepared by reacting the cadmium chloride complex of *sn*-glycero-3-phosphocholine (Baer & Buchnea, 1959) with the appropriate fatty acyl chloride or anhydride (Cubero Robles & van den Berg, 1969). All phospholipids were chromatographically pure when analyzed on 0.25-mm layers of silica gel HR developed in chloroform–methanol–acetic acid–water (50:25:7:3 by volume) (Skipski et al., 1963). Chloroform solutions of lipids were stored under nitrogen at −20 °C.

Acidic methanolysis of synthetic phosphatidylcholines (0.2–0.5 μmol) was carried out (Ways et al., 1963), and the resulting fatty acid methyl esters were analyzed by gas–liquid chromatography with a Hewlett-Packard Model 5830A instrument equipped with a flame ionization detection system and an electronic integrator. Dual glass columns (1/8 in. × 6 ft) of 15% diethylene glycol succinate on 80–100 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) were operated between 120 and 190 °C at 2 °C min^{-1} and isothermally thereafter, with nitrogen (20 mL min^{-1}) as carrier gas. The weight distributions of the principal fatty acids in each phospholipid were the following: dimyristoyl-PtdCho, 99.0%; dioleoyl-PtdCho, 98.5%; dielaidoyl-PtdCho, 99.6%.

Phospholipid Exchange Protein and Membrane Preparations. Previously published procedures were followed to purify the phospholipid exchange protein from fresh bovine cerebral cortex (Helmkamp et al., 1974). The protein was stored in 10 mM Hepes and 50 mM NaCl (pH 7.4) containing 50% glycerol at −20 °C. The preparation of rat liver microsomes labeled specifically with phosphatidyl-*myo*-[2-^{3}H]inositol has been described (Demel et al., 1977).

Liposomes were prepared in 10 mM Hepes and 50 mM NaCl (pH 7.4) at a phospholipid concentration of 5 mM and sonicated (Heat Systems Model W185-F sonicator) under nitrogen at 60–80 W for 10–15 min. During sonication the lipid suspension was maintained at a temperature ~10 °C above the thermotropic transition temperature for the specific phosphatidylcholine. Following sonication, the vesicles were centrifuged at 48000*g* for 30 min to remove multilamellar aggregates and titanium fragments. In addition to PtdCho, all vesicles contained 2 mol % phosphatidic acid and 0.3 mol % cholesteryl [1-^{14}C]oleate. The latter, a nonexchangeable membrane component, permits an estimation of membrane recovery after incubation and extraction. Protein determinations (Lowry et al., 1951) used bovine plasma albumin as a standard. Microsomal and vesicle lipids were quantitatively extracted (Bligh & Dyer, 1959); phosphorus analyses were done on all membrane preparations (Rouser et al., 1970).

Assay of Phospholipid Transfer Activity. Phospholipid transfer activity was determined with donor membranes of rat liver microsomes (1.25 mg of protein, 60 nmol of PtdIns), acceptor membranes of PtdCho vesicles (1 μmol of phospholipid, unless otherwise stated), and 2.8 μg of exchange protein (Helmkamp et al., 1974). The buffer system was 10 mM Hepes and 50 mM NaCl (pH 7.4) with a final volume of 2.5 mL; incubation conditions were 30 min at 37 °C. Initial rates of PtdIns transfer are observed under these conditions. Activity is expressed as nanomoles of PtdIns transferred per hour from microsomes to vesicles. Incubations carried out in the absence of exchange protein allowed correction for noncatalyzed phospholipid transfer or undefined aggregation of microsomes and vesicles.

Fluorescence Polarization Measurements. Suspensions of phospholipid vesicles, 0.4–0.8 mM in 10 mM Hepes and 50 mM NaCl (pH 7.4), were treated with 1 mM DPH in tetrahydrofuran such that the mole ratio of phospholipid to DPH was in the range 700–1000. An equivalent volume of pure tetrahydrofuran was added to another portion of the vesicle suspension, to be used as a light-scattering blank. All samples were then incubated for 1 h at 37 °C so that DPH could equilibrate completely with the membrane preparations. The fluorescence polarization of DPH was measured (Shinitzky & Barenholz, 1978) with an SLM Instrument Model 8000 spectrofluorometer. DPH was excited at 357 nm; its fluorescence emission was detected at 435 nm in the presence of a Corning CS 3-73 cutoff filter. Irradiation of the samples was limited to 2 s, with at least 15 s between readings. All measurements were corrected for light scattering, although this step proved to be virtually unnecessary for these single bilayer vesicles. Polarization, P, was calculated from the fluorescence intensities (photon counts) detected parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) to the direction of polarization of the exciting radiation, according to the relationship

$$P = [(I_{\parallel}/I_{\perp}) - 1]/[(I_{\parallel}/I_{\perp}) + 1] \quad (1)$$

Temperature was controlled with a thermostated cuvette holder connected to a circulating refrigerator–heater unit. For measurements of fluorescence polarization as a function of

[1] Abbreviations used: PtdCho, phosphatidylcholine; PtdIns, phosphatidylinositol; PtdEtn, phosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene.

Table I: Activity Parameters of Phosphatidylcholine Vesicles of Various Fatty Acid Composition

membrane PtdCho[a]	transfer act. (nmol h^{-1})	K_m (mM)	V_{max} (nmol h^{-1})	Arrhenius activation energy (kJ mol^{-1})	fluorescence polarization[b]
egg	15.4	0.09	17.2	34.7	0.102 ± 0.001
dioleoyl	15.2	0.11	20.0	33.9	0.100 ± 0.001
dielaidoyl	9.6	0.93	26.3	60.2	0.137 ± 0.006
dimyristoyl (98 mol %)	1.6	_[c]	–	–	0.155 ± 0.004
dimyristoyl (60 mol%)	12.4	0.88	20.0	–	–
dimyristoyl (40 mol %)	13.8	0.22	21.9	–	–

[a] Membranes also contained 2 mol % phosphatidic acid, and in the case of dimyristoyl-PtdCho vesicles the balances were made up by egg PtdCho. [b] Values represent mean ± SD for at least five corrected determinations of DPH polarization. [c] Not determined.

temperature, samples were gradually heated to a new temperature and allowed to equilibrate completely before irradiation.

Materials. *myo*-[2-^{3}H]Inositol was purchased from Amersham Corp., Arlington Heights, IL, and cholesteryl [1-^{14}C]oleate was from New England Nuclear, Boston, MA. Fatty acids were purchased from Nu Chek Prep, Elysian, MN. Sepharose 4B was a product of Pharmacia Fine Chemicals, Piscataway, NJ. DPH was obtained from Aldrich Chemical Co., Milwaukee, WI. Organic solvents were freshly distilled, and water was distilled in an all-glass apparatus. All other chemicals were reagent grade.

Results

Characterization of Sonicated Liposome Dispersions. The morphology of the sonicated liposome dispersions used in this study was investigated by molecular sieve chromatography. Following centrifugation, a typical membrane preparation was layered on Sepharose 4B, previously equilibrated with 10 mM Hepes, 50 mM NaCl, and 0.2% NaN_3 (pH 7.4), and saturated with egg PtdCho (Huang, 1969). The elution profile of the vesicles is shown in Figure 1. The ratio of phospholipid to cholesteryl oleate in the fractions remained essentially constant, suggesting a stable interaction between these molecules upon vesicle formation. Furthermore, the membranes were retained by the column matrix, indicating that single bilayer vesicles were the predominant structure in the preparation. Some membrane preparations exhibited minor (<10%) contamination by multilamellar vesicles, which eluted with the void volume. There was, however, generally no difference between the sonicated dispersions before and after molecular sieve chromatography when used as acceptor membranes in phospholipid exchange systems.

The thermotropic phase transition of several of the vesicle preparations was investigated by measuring the fluorescence polarization of DPH embedded in the lipid bilayer and recording the changes in polarization that occurred as the bilayer underwent a gel to liquid-crystalline phase transition. Both dimyristoyl-PtdCho and dielaidoyl-PtdCho were investigated in this manner. For dimyristoyl-PtdCho vesicles the transition occurred between 12 and 30 °C with a midpoint of 21 °C; for dielaidoyl-PtdCho the transition range was 6–15 °C with a midpoint of 11 °C. These values compare favorably with transition temperatures determined for the pure phospholipids, namely, 23 °C for dimyristoyl-PtdCho (Phillips et al., 1969) and 12 to 13 °C for dielaidoyl-PtdCho (Norman et al., 1972; Wu & McConnell, 1975). The slightly lower values of the transition temperatures reported here may be influenced by the inclusion of egg phosphatidic acid in the vesicle preparations (Jacobson & Papahadjopoulos, 1975), while the broad transition range reflects the analysis of small, single bilayer vesicles (Sheetz & Chan, 1972; Lentz et al., 1976). Transition temperatures have been reported to be −5 to −15 °C for egg PtdCho (Ladbrooke et al., 1968) and −22 °C for dioleoyl-PtdCho (Phillips et al., 1972). It is clear that at 37 °C all membranes have completed their phase transition and exist only in the liquid-crystalline orientation.

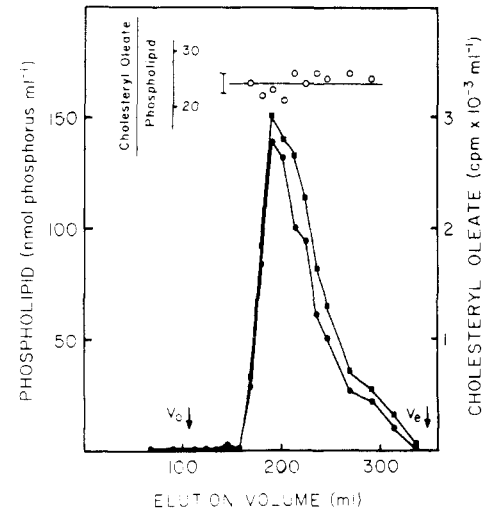

FIGURE 1: Chromatography of sonicated phosphatidylcholine liposomes. Hand-shaken liposomes were prepared in 10 mM Hepes and 50 mM NaCl (pH 7.4) from the following lipid mixture: dielaidoyl-PtdCho (98 mol %), egg phosphatidic acid (2 mol %), and cholesteryl [1-^{14}C]oleate (0.3 mol %). The membranes were sonicated at 25 °C and then centrifuged at 48000*g* for 30 min. The supernate was applied to a 2.5 × 50 cm column of Sepharose 4B and eluted with the above buffer. Column characteristics were determined with Blue Dextran (v_0) and riboflavin (v_e). Fractions were analyzed for phospholipid (lipid phosphorus, ●), cholesteryl oleate (^{3}H radioactivity, ■), and the ratio of cholesteryl oleate to phospholipid (○). If necessary, peak fractions were concentrated by ultrafiltration with an Amicon XM100A membrane prior to use in phospholipid exchange systems.

Variation of Phospholipid Fatty Acid Composition in Single Bilayer Vesicles. Single bilayer vesicles were prepared from phospholipids containing monounsaturated or saturated fatty acyl residues. The vesicles were then evaluated as acceptors of microsomal PtdIns in the standard microsome–vesicle assay system. As summarized in Table I, transfer activity was 15.2 nmol h^{-1} with dioleoyl-PtdCho and 9.6 nmol h^{-1} with its trans isomer dielaidoyl-PtdCho. The activity in the presence of saturated dimyristoyl-PtdCho vesicles was only 1.6 nmol h^{-1}. By comparison, egg PtdCho vesicles, in which the major unsaturated fatty acid is oleic acid (White, 1973), supported an activity of 15.4 nmol h^{-1}. A series of vesicles was prepared which contained varying proportions of egg PtdCho and dimyristoyl-PtdCho in order to examine more fully the effect of saturated fatty acyl residues on phospholipid transfer. The highest transfer activity occurred with egg PtdCho vesicles and the lowest with pure dimyristoyl-PtdCho vesicles (Figure 2). With vesicles prepared from mixtures of the unsaturated and saturated phospholipids, transfer activity decreased dramat-

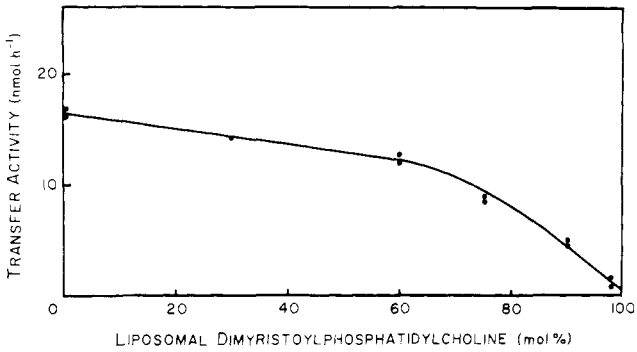

FIGURE 2: Effect of dimyristoyl-PtdCho on phospholipid exchange protein activity. Liposomes were prepared from egg PtdCho, dimyristoyl-PtdCho, or the indicated mixtures of these PtdCho's; they also contained 2 mol % phosphatidic acid. Following sonication and centrifugation, the vesicles (1 μmol of lipid phosphorus) were tested as acceptors of microsomal PtdIns in the presence or absence of bovine brain exchange protein.

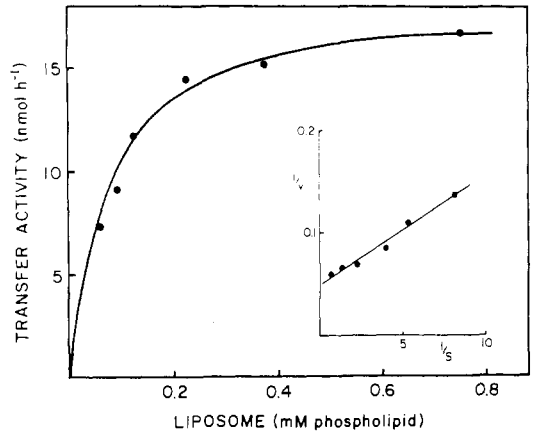

FIGURE 3: Phosphatidylinositol transfer activity as a function of sonicated liposome concentration. The transfer of PtdIns is measured from rat liver microsomes to the indicated quantity of dioleoyl-PtdCho vesicles containing 2 mol % phosphatidic acid in a total volume of 2.5 mL. The inset depicts the data in a double-reciprocal plot, where S is vesicle phospholipid millimolarity and v is nanomoles of PtdIns transferred per hour.

ically as the content of dimyristoyl-PtdCho increased. These measurements were made at 37 °C, well above the phase transition temperatures of all the phospholipids employed. It is concluded that bovine brain phospholipid exchange protein can interact with both cis- and trans-monounsaturated phospholipid bilayers, with the geometry of the cis species providing a more favorable catalytic environment. On the other hand, saturated phospholipid bilayers appear to be inactive in the protein-catalyzed exchange of phospholipids.

Kinetic Parameters for PtdCho Vesicles. Transfer activity was measured as a function of sonicated liposome phospholipid concentration to investigate more fully the interaction between phospholipid exchange protein and a particular vesicle population. Typical results are shown in Figure 3. Transfer activity exhibited a hyperbolic relationship with the concentration of each of the membranes studied. Transposition of the data to double-reciprocal plots and curve fitting by least-squares analysis produced linear relationships from which values of an apparent maximum catalytic velocity, V_{max}, and an apparent Michaelis constant, K_m, were calculated (Table I). Membranes were prepared from mixtures of dimyristoyl-PtdCho and egg PtdCho based upon the results shown in Figure 2 in order to obtain measurable rates of transfer with dimyristoyl-PtdCho. The values of V_{max} remained relatively constant for the various vesicles, in a range

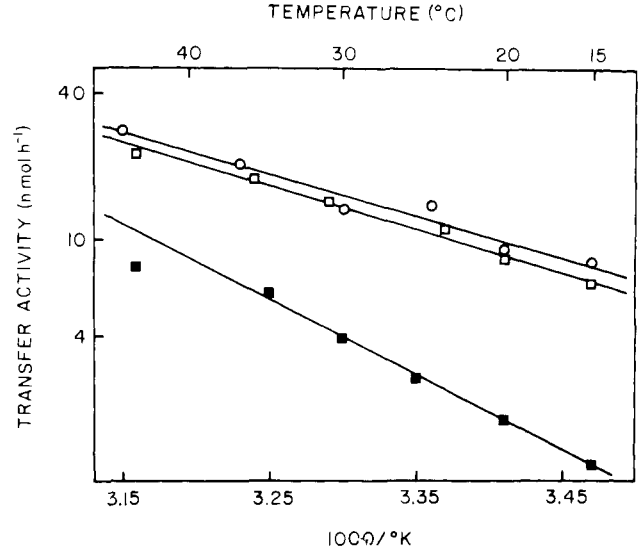

FIGURE 4: Temperature dependence of phosphatidylinositol transfer from microsomes to vesicles. Vesicles were prepared from egg PtdCho (□), dioleoyl-PtdCho (○), or dielaidoyl-PtdCho (■).

from 17.2 to 26.3 nmol h^{-1}. On the other hand, rather striking differences in K_m were found. Egg PtdCho and dioleoyl-PtdCho gave Michaelis constants of 0.09 and 0.11 mM, respectively. The K_m increased to 0.83 mM for the trans isomer dielaidoyl-PtdCho. Finally, for egg PtdCho vesicles containing 40 mol % dimyristoyl-PtdCho a K_m of 0.22 mM was determined, while for those containing 60 mol % dimyristoyl-PtdCho the K_m was 0.88 mM. By extrapolation, the K_m for pure dimyristoyl-PtdCho would be expected to be considerably greater.

Activation Energies of Phospholipid Exchange. The transfer of PtdIns from microsomes to various PtdCho vesicles was investigated over the temperature range 15–45 °C. Arrhenius plots were constructed by using the observed rates of transfer. In all cases the curves were linear and without discontinuity (Figure 4). The activation energies for transfer to egg PtdCho and dioleoyl-PtdCho membranes were 34.7 and 33.9 kJ mol^{-1}, respectively. The activation energy with dielaidoyl-PtdCho vesicles was 60.2 kJ mol^{-1}, nearly twice that for the cis isomer (Table I). Thus, the geometry of unsaturation in the membrane phospholipid fatty acyl residue plays a critical role in the phospholipid exchange process. On the basis of the observed differences in Arrhenius activation energies, the rate-limiting step most likely involves an event at or within the vesicle bilayer membrane.

Fluorescence Polarization of DPH in PtdCho Vesicles. The ability of a vesicle to participate in intermembrane phospholipid transfers has been, thus far, characterized by the rate at which it accepted PtdIns from rat liver microsomes and by the magnitude of the apparent Michaelis constant for the interaction between phospholipid exchange protein and the membrane. For all vesicles studied the assay temperature of 37 °C was well above the gel to liquid-crystalline phase transition temperature. Nevertheless, because of significant differences in the nature of the fatty acyl residues in the phospholipids, it is not unlikely to expect differences, however subtle, in the nature of the liquid-crystalline state of these molecules. For this reason the rotational mobility of the fluorescent probe DPH, which partitions to the hydrophobic interior of a lipid bilayer but perferentially into neither gel nor liquid-crystalline phases (Lentz et al., 1976), was investigated. Rotational mobility and membrane fluidity were approximated by the degree of fluorescence polarization; i.e.,

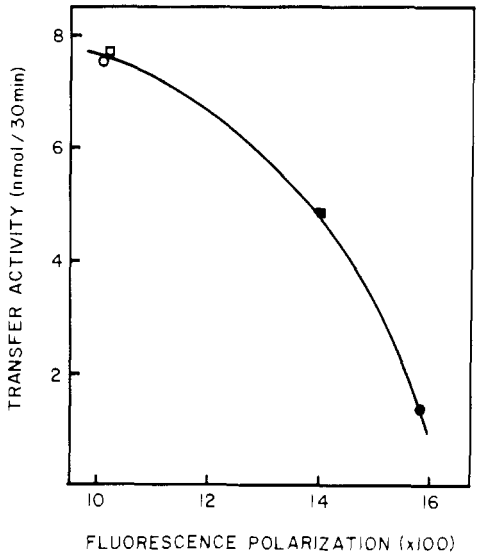

FIGURE 5: Correlation between fluorescence polarization of diphenylhexatriene and rates of phosphatidylinositol transfer to different vesicle preparations. All measurements were performed at 37 °C as outlined under Experimental Procedures. Vesicles were prepared from egg PtdCho (□), dioleoyl-PtdCho (○), dielaidoyl-PtdCho (■), or dimyristoyl-PtdCho (●).

the lower the polarization, the greater the rotational mobility and the greater the fluidity (Shinitzky & Barenholz, 1978). As summarized in Table I, DPH in vesicles prepared from egg PtdCho or dioleoyl-PtdCho displayed polarizations of 0.102 and 0.100, respectively. Polarization increased to 0.137 for dielaidoyl-PtdCho and to 0.155 for dimyristoyl-PtdCho. By comparison, the polarization of DPH in dipalmitoyl-PtdCho vesicles was 0.251, at a temperature probably within the gel to liquid-crystalline phase transition range. The polarization of DPH in the absence of rotational motion is 0.460 (Andrich & Vanderkooi, 1976). Clearly, DPH encounters a spectrum of physical environments in these various lipid bilayers and exhibits a more hindered rotational behavior in those membranes prepared from phospholipids containing saturated or trans-unsaturated fatty acids.

The same spectrum of physical environments described by the rotational mobilities of DPH may also contribute to the differences among these vesicles observed in phospholipid transfer. Indeed, there was a reasonable correlation between transfer activity for various vesicle preparations and the fluorescence polarization of DPH in those same vesicles (Figure 5). That is to say, transfer rates of PtdIns were highest to membranes which presented the least constrained (most fluid) bilayer interior to the phospholipid exchange protein. It is significant that maximum and minimum rates of PtdIns transfer occurred over a range of DPH polarization that was much narrower than the range accompanying the typical gel to liquid-crystalline phase transition. This suggests that the physical events within the lipid bilayer which are responsible for altering phospholipid exchange activity may involve changes in fatty acyl chain mobility and organization different from those described for a thermotrophic phase transition.

Discussion

In previous studies the mechanism by which phospholipid exchange proteins catalyze the transfer of phospholipids between membranes has been examined in detail (van den Besselaar et al., 1975; Helmkamp et al., 1976; Wirtz et al., 1979). What has emerged from this work is a model based on the following assumptions: (1) the formation of complexes between the exchange protein and a membrane and between the exchange protein and a phospholipid and (2) the deposition of a protein-bound phospholipid into the membrane and/or the acquisition of a membrane-bound phospholipid by the protein. Each detectable catalytic cycle can, therefore, be thought to consist of two distinct and sequential half-reactions, namely, a protein–donor membrane interaction followed by a protein–acceptor membrane interaction. During each interaction the possibility of phospholipid exchange exists. In support of this model, complexes between phospholipids and a number of phospholipid exchange proteins have been demonstrated (Kamp et al., 1973; Johnson & Zilversmit, 1975; Helmkamp et al., 1976). More recently, Wirtz et al. (1979) provided clear evidence for complexes between phospholipid vesicles and bovine liver phospholipid exchange protein.

Kinetically the overall exchange process has been treated as an enzyme (exchange protein)–bireactant (donor membrane and acceptor membrane) system and has been analyzed by appropriate initial velocity studies. With bovine brain phospholipid exchange protein, for example, the series of parallel lines obtained in double-reciprocal plots of transfer activity vs. varying concentrations of both donor and acceptor membranes was interpreted as supporting a ping-pong bi-bi mechanism and arguing against any mechanism which involved a ternary complex among the protein, donor membrane, and acceptor membrane (Helmkamp et al., 1976). While there is little knowledge available as to which step in the exchange sequence is rate limiting, such information does not preclude the calculation of apparent Michaelis constants from individual double-reciprocal plots for any given protein–reactant combination (Cleland, 1970). Keeping in mind that an apparent K_m is strictly an experimentally determined parameter, we nevertheless feel that this term provides a useful means by which an exchange protein–membrane interaction may be quantitated and different membranes may be compared.

In experimental systems where one or more of the reactants does not exist in true molecular solution, e.g., a phospholipid membrane, the manner in which reactant levels and apparent K_m are expressed must be considered. The concept of *surface concentration* has been elegantly described by Verger et al. (1973) and Deems et al. (1975) for the action of phospholipase A_2 toward phospholipid monolayers or mixed phospholipid–detergent micelles. This approach permits a precise estimation of not only the quantity of phospholipid available for hydrolysis but also the surface area to which an enzyme can bind. These two systems are characterized by a single, readily measurable pool of phospholipid, all of which is exposed to the protein-containing medium. On the other hand, the use of *bulk concentration* of membrane phospholipid in the calculation of apparent K_m for the action of phospholipase A_2 toward single bilayer vesicles and multilamellar liposomes has been noted (Kensil & Dennis, 1979). In the current investigation, as well as in earlier ones (Helmkamp et al., 1974, 1976; Helmkamp, 1980), we have chosen to employ total membrane phospholipid in all concentration expressions. The interactions between phospholipid exchange protein and a vesicle membrane can be envisaged as occurring at discrete surface loci, the concentration of which is proportional to the total membrane phospholipid. That such a proportionality holds is indicated by the following observations on certain structural aspects of egg PtdCho and dioleoyl-, dielaidoyl-, and dimyristoyl-PtdCho. (1) The unsaturated PtdCho vesicles were essentially identical in size (outer diameter and bilayer thickness), while the saturated PtdCho vesicles were only somewhat smaller (de Kruijff et al., 1976; Laggner et al., 1979). (2) Above the gel to

liquid-crystalline phase transition all PtdCho molecules exhibited similar membrane surface areas (Huang & Mason, 1978; Watts et al., 1978). For PtdCho vesicles containing 2 mol % phosphatidic acid, the phospholipids were distributed symmetrically across the outer and inner faces of the bilayer (Massari & Pascolini, 1977; Koter et al., 1978). These observations suggest that the differences in the activity of the vesicle preparations in phospholipid exchange systems cannot be attributed to variations in the size, surface area, or surface chemistry of the membranes.

Our studies have demonstrated significant differences in fluidity between saturated and unsaturated phospholipid vesicles as well as between cis- and trans-unsaturated vesicles. To interpret the observed correlation between the participation of a vesicle membrane in phospholipid exchanges and the fluidity of lipid bilayer in terms of protein–lipid interactions, one may consider several possibilities: penetration of the phospholipid exchange protein into and subsequent expulsion from the lipid bilayer, insertion of a PtdIns molecule into and extraction of a PtdCho molecule from the lipid bilayer, or a combination of these two. These processes share a common determinant in that the phospholipid bilayer presents a barrier to molecular movements into and out of the membrane; such movements may be facilitated by a more fluid environment. Thus, the physicochemical properties of phospholipids and the membranes prepared from them dictate to a very significant extent the catalytic activity of bovine brain phospholipid exchange protein.

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Paramagnetic Isoprenoid Carrier Lipids. 1. Chemical Synthesis and Incorporation into Model Membranes†

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ABSTRACT: The synthesis, purification, and characterization of two types of spin-labeled glycosyl carrier lipids and shorter chain isoprenols are described. As models for phosphorylated lipid intermediates, phosphodiesters of tempol and the prenols dolichol, ficaprenol, solanesol, phytol, and farnesol were prepared. For analogues of neutral species each prenol was esterified with a pyrrolidinecarboxylic acid based label. Tripropylbenzenesulfonyl chloride was used as the condensing agent in both cases. Phosphodiester yields ranged from 36% for the 55-carbon compound to >66% for the 95-carbon prenol. Both types of probes were incorporated into phospholipid bilayers, where each became oriented with the artificial head group at, or very close to, the water–hydrocarbon interface. Electron spin resonance spectra of the phosphodiesters are matrix dependent, indicating rapid isotropic tumbling in chloroform but highly anisotropic reorientation in unsaturated phosphatidylcholine (PC) hosts. Rotation or large amplitude oscillation about either or both the tempo C_4–O linkage and the P–O (chain) bond as well as whole molecule rotation within the bilayer could account for the observed *x*-axis anisotropy. Segmental motion within the polyprene chain does not appear to be a determinant.

Dolichyl phosphate and undecaprenyl phosphate are poly-cis-isoprenoid lipids which function as chemical carriers of saccharide units during the membrane-directed assembly of mammalian glycoproteins and a variety of bacterial surface polysaccharides. During the past 10 years a substantial amount of information concerning their biochemistry has been amassed (Hemming, 1974; Lennarz, 1975; Waechter & Lennarz, 1976; Parodi & Leloir, 1979). Reactions leading to their biosynthesis and acylation as well as the detailed sequences of transfer reactions they mediate are being mapped out. Subcellular locations, nucleotide and polyisoprenoid specificities, antibiotic sensitivities, and detergent and metal effects for the complementary transferases are continually being defined. Singularly lacking, however, is an understanding of the organizational and dynamic parameters of the lipid carriers and transferases within either model or biological membranes. To our knowledge only one group has attempted to directly probe the immediate membrane environment of a "lipid-linked sugar" (Johnston & Neuhaus, 1975; Weppner & Neuhaus, 1978). After studying the fluorescence spectra of an endogenous dansylated intermediate of peptidoglycan synthesis in *Staphylococcus aureus*, they concluded that the dansyl moiety was located near the membrane surface and that the intermediate was immobilized within the membrane.

It is conceivable that the length and poly-cis geometry of these compounds could endow them with some unique physicochemical properties that are important to their biological function(s). For the purpose of investigating certain of these characteristics in model systems, we have chemically synthesized two groups of spin-labeled polyisoprenols. One group was designed to mimic the behavior of phosphorylated intermediates, and a second series of carboxylate esters was intended to model properties of the abundant neutral species found in vivo (Keenan et al., 1977a; Bohnenberger & Sandermann, 1976). Human dolichol and plant undecaprenol were chosen as the primary starting materials. In order to compare cis/trans and chain-length effects, analogues from farnesol, phytol, and solanesol were also synthesized. This report outlines details of the synthesis and purification of these probes and also gives a preliminary account of their spectral properties in various systems.

Experimental Section

General Methods and Materials. Anhydrous solvents were prepared as described elsewhere[1] and dispensed via a repipet with an attached drying tube. In condensation reactions where anhydrous conditions were required, the separate or combined reagents were dried in a small (5–50 mL) round-bottom flask

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[1] The details of these experiments are described fully in McCloskey (1979).

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