Aust. J. Plant Physiol., 1987, **14**, 311-18

Lateral Stresses in Membranes at Low Water Potential

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Abstract

Severe dehydration of biological cells can produce large stresses and substantial strains in the membranes of some organelles. Water contents and water potentials which are critical for cellular damage in rather dry tissues may reflect a limiting stress characteristic of cell membranes. Different levels of tolerance of extreme dehydration may therefore be a result of different cellular osmotic pressures or of different stress-strain characteristics of the cellular membranes. In this paper, data from phospholipid osmotic stress measurements are used to model the stresses generated in membranes in dehydrated phases.

Although most biological tissues are predominantly composed of water, some plant tissues (such as seeds) and animals (such as soil nematodes) can survive a range of dehydrations to water contents as low as several percent (Crowe and Clegg 1973; Leopold 1986). (In these studies 'water content' means weight ratio of water to dry matter.) Several important effects are reported at water contents in the region below about 25% by weight. For example, when dry seeds or dry nematodes are plunged into water, solutes are lost into the external medium if the initial water content is less than 20%, but the leakage rate is abruptly reduced at higher water contents (Crowe and Crowe 1986). A sudden reduction in the rate of O_2 uptake by soybean seeds below about 24% water content is reported by Vertucci and Leopold (1984).

Mechanical damage to the cell membranes has been correlated with injury at low hydration (reviewed by Gaff 1980). It has been suggested that the mechanical stresses* in biological membranes become large during dehydration at such low water contents (Wolfe *et al.* 1986) and that these stresses may therefore be responsible for membrane deformations which are physiologically deleterious. This paper describes a simple model of the lateral stress in a bilayer of a lamellar phase of lipid and aqueous solution in order to give a rough estimate of the water content at which such stresses cease to be negligible. The limited relevance of such a simple system to biological membranes is acknowledged, but it is noted also that lateral segregation of membrane particles has been reported in dehydrated plant protoplasts and that such lamellar phases (as well as hexagonal$_{II}$ phases) have formed in the particle-free regions (Gordon-Kamm and Steponkus 1984).

Degrees of hydration of biological cells can be divided into two qualitatively different regimes. At high or intermediate hydration, there exist volumes of solution which have dimensions of nanometres or more, and these may have non-negative pressures. In this

* The term 'stress' is often used as a metaphor in biology. In this paper it is used only in its physical meaning of force per unit area (in two dimensions) and force per unit length (in one dimension).

0310-7841/87/030311$02.00

regime, the volume of aqueous solution is determined by osmotic pressure and external mechanical constraints such as those imposed by the cell wall of plant cells. It has previously been argued that dehydration in this range does not produce large stresses in membranes (Wolfe and Steponkus 1983*a*; Wolfe *et al.* 1986).

At low hydration, cellular components (membranes and macromolecules) are concentrated — brought closer together — until further approach is opposed by large, short-range, repulsive forces among these structures. The range of these forces is of order 1 nm. This regime is therefore defined by the absence of water volumes more than a few nanometres thick. Further dehydration in this state is opposed by the suction (negative pressure or bulk tension) in the remaining small volumes of solution with relatively small further changes in the osmotic pressure. The equilibrium conditions necessary to produce this state of dehydration are very different for different organelles. The vacuole(s) in a plant cell would require a very large reduction in volume before the tonoplast was pressed against itself, and such a reduction would be opposed by the increasing osmotic pressure inside the vacuole(s). Membranous organelles such as mitochondria, chloroplasts, the Golgi body and some regions of the cytoplasm, on the other hand, require a much smaller reduction in water volume before membranes are closely opposed. Layers of membranes thus oriented may have similar geometry to that of the liquid crystal phases of lipids in water. For example, in the cytoplasm of rye protoplasts dehydrated by suspension in 5·37 osmolal sorbitol solution, aparticulate lamellae, lamellar phases and hexagonal$_{II}$ phases are observed near the plasma membrane (Gordon-Kamm and Steponkus 1984). A portion of bilayer lamellar phase — which probably resembles the aparticulate lamellae mentioned above — is sketched in the inset in Fig. 1. l is the lipid volume per unit membrane area (the volume-weighted membrane thickness) and x is the water volume per unit membrane area (the volume-

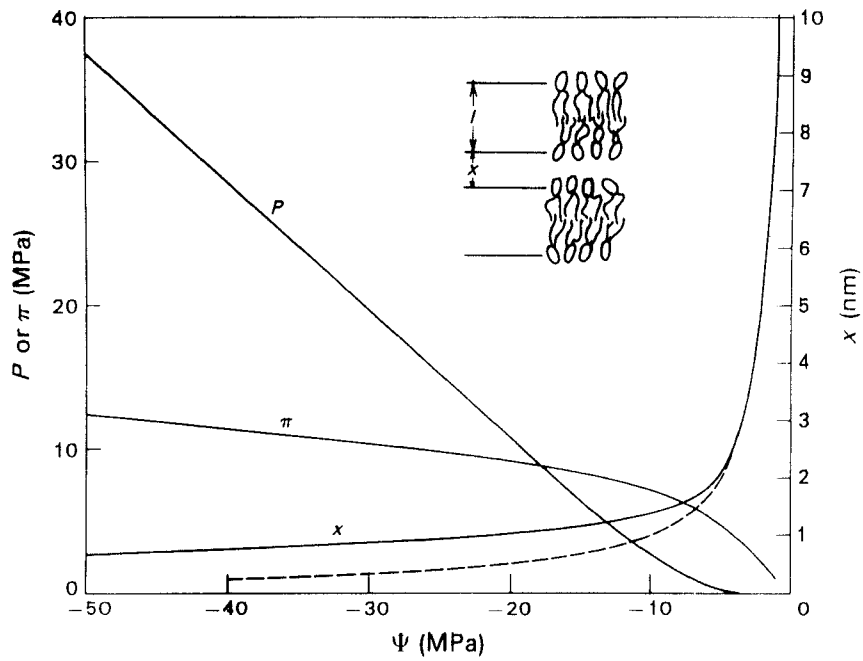

Fig. 1. The inset is a sketch of a lamellar lipid phase showing the density-weighted water and lipid thicknesses x and l. The graphs plot, as a function of water potential Ψ, the density-weighted average intermembrane spacing x, the osmotic pressure π of the solution in the lamellar phase, and P, the intermembrane 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.

weighted water thickness). Molecules which adsorb at the interface have been omitted; these would increase l, and may also affect the membrane stress-strain relation.

The suction acts to reduce the aqueous volume: it acts to draw the membranes closer together but is opposed by a repulsive force per unit area P which the membranes exert on each other. The suction also acts to compress the system in the plane of the membrane. This 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 where γ is the surface tension of both faces of the membrane (its bifacial tension). It will be assumed for simplicity that the intermembrane repulsive force acts at the volume-weighted average surface between membrane and solution. At mechanical equilibrium, the compressive force per unit length in the membrane is balanced by the suction which acts over an area of unit length times x. The suction is equal in magnitude to the intermembrane repulsion P so

$$-\gamma = Px. \tag{1}$$

Lis *et al.* (1982) and Horn (1984) have measured the intermembrane repulsion P as a function of x for a variety of lipid-water systems. For all systems studied, there is a range of x (typically 1·0–2·5 nm) over which P decreases approximately exponentially with x with a characteristic length of typically 0·2–0·3 nm. At $x = 1{\cdot}0$ nm, P is typically 5·0–20 MPa. Writing

$$P = P_c \exp(-x/\lambda)$$

gives

$$-\gamma = P_c\, x \exp(-x/\lambda). \tag{2}$$

Using equations (1) and (2), relations among P, $-\gamma$, x and the volume ratio of water to lipid may be calculated. The strong dependence of P and $(-\gamma)$ on x or concentration is demonstrated by the following table, calculated using the values for dilaurylphosphatidylcholine (DLPC) cited by Lis *et al.* (1982), and $l = 4{\cdot}1$ nm at zero stress.

x (nm)	P (MPa)	$-\gamma$ (mN m^{-1})	soln/lipid
1·6	1·1	1·8	0·39
1·3	3·5	4·6	0·31
1·0	11	11	0·22
0·8	24	19	0·17
0·6	52	31	0·12

First note the variation in the normal stress: a reduction in water/lipid ratio from 0·39 to 0·12 increases P from a value comparable with turgor pressure in a typical plant cell to a value 50-fold larger. Nevertheless, these pressures are much smaller than the bulk moduli of typical fluids (e.g. 2 GPa for water), and an isotropic stress of this magnitude would be expected to have relatively little effect on the geometry of a condensed phase. It is more likely that the non-isotropic effects of the interaction produce substantial deformations, so the lateral stress will be considered next.

Sufficiently large $(-\gamma)$ may have direct effects on proteins in the membrane, or they may cause deformations in the membrane which disrupt its integrity. At what value of x does $(-\gamma)$ cease to be negligible? The appropriate quantity with which to compare lateral stresses is the area elastic modulus which is defined as

$$k_A = A\,\mathrm{d}\gamma/\mathrm{d}A, \tag{3}$$

where A is the area. k_A is 140 mN m^{-1} for a phosphatidylcholine bilayer (Kwok and

Evans 1981). It is rather higher — about 230 mN m^{-1} — for a complete plant membrane including proteins (Wolfe and Steponkus 1983*b*).

Tensile stresses of about $\gamma = 5$ mN m^{-1} cause lysis, i.e. the membrane ruptures after an expansion of a few percent. Membranes are not lysed directly under compressive lateral stress, but they may be deformed. In studies on dilauryl-, dipalmitoyl- and dimyristoylphosphatidylcholine, Lis *et al.* (1982) report a phase transition induced by lateral stress at temperatures well above those of the unstressed phases: e.g. an abrupt change in area per molecule occurs at a (bifacial) lateral stress of 24 mN m^{-1} ($-\gamma/k_A = 0{\cdot}17$) in dilaurylphosphatidylcholine (DLPC) lamellae at room temperature. The value of $(-\gamma)$ at which stress-induced transitions occur in other membranes is likely to vary substantially with composition and temperature, but this value gives an order of magnitude for the stress which causes substantial change in membrane morphology. It will be argued later that lateral stress is a strong function of both water content and chemical potential of water.

A more spectacular deformation is the transition from lamellae to the inverted cylindrical micelles of the hexagonal$_{II}$ phase; this transition is induced in some biological membrane systems at low hydration (e.g. dehydrated protoplasts: Gordon-Kamm and Steponkus 1984). In simple lipid–water mixtures, this transition occurs (if at all) at water/lipid ratios of typically 15–25% (e.g. Luzzati and Husson 1962; Gulik-Krzywicki *et al.* 1967).

There is evidence that some solutes which are accumulated in the cells of plants grown in dry environments change the two-dimensional stress–strain relation of lipid monolayers at the solution–air interface (Crowe and Crowe 1986). Such solutes may also act on membranes to reduce the strain produced by the stresses due to intermembrane repulsion.

Finally, the application of relatively modest lateral stress may cause demixing of phases with different composition. I know of no detailed measurements of phase properties of mixed lipid lamellae as a function of $(-\gamma)$ and T; however, phase diagrams have been calculated for ideal mixtures using monolayer data (Marčelja and Wolfe 1979). Briefly, the importance of lateral stress may be emphasised by noting that in these calculations an increase in $(-\gamma)$ of 1–2 mN m^{-1} has an effect on the phase equilibrium similar to that of a decrease in T of 1°C.

Thus the range of water contents 25–10% represents a range over which the lateral stress in lamellar phases varies from very much less than k_A to comparable with k_A, includes the range of lateral stress-induced phase transitions of two types, and may involve substantial lateral demixing of lipid species. Under which conditions are such water contents achieved?

The thermodynamics of water in plants are usually discussed in terms of the water potential, Ψ, defined as the chemical potential of water divided by the molar volume $\bar{V}$ of water at atmospheric pressure. If changes of phase are not involved, and for pressures much less than the bulk modulus of water, $\bar{V}$ is approximately constant, and the water potential $\Psi = P - \pi$. Here P is the hydrostatic pressure, π is the osmotic pressure and Ψ is taken to be zero in pure water at atmospheric pressure (Slatyer 1967). π may be written as $\Gamma nRT/V$, where V is the volume of solution, n is the number of moles of solute dissolved therein, R is the gas constant and Γ is the average activity coefficient of those solutes ($\Gamma = 1$ for an ideal solution). (I shall neglect any specific interactions between solutes and the lamellae. Very large molecules may be excluded from the intermembrane solution into a highly concentrated solution phase; there will be a positive very-short-range interaction energy between ions and a neutral membrane due to Born energies, and of course electric interactions with charged membranes.)

The volume of aqueous solution and thus the intermembrane spacing are determined by the hydrostatic and osmotic pressures. At high levels of hydration where the

intermembrane forces are negligible, the thickness x of an aqueous volume separating two membranes may vary widely over different regions of the membrane. At very low levels of hydration, however, the hydrostatic pressure in any aqueous solution is determined by the intermembrane repulsion. Uniform pressure in the solution then requires near uniform intermembrane spacing for that particular volume of solution. Setting $V = Ax$, where A is one-half of the total area of membrane bounding an aqueous phase of volume V, and using the subscript 0 to represent some reference state, the osmotic pressure becomes

$$\pi = (\Gamma/\Gamma_0)\ \pi_0\ (V_0/V). \tag{4}$$

(V_0/V) may be written as V_0/Ax and is approximately x_0/x.

Rearranging equations (2) and (3) gives an expression for the area A as

$$A = A_0\ [1 - \frac{xP_c\ \exp(-x/\lambda)}{k_A}]. \tag{5}$$

For the solution between two lamellae the pressure P_s is $-P_c \exp(-x/\lambda)$ and so, using equations (2), (4) and (5), we may write

$$\Psi = P_s - \pi = -P_c \exp(-x/\lambda) - \frac{(\Gamma/\Gamma_0)\pi_0\ V_0}{A_0\ x[1 - \frac{xP_c\ \exp(-x/\lambda)}{k_A}]}. \tag{6}$$

(Note that while $(-\gamma)$ is rather less than k_A, the second term in the denominator of equation (6) is small and so equation (6) is not very sensitive to the value of k_A.)

At high hydration (large x), the first term is negligible and the water potential in the membranous phase is equal in magnitude to its osmotic pressure. At low hydration (small x), the first term dominates, and further reductions in Ψ cause increases in the suction with relatively little change in π.

Equation (6) is used to plot x as a function of Ψ in Fig. 1. The area modulus k_A is taken as 140 mN m^{-1} and P_c and λ are again for DLPC. Γ/Γ_0 has been set at 1 because of my ignorance of the detailed non-ideal behaviour of biological solutions. (This ideal solution approximation is likely to be severely in error at low x. The effect of considering activity coefficients which increase with concentration is to decrease the slope in $x(\Psi)$ and to cause lower γ.) V_0/A will vary markedly among different cells and among different membrane-bound regions of the same cell: for largely aqueous organelles such as the vacuole of a plant cell it may be several micrometres, while for the cytoplasm and membranous organelles it is several to tens of namometres. The osmotic pressure π_0 at the reference state may vary from several megapascals for dry-adapted plants to a few tenths of a megapascal for animal cells. For $\pi_0 = 1{\cdot}0$ MPa and $V_0/A_0 = 10$ nm (typical values for the cytoplasm of a plant cell), $(\pi_0\ V_0/A_0)$ is 0·01 Pa m. Fig. 1 also plots the intermembrane repulsion P (equal in magnitude to 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.

Fig. 2 represents $x(\Psi)$ and $-\gamma$ from equation (6) on a logarithmic scale. In these calculations a range of $(\pi_0\ V_0/A_0)$ is used: 0·1, 0·02, 0·01 and 0·005 Pa m. If the membrane–water phase is in equilibrium with an aqueous solution at atmospheric pressure, then $-\Psi$ is the osmotic pressure of that solution. The concentration C in osmolal of such a solution is also shown on the abscissa of Fig. 2. If the water in the membrane–water phase is in equilibrium with an atmosphere with relative humidity r.h., then $\Psi = RT \ln$ (r.h.). In Fig. 2, r.h. is also represented on the abscissa. If the phase is in equilibrium with pure water, then that water must have a (negative) pressure equal

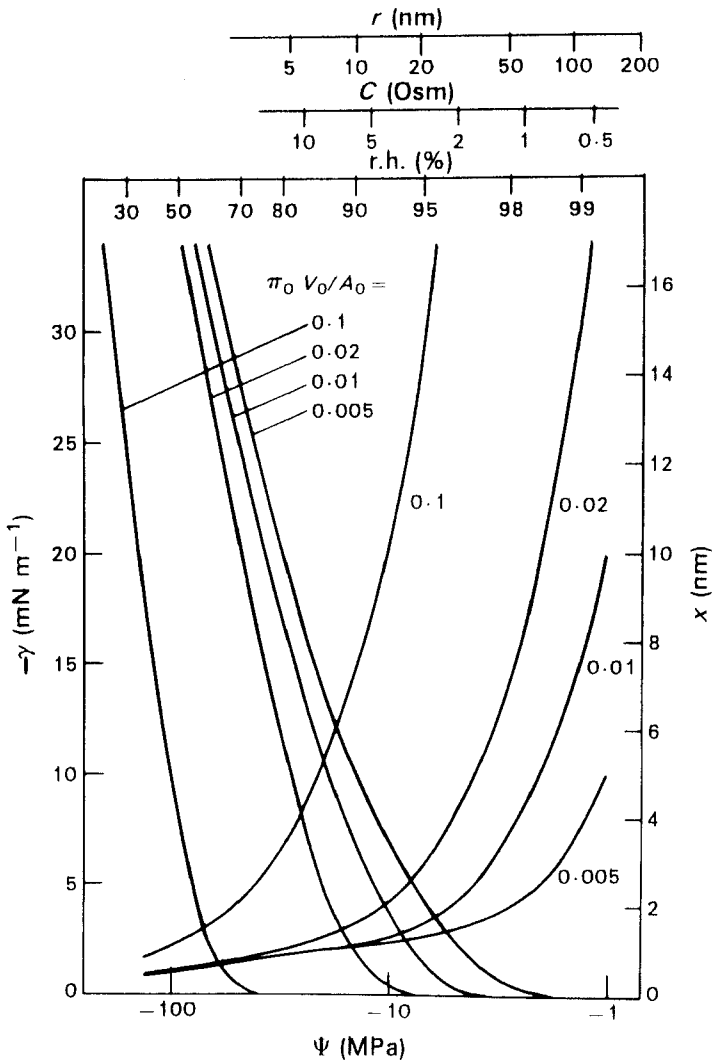

Fig. 2. The abscissa gives Ψ on a log scale. Also on the abscissa (above the graph) are properties of various phases with this Ψ. For an air-water vapour atmosphere, the relative humidity is given. For an aqueous solution, the composition (in osmolal) is given. For a pure water phase, the (negative) pressure is just Ψ. The radius, r, of a spherical meniscus which would support this pressure difference across an air-water interface is given. The set of curves with positive slope shows the average intermembrane spacing x for different values of the parameter $(\pi_0 V_0/A_0)$, given in Pa m for each curve. The set of curves with negative slope shows the lateral compressive stress $-\gamma$ exerted on the membranes for the same parameter values.

to Ψ; this negative pressure could be supported in a colloidal phase by microscopic menisci with curvature $\xi = \Psi/\gamma_w$, where γ_w is the surface tension of water. In Fig. 2, $2/\xi$ (the radius of a spherical meniscus) is also represented on the abscissa. For the parameter range represented here, 'large' lateral stresses are predicted for Ψ more negative than about -50 MPa. Such a value is rare in a transpiring plant, but is rapidly reached in a tissue that is allowed to equilibrate with a moderately dry atmosphere.

Many plants grown in dry or cold environments have cells whose osmotic pressure is greater than that of plants grown under standard conditions. It is therefore of interest to ask how the water content depends on the initial osmotic pressure. 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 (I do not know how this parameter is affected by growth in dry or cold environments), but with values of π_0 of 10, 2·0, 1·0 and 0·5 MPa. At any given Ψ, the higher osmotic pressure maintains of course a higher water content, although the effect is smaller than that predicted from simple osmotic behaviour. The higher osmotic pressure also preserves a lower stress in the membrane. To the extent that membranous regions of cells behave like the lipid phases of this model, this is likely to be physiologically important.

Further, it is important to note that, at any large negative Ψ, the membrane stress will be largest in organelles with relatively large dry weights or where the average intermembrane spacing is already small at Ψ near zero. Thus, assuming broadly similar intermembrane interactions, one would anticipate severe stresses in, for example, thylakoid and mitochondrial membranes at values of Ψ which had little effect on other membranes.

Conclusions

In membranous phases where the relative water content is less than about 25%, very large intermembrane forces are exerted (tens of megapascals). These cause:

(1) Similarly large suctions in the intermembrane solution, and thus give water contents much larger than expected from ideal osmotic behaviour; and

(2) Large lateral compressive stresses in the plane of the membrane (tens of $mN\ m^{-1}$), which may be responsible for membrane strains and phase transitions which cause physiological damage. The magnitude of the strain may be affected by the presence of solutes which alter the surface forces at the membrane–solution interface.

Such stresses occur at more negative water potentials if the solution has a higher osmotic pressure. The stresses are larger in organelles with higher membrane content or higher dry weight.

Acknowledgments

I thank D. F. Gaff, A. C. Leopold, V. A. Parsegian and P. L. Steponkus for helpful comments on the draft manuscript. This paper is in part based upon work supported by the United States Department of Energy under Grant No. DE-FG03-84ER13214.

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Manuscript received 23 September 1986, accepted 5 January 1987