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A MODEL FOR TRANSITION STATE DYNAMICS IN BILAYERS

IMPLICATIONS FOR THE ROLE OF LIPIDS IN BIOMEMBRANE TRANSPORT

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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

protein. If the transducer protein transports an ion down the field gradient, the system is electro-mechanically coupled.

The theory predicts that the action potential is a thinning (Na transport) followed by a thickening (K transport) of the bilayer; that the proton pumping of bacteriorhodopsin and other transport phenomena occur as standing compaction waves; and that rhodopsin, when it absorps a photon, generates a wave (perpendicular to the plane of the bilayer) spreading to the edge of the disc that contains it.

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RESOLUTION OF HETEROGENEOUS FLUORESCENCE BY PHASE-SENSITIVE FLUORESCENCE SPECTROSCOPY

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Fluorescence spectroscopic methods are widely utilized in studies of proteins, membranes and more complex biological samples. One hindrance to the interpretation of these data is the heterogeneous nature of the fluorescence emission. Frequently, emission occurs from more than a single fluorophore, e.g., the emission from tyrosine and tryptophan residues in proteins (1, 2). We report here the development of a new method, phase-sensitive fluorescence spectroscopy (PSFS), which allows the emission spectra of each fluorophore in a two-component mixture to be recorded directly. This method uses a phase fluorometer, modified by the addition of a lock-in amplifier. Emission spectra are recorded at various detector phase angles. The chosen angles can be out of phase with any given component, thereby suppressing the emission from this component. The lifetime of the suppressed component may be calculated from the detector phase angle. Phase-sensitive fluorescence spectroscopy can thus resolve the spectra or lifetimes of a heterogeneous sample. In this report we describe our studies with mixtures of fluorophores that simulate the heterogeneous emission from proteins.

THEORY

The sample is excited with light whose intensity is modulated sinusoidally

$$l(t) = 1 + m_l \sin \omega t \qquad (1)$$

where ω is the circular modulation frequency and m_l is the degree of modulation. The emission is then

$$F(t) = 1 + m_F \sin (\omega t - \phi) \qquad (2)$$

where m_F is the modulation of the emission and ϕ is the phase shift (3). This shift is related to the fluorescence lifetime (τ) by

$$\tan \phi = \omega\tau. \qquad (3)$$

When quantified using a lock-in amplifier, the modulated emission yields a DC signal which depends upon the phase angle of the detector (ϕ_D)

$$F(\phi_D) = k \cos (\phi_D - \phi). \qquad (4)$$

The constant k contains factors due to concentration, lifetime and other molecular and instrumental parameters.

Suppose the sample contains two fluorophores, A and B, with different lifetimes, τ_A and τ_B, and spectral distributions, $I_A(\lambda)$ and $I_B(\lambda)$. The modulated emission then consists of two sine waves of the same frequency but different phase angles (Eq. 3). Phase-sensitive detection yields

$$F(\phi_D) = k_A I_A(\lambda) \cos (\phi_D - \phi_A) + k_B I_B(\lambda) \cos (\phi_D - \phi_B) \qquad (5)$$

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