INTERFACIAL FREE ENERGIES AND THE CONTROL OF THE POSITIONING AND AGGREGATION OF MEMBRANE PROTEINS

DONALD F. GERSON
Basel Institute for Immunology, CH-4005 Basel, Switzerland

Membrane proteins are imbedded in the lipid bilayer which is the predominant feature of the cell membrane. Some membrane proteins are completely immersed in the lipid bilayer, while others protrude into either intracellular or extracellular regions (Fig. 1). The degree to which some membrane proteins protrude into the extracellular medium depends on the lipid composition of the bilayer (Borochov and Shinitzky, 1976; Shinitzky and Souroujon, 1979). These variations in the vertical placement of membrane proteins have been correlated with differences in the microviscosity of the lipid bilayer resulting from altered lipid composition. It is difficult to develop a physical model which relates the static vertical placement of membrane proteins to bilayer microviscosity. It is possible, however, to develop a physical model relating membrane protein placement at the external surface of the bilayer to the interfacial free energies between the bilayer, the protein, and the extracellular medium. The following is a brief presentation of such a model, and a description of its applicability to the vertical placement and aggregation of membrane proteins.

RESULTS AND DISCUSSION

Interfaces between immiscible phases have an associated interfacial free energy (γ, Joules/m^2). A simple model of the membrane-protein system consists of three isotropic, homogeneous phases: the membrane, the membrane protein, and the extracellular medium. There are three interfacial free energies, each between a pair of phases: the membrane (m) and the extracellular medium (ex), γ_{mex}; the membrane protein (p) and the extracellular medium, γ_{pex}; and the membrane and the membrane protein, γ_{pm}. If we further model the membrane as a planar, hydrophobic bulk phase which is thick relative to the membrane protein, and the membrane protein as a sphere of radius r, then it is possible to obtain a simple expression for the relative placement of the membrane protein at the boundary between the external medium and the membrane. This model and the nomenclature used are summarized in the lower frame of Fig. 1.

Qualitatively, the position of the membrane protein at the interface between the membrane and the exterior medium depends on its relative hydrophobicity: a hydrophilic protein will protrude into the external aqueous medium more than a hydrophobic one. Quantitatively, an equilibrium position is reached when the interfacial free energies balance. The distance of protrusion of the protein (h), relative to its radius, is given by H in Eq. 1.

$$H = \frac{h}{r} = \frac{\gamma_{pex} - \gamma_{pm}}{\gamma_{mex}}. \quad (1)$$

Experimental techniques exist for the determination of both γ_{pex} and γ_{mex} (Gerson, 1980, 1981*a*), but γ_{pm} must be calculated from these with a semiempirical equation-

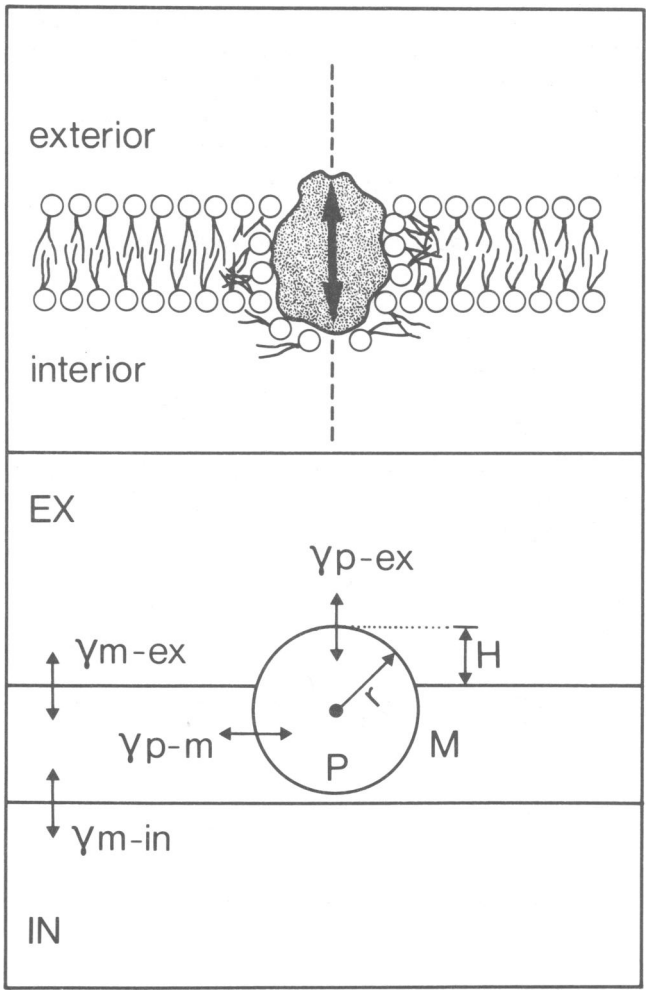

FIGURE 1 Simplified model for the positioning of membrane proteins at the membrane surface. The locations of the interfacial free energies, γ, are indicated by double-headed arrows. Symbols are explained in the text.

of-state for interfacial free energies (Gerson, 1981*a*, *b*), given by Eq. 2.

$$[\gamma_{pm} + \gamma_{mex} - \gamma_{pex}]\,[0.5(\gamma_{pm}\gamma_{mex})^{-0.5}] = \exp[\gamma_{pex}(0.00007\gamma_{mex} - 0.01)] \quad (2)$$

Figs. 2 and 3 give H as a function of γ_{mex} for various values of γ_{pex}. For a constant membrane hydrophobicity (γ_{mex}), decreasing the hydrophobicity of the protein (γ_{pex}) results in larger values of H, in agreement with qualitative expectation. Alternatively, for a constant γ_{pex} ($<\gamma_{mex}$), H increases as γ_{mex} increases. This agrees with the results of Shinitzky and Souroujon (1979) showing that the D blood group antigens of Rh^+ human erythrocytes protrude more from cell membranes high in cholesterol than from membranes high in phosphilipid. Bilayers rich in cholesterol are more hydrophobic than bilayers rich in phospholipid. The dependence of lymphocyte cytotoxicity on membrane cholesterol content suggests the possibility of a membrane-bound receptor protein essential for cytotoxicity which behaves similarly (Dabrowski et al., 1980). Fig. 3 diagrams the relations between H, γ_{mex} and γ_{pex} for situations close to the critical emergence of the membrane protein from the membrane into the external medium.

The interfacial free energies and the surface areas of the protein exposed to each phase allow calculation of equilibrium constants for the aggregation of membrane proteins in the plane of the membrane. For constant γ_{mex}, there is

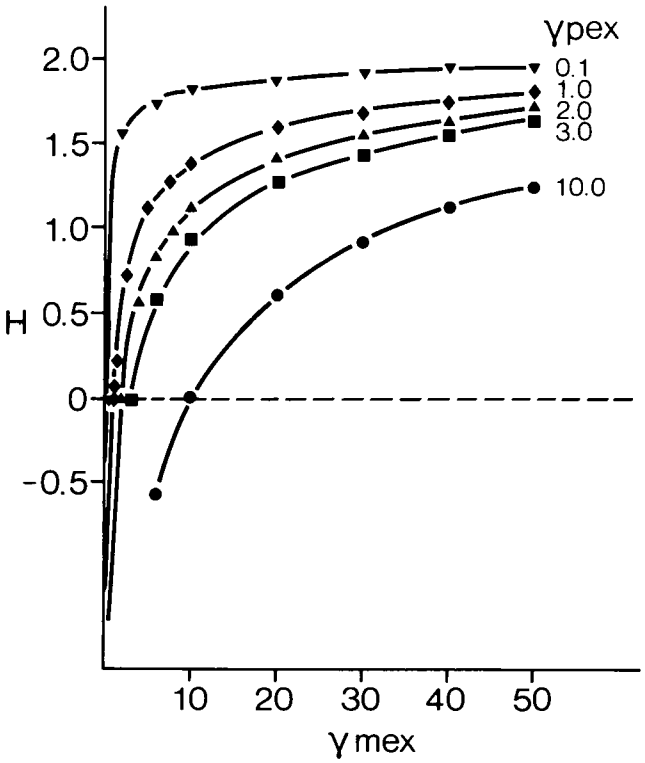

FIGURE 2 H as a function of γ_{mex} for various values of γ_{pex}.

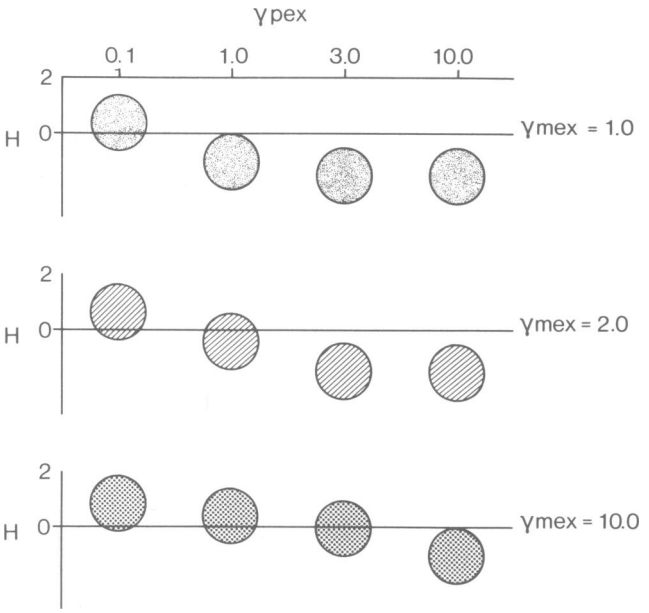

FIGURE 3 Diagrammatic representation of H for proteins of various γ_{pex} in membranes of 3 different γ_{mex}.

an optimal γ_{pex} for aggregation in the plane of the membrane which occurs when half of the membrane protein is exposed to the external medium ($H = 1$). It is thus possible to imagine membrane protein receptors which, upon combination with the appropriate ligand, approach the optimal γ_{pex} for aggregation. Hydrophilic membrane proteins would then tend to submerge into the membrane, while hydrophobic proteins would then tend to emerge from the membrane. Aggregation plus either emergence or submergence is commonly observed in receptor-hormone interactions at the cell surface (Schlessinger, 1979).

In summary, a model has been presented for the positioning of membrane proteins at the membrane surface in terms of interfacial free energies. The model could be extended to less ideal but more realistic geometries and for heterogeneous membrane proteins, using analyses similar to those of Neumann et al. (1979).

The Basel Institute for Immunology was founded and is supported by F. Hoffman-La Roche and Co., Ltd., CH-4002 Basel, Switzerland.

Received for publication 28 May 1981.

REFERENCES

1. Borochov, H., and M. Shinitzky. 1976. Vertical displacement of membrane proteins mediated by changes in microviscosity. *Proc. Natl. Acad. Sci. U.S.A.* 73:4526–4530.
2. Dabrowski, M. P., W. E. Peel, and A. Thompson. 1980. Plasma membrane cholesterol regulates human lymphocyte cytotoxic function. *Eur. J. Immunol.* 10:821–827.
3. Gerson, D. F. 1980. Cell surface energy, contact angles and phase partition. *Biochim. Biophys. Acta.* 602:269–280.
4. Gerson, D. F. 1981*a*. Methods in surface physics for Immunology. *In*

Immunological Methods. I. Lefkovits and B. Pernis, editors. Academic Press, New York. Vol. II. 105–139.
5. Gerson, D. F. 1981*b*. An empirical equation-of-state for solid-fluid interfacial free energies. *J. Colloid Interface Sci.* In press.
6. Neumann, A. W., O. Economopoulos, L. Boruvka, and A. U. Rapacchietta. 1979. Free energy analysis of heterogeneous cylindrical particles at fluid interfaces. *J. Colloid Interface Sci. 71:293–300.*
7. Ostro, M., B. Bessinger, J. Summers, and S. Dray. 1980. Liposome modulation of surface immunoglobulins on rabbit spleen cells. *J. Immunol.* 124:2956–2965.
8. Schlessinger, J. 1978. Receptor aggregation as a mechanism for transmembrane signaling. *In* Physical Chemical Aspects of Cell Surface Events in Cellular Regulation. C. Delisi and R. Bluenenthal, editors. Elsevier, New York. 89–115.
9. Shinitzky, M., and M. Souroujon. 1979. Passive modulation of blood-group antigens. *Proc. Natl. Acad. Sci. U.S.A.* 76:4438–4440.

A MODEL FOR TRANSITION STATE DYNAMICS IN BILAYERS

IMPLICATIONS FOR THE ROLE OF LIPIDS IN BIOMEMBRANE TRANSPORT

THOMAS H. HAINES
Department of Chemistry, City College of The City University of New York, New York 10031, U.S.A.

Remarkable agreement has emerged from both static (x-ray crystal) and dynamic (primarily NMR) approaches to the study of polar lipid headgroup conformations in membranes. This agreement on the similarity of the basic outlines of the conformations of lipid headgroups in both the static and dynamic states is analogous to that found for the globular proteins. Present theories of lipid dynamics (1–4) assume that the headgroups are mutually repulsive, whereas hydrocarbon chain motions are frequently viewed as cooperative (4). Below the transition temperature these cooperative motions are tilting motions (5). Above the transition state, rotational isomerization dominates chain dynamics distal to the carbons 9–10 on the akyl chains, whereas tilt continues to be important proximal to carbons 9–10. Each of these motions effectively thins the bilayer. Cooperative motions between adjacent lipid chains wherein the motions also alter the bilayer thickness imply a wave motion perpendicular to the plane of the bilayer.

DISCUSSION

A model of membrane dynamics is proposed in which the transition state is described as thermal compaction waves. The latter are derived from an unusually large component of translational motion in the thermal energy of water (Brownian Motion). Such waves are small in comparison to the proposed protein-generated waves discussed below. The headgroups are suggested to be attractively interacting with several neighbors in the plane of the bilayer, exchanging rapidly. Zwitterionic phospholipids may do this through ionic interactions of a flexible cation (e.g., choline) with neighboring phosphates. X-ray crystallography (6) shows glycolipids each forming multiple hydrogen bonds with three neighboring lipid molecules. Anionic lipids may attractively interact by acid-anion complexes analogous to the maleic acid-anion. Such attractive headgroup interactions are suggested to act as a restoring force when the headgroup sheet is pushed out of plane. This restoring force enhances wave motions (due to hydrocarbon chain cooperativity) that are perpendicular to the plane of the bilayer in each monolayer.

A theory is put forward in which a variety of membrane proteins utilize the wave motions just described to couple their activities to those of other proteins in the same bilayer. Thus a protein that abruptly alters the thickness of the bilayer by an energy input generates a wave in either or both monolayers simultaneously. A second protein (not in contact with the first) then alters its conformation as the wave passes. Sequence analyses of transmembrane proteins suggest that such proteins have discrete domains in the hydrocarbon region and discrete domains in the aqueous environment of the membrane. It is suggested therefore that a protein that alters its conformation as a cooperative wave passes will do so in the hydrocarbon domain.

Parsegian pointed out (7) that a cation passing through the low dielectric of a bilayer must cause a dimpling of the bilayer due to charge imaging in the high dielectric (water). Since the cation transport exceeds the breakdown voltage of the bilayer, a vigorous compaction wave will be initiated. As the wave passes proteins that surround the initial transport protein, these surrounding proteins (i.e., transducer proteins) will thin (undergo a responsive conformational change) in their hydrophobic domains.

Two types of proteins conduct cations across biomembranes, those coupled to an external energy source (pumps) and those that are not (transducer proteins). It is suggested that the pumps utilize external energy (redox, photons, ATP), to push the cation into the low dielectric, generating a compaction wave and coincidentally create a local field (rapidly spread) across the bilayer. The transducer protein is suggested to respond by undergoing a conformational change in its hydrophobic domain due to the compaction wave simultaneously responding to the field generated by the cation transport event of the pump