

STRESSES IN MEMBRANES OF RATHER DRY PHASES

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ABSTRACT

At low hydration, the separation between the membranes of some organelles is limited by the repulsive forces between them, and the relation between water content and water potential is determined largely by these forces. The large intra-membrane mechanical stresses and strains produced by these forces may be responsible for physiological damage. I present a simple model of the lateral stress in a bilayer of a lamellar lipid-aqueous solution phase in order to give an estimate of the water content at which such stresses cease to be negligible.

Some plant tissues survive dehydration to water contents (weight ratio of water to dry matter) as low as several percent [1,2]. Several important effects are reported at water contents in the region below about 25% by weight (e.g. solute loss during rehydration by seeds [1]; reduction in O₂ uptake by soybean seeds [3].)

It has been suggested that the large mechanical stresses and strains in membranes may be responsible for damage in dry tissues [4]. Further, the loss of semipermeability correlates with the observed lateral segregation of proteins and the formation of lamellar and hexagonal II phases in dehydrated plant protoplasts [5].

Dehydration brings membranes and macromolecules closer together until further approach is opposed by large (MPa–GPa), short-range (nm), repulsive forces among these structures. Further dehydration is opposed by the suction in the remaining small volumes of solution, rather than by substantial increases in the osmotic pressure. In rye protoplasts dehydrated in 5.37 osmolal sorbitol solution, aparticulate lamellae, lamellar phases and hexagonal II phases are observed near the plasma membrane [5]. The conditions which produce these states of dehydration are different for different organelles.

Let t be the volume-weighted membrane thickness and x the volume-weighted water thickness, and assume that the repulsive force acts at this interface. The suction equals the repulsive force per unit area. The suction acts to compress the system in the plane of the interface but is opposed by a compressive stress in the membrane. Let $(-\gamma)$ be the (compressive) force per unit length in the plane of a single membrane. At mechanical equilibrium $-\gamma = Px$.

The inter-membrane repulsion P decreases approximately exponentially with x with a characteristic length λ of typically 0.2 nm. At $x = 1.0$ nm, P is typically 5.0 MPa [6]. Thus :

$$-\gamma = Px = P_0 x \exp(-x/\lambda). \quad (1)$$

Sufficiently large $(-\gamma)$ may have direct effects on proteins in the membrane, or disrupt membrane integrity. It is convenient to compare γ with the area elastic modulus defined by $k_A = A d\gamma / dA$ where A is the area. k_A is 140 mN/m for a phosphatidylcholine bilayer [7].

Dipalmitoyl- and dimyristoyl- phosphatidylcholine bilayers undergo a phase transition induced by a lateral stress of 24 mN/m ($-\gamma/k_A = 0.17$) at temperatures well above those of the unstressed [6]. The more spectacular transition from lamellae to the inverted cylindrical micelles of the hexagonal II phase occurs in some simple lipid-water mixtures at water/lipid ratios of typically 15 to 25% [8].

Thus the range of water contents 25 to 10% represents a range over which the lateral stress in lamellar phases varies from very much less than k_A to comparable with k_A , and includes the range of lateral stress-induced phase transitions of two types. How do such water contents occur?

The osmotic pressure π may be written as $\Gamma n RT / V$ where V is the volume of solution, n is the number of moles of solute dissolved therein, and Γ is the average activity coefficient of those solutes. (We shall neglect for the moment any specific interactions between solutes and the lamellae.) Setting $V = Ax$ where A is the total area of membrane present in a phase, and using the subscript 0 to represent some reference state, this becomes

$$\pi = (\Gamma/\Gamma_0) \cdot \pi_0 \cdot (V_0/V) \quad (2)$$

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For the solution between two lamellae, the pressure $P_S = -P_C \exp(-x/\lambda)$ and so the water potential ψ is

$$\psi = P_S - \pi = -P_C \exp(-x/\lambda) - \frac{(\Gamma/\Gamma_0)\pi_0 V_0}{A_0 x \left[1 - \frac{x P_C \exp(-x/\lambda)}{k_A} \right]} \quad (3)$$

At high hydration (large x) the first term is negligible and the water potential in the membranous phase is $-\pi$. At low hydration (small x) the first term dominates, and further reductions in ψ cause increases in the suction rather than increases in π .

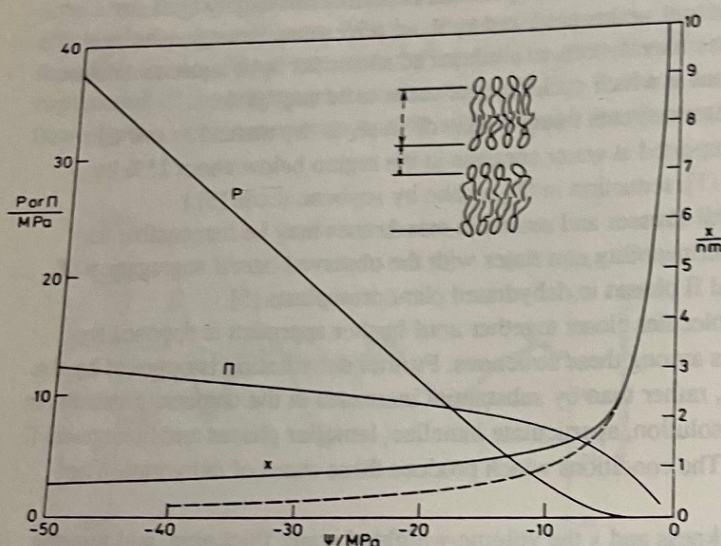


Fig. 1 Against the water potential ψ are plotted the density-weighted average inter-membrane spacing x , the osmotic pressure π of the solution in the lamellar phase and P , the inter-membrane repulsive force per unit area. $-P$ is the (suction) pressure in the aqueous phase. The ratio of solution volume to membrane volume is just x/l where l is the density weighted membrane thickness, or $(x/l_0)[1 - (xP_C/k_A)\exp(-x/\lambda)]$ where l_0 is the membrane thickness in excess solution. Ideal osmotic behaviour is shown by the dashed line.

Eq. 4 is used to plot x against ψ in figure 1. (Γ/Γ_0) has been set at 1, which is in error at low x . The effect of activity coefficients which increase with concentration is to decrease the slope in $x(\psi)$ and to cause lower γ . V_0/A varies markedly among different cells and among different membrane-bound regions of the same cell: several microns for vacuoles; several to tens of nm in cytoplasm and membranous organelles. The osmotic pressure π_0 at the reference state, may vary from several MPa for dry-adapted plants to a few tenths of MPa for animal cells. For $\pi_0 = 1.0$ MPa and $V_0/A = 10$ nm (typical values for the cytoplasm of a plant cell), $(\pi_0 V_0/A_0)$ is 0.01 Pa.m. Figure 1 also plots the inter-membrane repulsion P (equal to minus one times the suction in the aqueous phase) and the osmotic pressure π . the dashed line represents $x(\psi)$ for osmotic behaviour: i.e. the behaviour if $P_S = 0$. Note that below about -15 MPa the water content of the lamellar phase is about 2 to 3 times higher than that of an ideal osmometer, even without including non-ideal solution effects.

Fig. 2 represents $x(\psi)$ and $-\gamma$ from eq. 3 on a logarithmic scale for a range of $(\pi_0 V_0/A)$. For the parameter range represented here, "large" lateral stresses are predicted for ψ less than about -50 MPa.

Many plants grown in dry or cold environments have cells whose osmotic pressure is greater than that of plants grown under standard conditions. The curves of Fig. 2 can be considered as representing the water content and lateral membrane stress in phases with V_0/A_0 constant at 10 nm but with values of π_0 of 10, 2.0, 1.0 and 0.5 MPa. The higher osmotic pressure preserves a lower stress in the membrane.

At any large negative ψ , the membrane stress will be largest in organelles with relatively large dry weights where the average inter-membrane spacing is already small at high ψ . Thus one would anticipate severe stresses e.g. thylakoid and mitochondrial membranes at values of ψ which had little effect on other membranes.

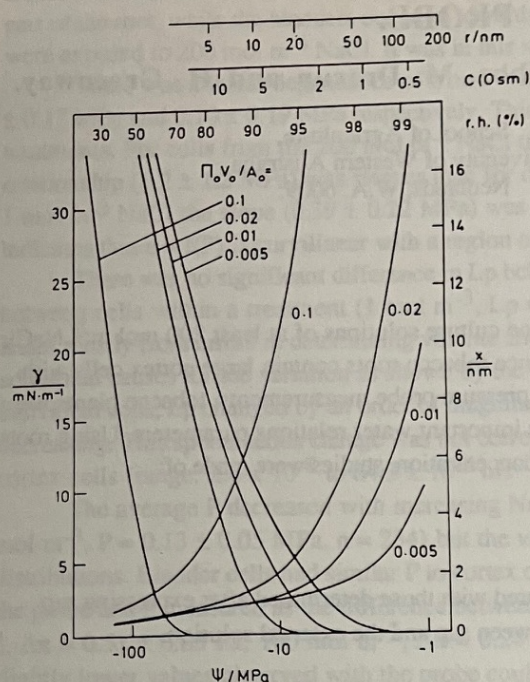


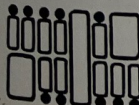
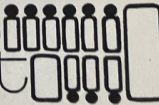
Fig.2 The abscissa gives ψ on a log scale. Above the graph are properties of various phases with this ψ : the relative humidity of an air-water vapour atmosphere; the composition (in osmolal) of an aqueous solution with $P_s = 0$. The radius of a spherical meniscus which would support this pressure difference across an air-water interface is given. The set of curves with positive slope show the average inter-membrane spacing x for different values of the parameter $(\Pi_0 V_0 / A_0)$ given in Pa.m. The set of curves with negative slope show the lateral compressive stress γ exerted on the membranes for the same parameter values.

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