PNP:basal ratios are found in the lipidated form of the enzyme.

To obtain a lipid-replete adenylate cyclase, the solubilization of rat liver plasma membrane adenylate cyclase by octyl-β-D-glucopyranoside, a nonionic detergent, was investigated. We have found that octyl-glucoside solubilizes an active and fully ligand-stimulable (F^- GMP-PNP) adenylate cyclase with extensive retention of annular phospholipids which can be separated as lipid-protein-detergent complexes. Membranes were solubilized with 30 mM octyl-glucoside at a detergent:protein ratio of 3.25. Further increase of the detergent:protein ratio yields progressively delipidated proteins and a rapid loss of activity, suggesting that, unlike Lubrol-PX, octyl-glucoside does not mimic the phospholipid milieu in supporting the enzyme activity. Solubilization of the liver plasma membranes with 30 mM octyl-glucoside releases only 22% of the phospholipids as free micellar lipid. The remaining phospholipid released from the membrane by octyl-glucoside is strongly associated with soluble membrane proteins to the extent of 30 nmol phospholipid per 100 μg of soluble protein. In contrast to minimally lipidated and delipidated forms of the enzyme solubilized with Lubrol-PX, the enzyme solubilized with octyl-glucoside is more stable and the loss of sensitivity to ligands and basal activity declines in parallel during prolonged incubation at 4°C.

The data obtained with octyl-glucoside suggest the preservation of the boundary lipids that are in residence with integral membrane proteins at the time of solubilization. Our results show that octyl-glucoside is preferred to Lubrol-PX, since the former detergent solubilizes a more stable enzyme complex and has the advantage of retaining the lipid-protein nature of adenylate cyclase in a soluble form. This synthetic detergent thus provides a soluble and fully active enzyme which is more nearly native in character than previously possible with other detergent solubilizations, and will allow further studies of the proposed lipid dependence of the hormonally stimulated adenylate cyclase.

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AN INTEGRATED VIEW OF THE DYNAMICS OF LIPID-PROTEIN INTERACTIONS AS DERIVED FROM SEVERAL SPECTROSCOPIC TECHNIQUES

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Over the last several years, spectroscopic studies of the effect of integral membrane proteins on the reorientational motions of membrane phospholipids have yielded two classes of results: those that indicate that protein increases the average orientational order of the system (EPR, static and time-resolved fluorescence depolarization, and Raman) and those that indicate that protein decreases or has no effect on this order (predominantly ^2H NMR). Superficially, these two classes of results appear to contradict each other. However, if one recognizes that these techniques are sensitive to motions in different, distinct, time regimes, it is possible to construct models that account for these apparently contradictory results and provide a common, unified picture of the effect of protein on the reorientational motions of phospholipids. One such model has been schematized in Fig. 1. Here we indicate how the orientational order parameter of a phospholipid might be reduced from its no-motion limit of 1.0 to the value it has after ~ 1 ms by a series of discrete motions, each with a characteristic rate and amplitude. Fig. 1 shows the two hypothetical cases of phospholipid in the presence and absence of an integral membrane protein and indicates the

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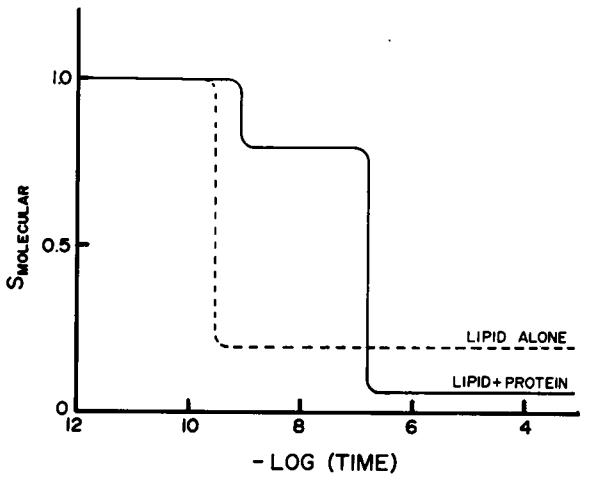

FIGURE 1 Hypothetical changes in the orientational order parameter ($S_{molecular}$) of membrane phospholipids as a function of time which are consistent with known experimental evidence. Two cases are shown: phospholipids in the absence of an integral membrane protein (dashed line) and phospholipids presumed to be associated with the protein surface in the presence of an integral membrane protein (solid line). Reductions in $S_{molecular}$ occuring at a rate of $\sim 10^9\ s^{-1}$ are indicated by time-resolved fluorescence depolarization (see Wolber and Hudson, 1982, this volume). Increased values of $S_{molecular}$ in the presence of protein relative to the lipid alone in the region of 10^{-9} to 10^{-8} s are derived from EPR and time-resolved fluorescence depolarization. Motion reducing $S_{molecular}$ at a rate of 10^6–$10^7\ s^{-1}$ in the presence of protein is inferred from ^2H NMR relaxation times and preliminary nitroxide electron T_1 results (see text). Decreased values of $S_{molecular}$ in the presence of protein relative to the lipid alone at times 10^{-5} s come from ^2H NMR.

qualitative differences in motional properties deduced on the different time scales of EPR, time-resolved fluorescence depolarization, and ^2H NMR. Also shown is a motion corresponding to the new phospholipid motion introduced by protein that is presumed to occur at a rate between the EPR and ^2H NMR time regimes.

We have used a variety of spectroscopic techniques on a single, well-defined system to collect orientational order parameters over as much of the time domain of Fig. 1 as possible. Further, we have attempted to place limits on the correlation times of motions presumed to occur between these order parameters at the different spectroscopic time regimes.

In collaboration with James Davis and Myer Bloom of the University of British Columbia, we have recently shown that the effect of protein concentration on the average orientational order of the system does indeed have the same form for EPR and ^2H NMR if one assumes these techniques are in the limit of slow and rapid exchange, respectively, of lipid on and off the protein surface (Paddy et al., 1981). In both techniques, the change in orientational order produced by the protein fits a two-state model in which phospholipid occupies sites either on or off the protein surface. Further, both EPR and ^2H NMR yield similar values for the amount of phospholipid necessary to cover the membranous surface of cytochrome *c* oxidase. Hence, there appears to be excellent agreement in the concentration dependence of the effect of protein on phospholipid orientational order at these two locations along the time axis of Fig. 1.

Using the measured values of average orientational order in the EPR and ^2H NMR time domains, we interpret the ^2H NMR relaxation times to indicate a new motion in the presence of protein with a correlation time of at least 10^6–$10^7\ s^{-1}$ (Paddy et al., 1981). This new motion may represent the chemical exchange of phospholipids on and off the protein surface. Alternatively, it may reflect a motion of the protein surface itself. We have represented this new motion in Fig. 1 as the line connecting the values of average orientational order at 10^{-8} s and 10^{-4} s.

It is important to establish the physical nature of this new motion. Though NMR methods can, in principle, be used to estimate the amplitudes and rates of all the motions along the time axis of Fig. 1, these methods do not necessarily identify the physical nature of these motions. One promising technique for determining the rate at which spin-labeled phospholipid analogues exchange on and off the protein surface is the use of nitroxide electron spin-lattice (T_1) relaxation times. Conventional EPR spectra of the samples used for these measurements would be sensitive to motions occurring at rates faster than $\sim 10^8\ s^{-1}$ (see Fig. 1), and thus should show the usual free and protein-associated components in their spectra. However, nitroxide electron T_1 values are $\sim$ 1–10 $\times$ 10^{-6} s in membrane systems, considerably longer than the time scale of conventional EPR. If exchange between free and protein-associated components were rapid relative to 1–10 $\times$ 10^{-6} s, as is suggested by the ^2H NMR relaxation data, one might expect T_1 for the lipid-protein system to be a single exponential whose value is the weighted average value of T_1 in the two environments. If exchange is slow relative to 1–10 $\times$ 10^{-6} s, one might expect a double exponential T_1 comprised of weighted amounts of the two rate constants for the free and protein-associated environments alone.

We have recently completed a preliminary series of such nitroxide electron T_1 measurements on a reconstituted cytochrome *c* oxidase system in collaboration with Akihiro Kusumi and James Hyde at the National Biomedical ESR Facility in Milwaukee. Values of T_1 were determined on their home-built instrument using a saturation recovery method (Hyde, 1979). Preliminary results in the fluid phase indicate a three- to fourfold difference in T_1 in going from phospholipid dispersions to a reconstituted system containing about half the phospholipid necessary to cover the membranous surface of cytochrome *c* oxidase. Samples at intermediate lipid:protein ratio appear to show single exponential decays with values of T_1 proportional to their protein:lipid ratio. Preliminary plots of $1/T_1$ vs. P/L appear to have the same general shape of the M_1 vs. P/L plot of Fig. 7 in Paddy et al. (1981), showing linear behavior at low values of P/L and a break in the general

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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