region of four $\leq P/L \leq 6$. If these preliminary results are supported by further work, it should be clear that the exchange of phospholipids on and off the protein surface is fast relative to $1-10 \times 10^{-6}$ s. This exchange would thus appear likely to be the new motion in the presence of protein occuring at a rate of 10^6-10^7 s^{-1} or greater that is detected in the ^2H NMR relaxation measurements.

In summary, our results to date show that: (*a*) in both the EPR and ^2H NMR time regime, the effect of protein on the orientational order of the system fits a two-state model; (*b*) ^2H NMR relaxation measurements show a new motion in the presence of protein with a correlation time of $10^{-6}-10^{-7}$ s or shorter; and (*c*) preliminary nitroxide electron T_1 results indicate this new motion may be the chemical exchange of phospholipids on and off the protein surface.

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ACTIVITY OF PHOSPHOLIPID EXCHANGE PROTEINS TOWARD GEL AND LIQUID-CRYSTALLINE PHOSPHOLIPID VESICLES

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Phospholipid exchange proteins catalyze the transfer of phospholipid molecules between a variety of biological and artificial membranes (1). A bovine brain protein transfers phosphatidylinositol (PtdIns) or phosphatidylcholine (PtdCho); another, isolated from bovine liver, transfers exclusively PtdCho. Thus, a high degree of selectivity exists in the interaction between phospholipid and phospholipid exchange protein. Of considerable importance also is the interaction between membrane and phospholipid exchange protein. The nature of this interaction is the focus of this investigation.

RESULTS AND DISCUSSION

Single bilayer vesicles are prepared from PtdChos of known fatty acid composition and are tested as acceptors of radiolabeled phospholipid in the presence and absence of phospholipid exchange protein. Donor membranes include rat liver microsomes and single bilayer vesicles; activity is expressed as the rate of protein-catalyzed transfer of PtdIns or PtdCho from donor to acceptor (2, 3). The fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) in PtdCho vesicles provides information about the phase behavior and hydrocarbon fluidity of the membranes (4).

When bovine brain phospholipid exchange protein is incubated at 37°C with rat liver microsomes and vesicles of egg, dioleoyl-, dielaidoyl-, or dimyristoyl-PtdCho, initial rates of PtdIns transfer are highest with the two *cis*-unsaturated species (15.4 nmol h^{-1} for egg PtdCho, 15.2 nmol h^{-1} for dioleoyl-PtdCho), intermediate with the *trans*-unsaturated species (9.6 nmol h^{-1}), and lowest with the saturated species (1.6 nmol h^{-1}). With vesicles prepared from mixtures of egg and dimyristoyl-PtdCho, transfer activity decreases as the proportion of saturated lipid increases. The brain protein-catalyzed transfer of PtdIns from microsomes to the various unsaturated PtdCho vesicles is investigated over the temperature range 15–45°C. The results are expressed as Arrhenius plots (Fig. 1). The curves are linear and without discontinuity; the apparent activation energies of PtdIns transfer to the *cis*-unsaturated vesicles are 34 kJ mol^{-1}, while that to the *trans*-unsaturated vesicles is 60 kJ mol^{-1}. These data demonstrate that the degree and geometry of unsaturation of membrane phospholipid fatty acyl residues have a profound influence on the catalytic activity of bovine brain phospholipid exchange protein. The observed differences among the membrane preparations cannot be attributed to variations in vesicle size or molecular surface area. Although the assay temperature of 37°C is well above the gel to liquid-crystalline phase transition temperatures of all the PtdChos used, significant differences in the fluorescence of DPH are noted for the several liquid-crystalline states. The inverse correlation between polarization and the rate of PtdIns transfer suggests that membrane fluidity is another important determinant of phospholipid exchange protein activity.

The interaction of bovine liver phospholipid exchange proteins with different phospholipid membranes is investigated by measuring the rates of PtdCho transfer from egg PtdCho donor vesicles to egg or dimyristoyl-PtdCho acceptor vesicles. For comparable amounts of protein at

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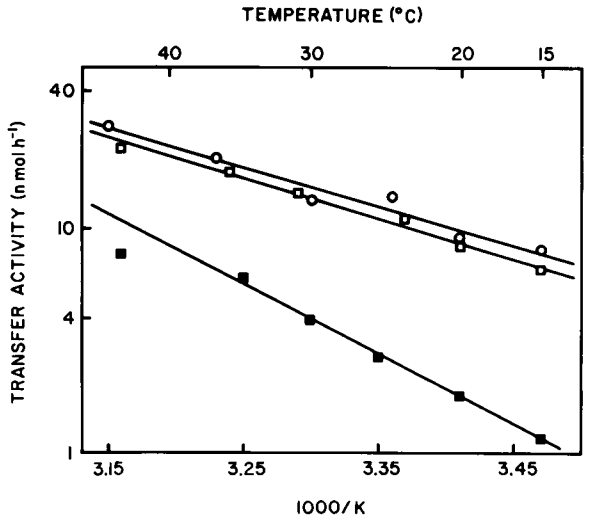

FIGURE 1 Temperature dependence of bovine brain phospholipid exchange protein-catalyzed transfer of PtdIns from microsomes to vesicles. The assay system contained, in a total volume of 2.5 ml of 10 mM Hepes, 50 mM NaCl, 1 mM Na_2EDTA (pH 7.4), 1.25 mg rat liver microsomes, 1 μmol PtdCho vesicles, and 2.8 μg exchange protein. Vesicles were prepared from egg PtdCho (□), dioleoyl-PtdCho (○), or dielaidoyl-PtdCho (■).

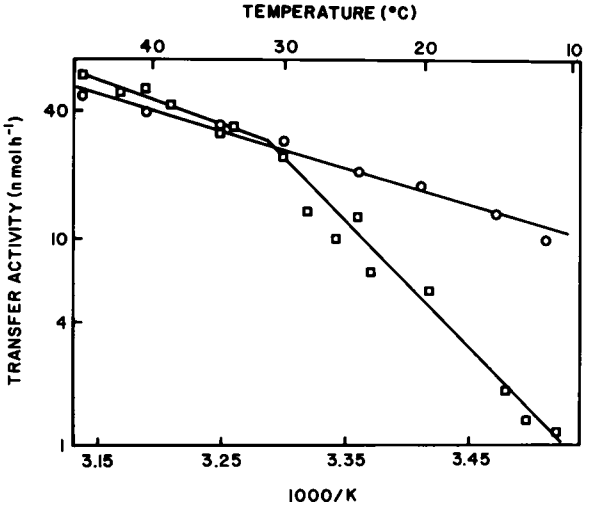

FIGURE 2 Temperature dependence of bovine liver phospholipid exchange protein-catalyzed transfer of PtdCho from vesicles to vesicles. The assay system contained, in a total volume of 0.5 ml of 10 mM Hepes, 50 mM NaCl, 1 mM Na_2EDTA (pH 7.4), 200 nmol egg PtdCho donor vesicles with 10 mol% lactosylceramide, 1 mg bovine plasma albumin, and the following: 200 nmol egg PtdCho acceptor vesicles and 0.12 μg exchange protein (○) or 200 nmol dimyristoyl-PtdCho acceptor vesicles and 0.30 μg exchange protein (□).

37°C, the unsaturated species supports a higher transfer activity (36.4 nmol h^{-1}) than the saturated species (14.3 nmol h^{-1}). The liver protein, in contrast to the brain protein, appears to be more tolerant of changes in phospholipid fatty acid composition. This is also indicated by apparent activation energies of 34 kJ mol^{-1} for both vesicles over the temperature range 31–45°C (Fig. 2). The dimyristoyl-PtdCho acceptor vesicles display a discontinuity in the Arrhenius plot at 31°C, below which temperature an apparent activation energy of 115 kJ mol^{-1} is calculated. This discontinuity corresponds with the onset of the liquid-crystalline to gel phase transition, as monitored by the fluorescence polarization of DPH. For dimyristoyl-PtdCho single bilayer vesicles, this thermotropic phase transition occurs between 12°C and 30°C. Thus, gel-state phospholipid bilayers function in protein-catalyzed PtdCho transfer systems, but with markedly altered kinetic and thermodynamic properties. The incorporation of cholesterol into dimyristoyl-PtdCho vesicles, at a concentration sufficient to abolish the gel to liquid-crystalline phase transition, yields a monophasic Arrhenius plot in the temperature range 20–41°C and an apparent activation energy of 81 kJ mol^{-1}.

These results provide further insight into the mechanism of action of phospholipid exchange proteins, specifically with respect to the interaction of this class of proteins with lipid bilayers. Preference is exhibited toward liquid-crystalline membranes and, within that category, toward membranes of increased hydrocarbon fluidity.

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