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[1]Litman, B. J. Unpublished results.

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REFERENCES

Helenius, A., E. Fries, and J. Kartenbeck. 1977. Reconstitution of Semliki forest virus membrane. *J. Cell Biol.* 75:866–880.

Stubbs, G. W., and B. J. Litman. 1978. Effect of alterations in the amphipathic microenvironment on the conformational stability of bovine opsin. 1. Mechanism of solubilization of disk membranes by the nonionic detergent, octyl glucoside. *Biochemistry.* 17:215–219.

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).

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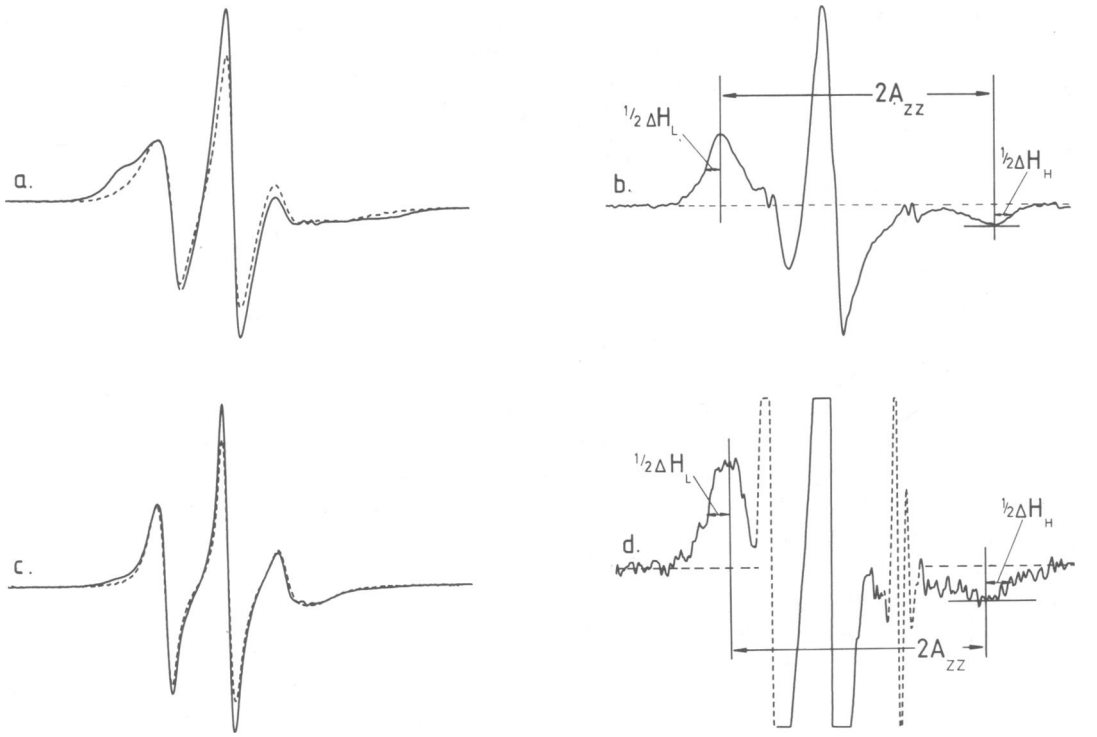

FIGURE 1 ESR spectra of 14-doxyl stearic acid in ROS disk membranes (solid lines), *a* at 3°C and *c*, at 24°C, and in bilayers of extracted lipid (dashed lines of *a* and *c*). Computer subtraction of the spectra in *a* gave *b*, and of those in *c* gave *d*. The outer hyperfine splitting, $2A_{zz}$ is readily measured in each spectrum to be 59G at 3°C in *b* and 54G at 24°C in *d*. Correlation times estimated from the outer linewidths (7), ΔH_L and ΔH, are ~50 ns at 3°C and ~20 ns at 24°C.

A qualitative analysis of the more mobile, fluid-like spectral component of 14-doxyl stearic acid in ROS disk membranes indicates that the protein also decreases the motional rate and/or the motional amplitude of the lipid chains not directly interacting with rhodopsin.

It now appears that there is no controversy about the interpretation of spin-label experiments on lipid-protein interactions in ROS disk membranes (5).

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REFERENCES

1. Watts, A., I. D. Volotovski, and D. Marsh. 1979. Rhodopsin-lipid associations in bovine rod outer segment membranes. Identification of immobilized lipids by spin-labels. *Biochemistry.* 18:5006–5013.
2. Marsh, D., A. Watts, P. F. Knowles, R. D. Pates, R. Uhl, and M. Esmann. 1982. ESR spin-label studies of lipid-protein interactions in membranes. *Biophys. J.*, 37:265–274.
3. Favre, E., A. Baroin, A. Bienvenue, and P. F. Devaux. 1979. Spin-label studies of lipid-protein interactions in retinal rod outer segment membranes. Fluidity of the boundary layer. *Biochemistry.* 18:1156–1162.
4. Davoust, J., B. M. Schoot, and P. F. Devaux. 1979. Physical modification of rhodopsin boundary lipids in lecithin-rhodopsin complexes. A spin-label study. *Proc. Natl. Acad. Sci. U.S.A.* 76:2775–2759.
5. Davoust, J., A. Bienvenue, P. Fellman, and P. F. Devaux. 1980. Boundary lipids and protein mobility in rhodopsin-phosphatidylcholine vesicles. Effects of lipid phase transitions. *Biochim. Biophys. Acta.* 596:28–42.
6. Watts, A., J. Davoust, D. Marsh, and P. F. Devaux. 1981. Distinct states of lipid mobility in bovine rod outer segment membranes. Resolution of spin-label results. *Biochim. Biophys. Acta.* 643:673–676.
7. Freed, J. H. 1976. Theory of slow tumbling ESR spectra for nitroxides. *In* Spin Labeling. Theory and Application. Academic Press, New York. 53–132.