membrane bilayer which involves a cross-linking of the membrane receptors.

These experiments indicate that cross-linking of integral membrane proteins can alter the observed bilayer rigidity. By changing the lipid bilayer structure, cross-linking of specific proteins may be a factor in altering the lateral arrangement of other membrane components.

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REFERENCES

1. Compans, R. W., and P. W. Choppin. 1975. Reproduction of myxoviruses. *In* Comprehensive Virology. H. Fraenkel and R. R. Wagner, editors. Plenum Press, New York. 4:179–252.
2. Choppin, P. W., and R. W. Compans. 1975. Reproduction of paramyxoviruses. *In* Comprehensive Virology. H. Fraenkel and R. R. Wagner, editors. Plenum Press, New York. 4:96–178.
3. Wagner, R. R. 1975. Reproduction of rhabdoviruses. *In* Comprehensive Virology. H. Fraenkel and R. R. Wagner, editors. Plenum Press, New York. 4:1–94.
4. Landsberger, F. R., D. S. Lyles, and P. W. Choppin. 1978. Enveloped viruses: structure and interaction with cell membranes. *In* Negative Strand Viruses and the Host Cell. B. W. J. Mahy and R. D. Barry, editors. Academic Press, Inc., London. 787–800.
5. Landsberger, F. R., and L. D. Altstiel. 1980. Lipid-protein interactions in enveloped viruses. *Annals N. Y. Acad. Sci.* 348:419–425.
6. Helenius, A., E. Fries, and J. Kartenbeck. 1977. Reconstitution of Semliki forest virus membrane. *J. Cell Biol.* 75:866–880.
7. Altstiel, L. D., and F. R. Landsberger. 1981. Structural changes in BHK cell plasma membrane caused by the binding of vesicular stomatitis virus. *J. Virol.* 39:82–86.
8. Landsberger, F. R., and R. W. Compans. 1976. Effect of membrane protein on lipid bilayer structure: a spin-label electron spin resonance study of vesicular stomatitis virus. *Biochemistry.* 15:2356–2360.
9. Kang, C. Y., and L. Prevec. 1971. Proteins of vesicular stomatitis virus. III. Intracellular synthesis and extracellular appearance of virus-specific proteins. *Virology.* 46:678–690.
10. Little, S. P., and A. S. Huang. 1977. Synthesis and distribution of vesicular stomatitis virus-specific polypeptides in the absence of progeny production. *Virology.* 81:37–47.
11. Little, S. P., and A. S. Huang. 1978. Shedding of the glycoprotein from vesicular stomatitis virus-infected cells. *J. Virol.* 27:330–339.
12. Lyles, D. S., and F. R. Landsberger, 1978. Enveloped virus- and lectin-induced structural change in erythrocyte lipid bilayer: dependence on species of erythrocyte and on the miltivalence of the agglutinin. *Virology.* 88:25–32.

PRESSURE EFFECTS ON PROTEIN-LIPID INTERACTIONS

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Biphasic Arrhenius plots of membrane-bound enzymes have been interpreted as phase changes or phase separations in the membrane lipid bilayer (1). However literature reports can also be found where biphasic Arrhenius plots are attributed to conformational changes of the enzyme without interference of lipids (2).

We have made use of the experimental observation that the effect of pressure on protein conformational transition temperatures is much smaller (2–5 K/kbar) than the effect of pressure on the phospholipid phase transitions (20–25 K/kbar), the latter being a true melting phenomenon (3). Our experiments do not exclude the occurrence of protein conformational changes but indicate whether changes in the activity of enzymes are controlled by the physical state of the lipid or whether they are controlled by the protein without the involvement of lipids.

RESULTS AND DISCUSSION

In our experiments, the activity of the enzymes is measured, under steady-state conditions, as a function of pressure. A break is observed at a characteristic pressure. The pressure at which the break occurs is dependent on temperature (dp/dT). The data are represented as pressure effects on breaks in Arrhenius plots (dT/dp).

As a first example, pressure effect on biphasic Arrhenius plots of Azotobacter nitrogenase was studied (4). The temperature at which the break occurs increases with increasing pressure (dT/dp = 20 K/kbar). This is in agreement with the pressure dependence of the transition temperature of several synthetic phospholipids (5). Detergents and phospholipase A remove the break. Reconstitution can be performed only with specific phospholipids. The conclusion is that lipids are involved in the break.

Similar pressure effects were found on the Arrhenius plots of Na-K-ATPase from pig kidney outer medulla when ATP is used as a substrate (dT/dp = 27.7 K/kbar). Interestingly, the *p*-nitrophenylphosphatase activity of the enzyme shows no biphasic Arrhenius plots and likewise no biphasic plots are observed for the activity as a function of pressure (6). This indicates that the activity of the enzyme towards this substrate is not controlled by the lipids.

The sarcoplasmic reticulum Ca-Mg-ATPase is of

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special interest since it is one of the most studied membrane enzymes. The break in the Arrhenius plot has been ascribed to a conformational change of the protein (2). We have measured the activity of the enzyme optically with a coupled enzyme assay up to 1,000 bar (7). At 25°C a break in the activity vs. pressure is observed at 300 bar. At 35°C this break occurs at 700 bar. From experiments at different temperatures we obtain $dT/dp = 27$ K/kbar. This is an indicator of the involvement of lipids in the Arrhenius discontinuities.

In conclusion, pressure effects on the breaks in Arrhenius plots provide evidence, at least for the systems studied here, for a mechanism whereby a change in the physical state of the lipids is the trigger for a consequent change in the protein such as conformational changes and/or association phenomena. Since in many cases the breaks in the Arrhenius plots are not related to changes in the fluidity of the bulk membrane lipid, the simplest interpretation of the observed pressure effect is the existence of a lipid annulus around the enzyme.

A more general conclusion is that temperature together with pressure are important parameters for the study of membrane phenomena. Because thermotropic lipid transitions are more sensitive to pressure than conformational changes in proteins, pressure is a useful tool for discrimination between both phenomena in more complex systems.

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REFERENCES

1. Brasitus, T. A., D. Schachter, and T. G. Mamouneas. 1979. Functional interactions of lipids and proteins in rat intestinal microvillus membranes. *Biochemistry.* 18:4136–4144.
2. Dean, W. L., and C. Tanford. 1978. Properties of a delipidated, detergent-activated CaATPase. *Biochemistry.* 17:1683–1690.
3. Nagle, J. F., and D. A. Wilkinson. 1978. Lecithin bilayers: density measurements and molecular interactions. *Biophys. J.* 23:159–175.
4. Ceuterick, F., J. Peeters, K. Heremans, H. De Smedt, and H. Olbrechts. 1978. Effect of high pressure, detergents and phospholipase on the break in the Arrhenius plot of Azotobacter nitrogenase. *Eur. J. Biochem.* 87:401–407.
5. De Smedt, H., H. Olbrechts, and K. Heremans. 1975. Phase transitions in artificial membranes: high pressure light scattering studies. *Europhys. Conf. Abstr.* 1*A*:98–99.
6. De Smedt, H., R. Borghgraef, F. Ceuterick, and K. Heremans. 1979. Pressure effects on lipid-protein interactions in NaKATPase. *Biochim. Biophys. Acta.* 556:479–489.
7. Heremans, K., and F. Wuytack. 1980. Pressure effect on the Arrhenius discontinuity in CaATPase from Sarcoplasmic Reticulum. Evidence for lipid involvement. *Fed. Eur. Biochem. Soc. Lett.* 117:161–163.