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A SOLUBILIZED AND ACTIVE ADENYLATE CYCLASE LIPIDATED WITH NATIVE ANNULAR LIPIDS

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The critical role of phospholipids in the modulation of adenylate cyclase in plasma membranes has often been speculated upon, but unequivocal experimentation has been lacking. The modulating role of the membrane phospholipid milieu upon the enzyme has been demonstrated in intact membranes and cells; however, a requirement for specific phospholipids has been difficult to investigate due to the ease of disruption of lipid-protein associations in detergent solution and the lability of the soluble enzyme during purification (1).

In this communication we present a solubilization of rat liver plasma membranes which liberates a population of membrane proteins surrounded by an annulus of the phospholipids that were in residence at the lipid-protein interface at the moment of solubilization. Unlike K^+-cholate-solubilized adenylate cyclase, which retains an annular phospholipid milieu yet is highly inactive and shows no ligand sensitivity (2), our solubilization procedure yields an enzymatically active and ligand-sensitive adenylate cyclase.

METHODS

Purified rat liver plasma membranes were isolated as described earlier (3). Adenylate cyclase was assayed as described previously (4). Membrane lipid-protein-detergent complexes were isolated by sucrose density gradient centrifugation at 360,000 *g* (5). Phospholipid-phosphorous was determined as a malachite green complex with phosphomolybdate (6) after predigestion of sucrose with nitric acid.

RESULTS AND DISCUSSION

The solubilization of rat liver plasma membrane adenylate cyclase by detergents was examined to determine the extent of lipid association with the liberated soluble membrane proteins. The protein-detergent and protein-lipid-detergent interactions of the soluble membrane components were evaluated. Lubrol-PX, a non-ionic detergent (an ethylene oxide-fatty alcohol adduct [C_{12}–E_9]), is widely used to solubilize membrane-bound adenylate cyclase from rat liver plasma membranes. Purified plasma membranes were solubilized with 100 mM, 50 mM, 10 mM and 1 mM Lubrol-PX at detergent:protein ratios of 12.5, 6.25, 1.25 and 0.125, respectively. We have found that all concentrations of Lubrol-PX used to solubilize adenylate cyclase releases > 89% of the phospholipid present in the soluble membrane fraction as free micellar lipid. Under these conditions, a delipidated enzyme was found in all cases except with 10 mM Lubrol-PX, which yielded a soluble enzyme partially (minimally) associated with lipid. The presence of this lipid-enzyme complex was demonstrated by extended centrifugation at high gravitational fields of the detergent-soluble membrane preparation across a sucrose boundary. The soluble membrane protein was found to bind 4 nmol of phospholipid per 100 μg of soluble protein. These boundary lipids may be associated with high affinity lipid binding sites of the membrane proteins, since they do not represent a complete annular shell of boundary lipids and are the most resistant to detergent substitution.

Since the isolated protein-detergent complex which lacks phospholipids is still active, we suggest that Lubrol-PX provides a supportive milieu for the enzyme, mimicking native phospholipids. The enzyme that is minimally associated with lipid is more stable than the delipidated form and possesses higher enzyme activity and sensitivity to fluoride and guanylyl 5′ imidodiphosphate (GMP-PNP). Maximal fluoride:basal ratios and GMP-

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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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