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RHODOPSIN-PHOSPHOLIPID RECONSTITUTION FROM OCTYL GLUCOSIDE-SOLUBILIZED SAMPLES

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Rhodopsin and phospholipid, solubilized in the detergent octyl glucoside (OG), were reconstituted by removal of the detergent by dialysis. Sucrose density gradient centrifugation of samples reconstituted with disk lipids or egg phosphatidylcholine (PC) resulted in the separation of a phospholipid-rich (high phospholipid to protein ratio) and a protein-rich (low phospholipid to protein ratio) vesicle fraction, both of which deviated markedly from the phospholipid to protein ratio of the starting material. The disk lipid system, which has an initial phospholipid to protein ratio of ~70:1, had a final phospholipid to protein ratio of ~30:1 in the protein-rich fraction, while that of the PC system, in which the initial ratio ranged from 100:1 to 300:1, was in the range of 30:1 to 50:1. The ratio of the protein-rich fraction of the reconstituted PC system could be increased to as high as 100:1 by increasing the rhodopsin concentration of the starting material, but was not significantly affected by the addition of 3 mM $MgCl_2$, the rate of dialysis, or the salt concentration of the buffer.

To determine the origin of the heterogeneity of the reconstituted vesicles, aliquots of an OG-solubilized sample of rhodopsin and phospholipid were dialyzed to various fixed detergent concentrations encompassing the region of vesicle formation. The concentration of OG was determined; subsequently, the samples were centrifuged, and the supernatants were analyzed for protein and phospholipid content. Data for the rhodopsin-disk lipid system indicate that as the detergent is initially removed during dialysis, lipid-rich vesicles form and pellet; as the level of detergent is decreased further, protein-rich vesicles are formed (Fig. 1). By contrast, when the PC system was analyzed, rhodopsin and phospholipid showed identical pelleting behavior, implying a single vesicle population. However, the inhomogeneity of this system could be demonstrated by density gradients, which still yielded two vesicle populations. The difference in pelleting behavior of the two systems can be attributed to the difference in density of the highly unsaturated disk phospholipids and more saturated PC.

Solubilization of disks with OG results in 25–30 mol of phospholipid being associated with the initially solubilized rhodopsin; this is in good agreement with the number of phospholipid molecules calculated to form a boundary layer around rhodopsin (Stubbs and Litman, 1978). It appears that the most favorable packing of the mixed micelles in this system is represented by rhodopsin, surrounded by a boundary layer of phospholipid, and OG. Our centrifugation experiments indicate that disruption of phospholipid-rich micelles precedes disruption of protein-rich micelles. Thus, the phospholipid to protein ratio that exists in the protein-rich mixed micelles seems to determine the phospholipid to protein ratio observed in the reconstituted vesicle population. Helenius et al. (1977)

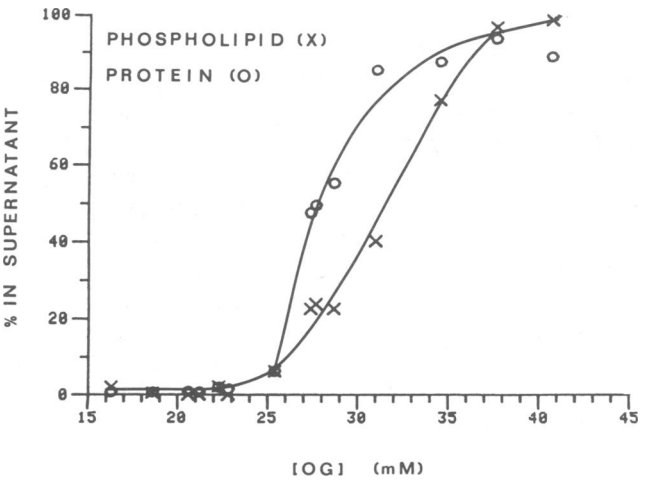

FIGURE 1 The percent phospholipid (×) and protein (O) in the supernatant as a function of the final, dialyzed OG concentration. Dialysis was carried out in 10 mM Tris, 10 mM sodium acetate, 50 mM potassium chloride (pH 7.0). The initial protein concentration was 1 mg/ml.

have also reported the formation of two major classes of vesicles during dialysis reconstitution of OG-solubilized spike glycoprotein of Semliki forest virus with egg PC. The phenomenon of an essentially limiting phospholipid to protein ratio for the protein-rich vesicle population is likely to be a general one, where the actual ratio obtained depends on the nature of the protein and detergent employed. When sodium cholate is used for rhodopsin-phospholipid reconstitution, variable phospholipid-to-protein ratios up to at least 250:1 can be achieved.[1] In some systems, a limiting ratio may not be detected. This may be due to either the ability of the protein-containing micelles to incorporate larger amounts of phospholipid or the mixing of protein-rich and lipid-rich micelles during vesicle formation. These experiments clearly indicate that there will not necessarily be a one-to-one correlation between the phospholipid to protein ratio initially solubilized and that obtained in vesicles produced by dialysis reconstitution.

[1]Litman, B. J. Unpublished results.

This work was supported by Public Health Service grant EY00548; National Science Foundation grant PCM-8012028 to Dr. Litman; Public Health Service training grant GM-07294 and a Pratt graduate fellowship to Dr. Jackson.

Received for publication 29 April 1981.

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SPIN-LABEL STUDIES OF RHODOPSIN-LIPID INTERACTIONS

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Rhodopsin-lipid interactions have been studied in bovine rod outer segment (ROS) disk membranes by using a variety of freely diffusing spin-labels. Spin-labeled fatty acid, sterol and phospholipid molecules all display an apparent two-component ESR spectrum at temperatures below ~30°C. The narrow component constitutes 60–65% of the total spectral intensity and is very similar to that recorded for the same label in non-protein-containing lipid bilayers. The other, broader component is characteristic of a markedly more motionally restricted environment and is attributed to lipids interacting directly with the major protein rhodopsin (90% of the total) of ROS disk membranes (1).

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RESULTS AND DISCUSSION

A detailed analysis of the 14-doxyl stearic acid spin-label spectra shows that the more immobilized component has a considerable temperature dependence with an outer splitting of 59G at 3°C and 54G at 24°C (see Fig. 1). This behavior shows that the mobility of the first shell or boundary lipids next to the protein is markedly less than that of the bulk lipids. Furthermore, the first shell lipids are not rigidly immobilized nor are they strongly bound to the protein, but they exchange readily with the remaining lipid phase (see Fig. 4 of reference 2). Effective rotational rates for these motionally restricted lipid chains are probably closer in correlation time (see Fig. 1) to the fluid lipids (τ_R~1 ns) than the protein backbone (τ_R~20 μs). This is in sharp contrast to the highly immobilized component which arises as a result of protein aggregation and shows little or no temperature dependence in the outer splitting (2–5).

BIOPHYS. J. © Biophysical Society · 0006-3495/82/01/94/02 $1.00
Volume 37 January 1982 94–95