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THE STRESS-STRAIN RELATION OF THE PLASMA MEMBRANE OF ISOLATED PLANT PROTOPLASTS

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Summary

Over periods of up to a few seconds the plasma membrane of isolated rye protoplasts behaves elastically with an area modulus of 230 $mN \cdot m^{-1}$. Over longer periods, the area increases with time under large tension and decreases under sufficiently small tension, suggesting that material is incorporated into or depleted from the plane of the membrane.

The stress-strain relation of the plasma membrane of isolated plant protoplasts has not, to our knowledge, been studied in detail hitherto. Previous studies of membrane stress-strain relations have considered only small or zero changes in area and principally have used sea urchin eggs [1] and red blood cells [2,3], the membranes of which are atypically rigid [4]. Experiments on protoplasts in which only the surface tension was measured [5] have implicitly suggested that under some conditions a surface energy law applied (surface tension constant) while analyses of experiments on red blood cells [6] have used visco-elastic relations. Large differences between the mechanical properties of the membranes of red blood cells and those of isolated protoplasts might be expected: the red blood cell membrane is closely attached to a rigid spectrin cytoskeleton [4], whereas the protoplast plasma membrane is deprived of its principal structural support (the cell wall) when the protoplasts are isolated.

Experiments [7,8] in which populations of isolated plant protoplasts were exposed to changes in the osmolarity of their suspending medium have suggested that the longer time-scale stress-strain relation of their plasma membrane

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is distinctly different from that of previously studied systems: (1) over a large range of osmolarities, isolated protoplasts behave as ideal osmometers; (2) following volumetric contraction or expansion, protoplasts are observed to be spherical (to the resolution of the light microscope), implying corresponding changes in the area of the plasma membrane; (3) the contraction in area may be as large as 3-fold; (4) after sufficiently large contractions, protoplasts may lyse on expansion before regaining their original volume and to any tissue may be ascribed a tolerable surface area increment which is characteristic for the genotype. These observations suggest that the membrane is subject to a tension over a large range of area and that the stress-strain relation has substantial hysteresis or time dependence. Here we report the measurement of the area stress-strain relation of the plasma membrane of protoplasts isolated (as described in Ref. 7) from leaves of rye (*Secale cereale* L. cv. Puma) using the micropipette elastimeter of Mitchison and Swann [1] which is described in Fig. 1.

A corollary of the ideal osmometric behaviour of protoplasts is that their volume is conserved at constant external concentration, and this conservation permits a greater resolution of small changes in area. The tension required to rupture the membrane is usually less than 4 $mN \cdot m^{-1}$ and tensions smaller than this produce large increases in area. Such tensions in the membranes of protoplasts of 35 μm diameter produce internal hydrostatic pressures of about 1 kPa. Since these are small compared to the osmotic pressure (about 1 MPa) of the suspending solution required to maintain their in vivo volume, cytoplasm and external solution are always very nearly in osmotic equilibrium and so the volume of the protoplast is conserved at constant external concentration. Assuming constant volume and neglecting high order terms in (a/R), it may be shown that the change in area of a protoplast of radius R which intrudes a distance D into a pipette of radius a is:

$$\Delta A = \begin{cases} \pi[D_0^2 + D^2 - D^3/3R - 2DD_0] & D \leqslant a \\ \pi[a(2D - a + 4D_0/3) + D_0^2 - 4DD_0] & D > a \end{cases} \quad (1)$$

where $D_0 = a^2/2R$.

The constancy of volume and the accuracy of Eqn. 1 during large deformations are demonstrated by the results in Fig. 2. Since the membrane slides freely over the lip of the pipette, we assume * that the tension in the delimited region equals that outside the pipette whence

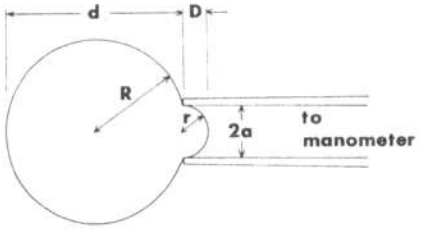
$$-P = \frac{2\gamma}{r} - \frac{2\gamma}{R} \quad (2)$$

where $(2/r)$ is the curvature of the deformed region of the membrane. From geometry:

$$r = \begin{cases} (D^2 + a^2)/2D & D \leqslant a \\ a & D > a \end{cases} \quad (3)$$

Thus the stress-strain relation may be determined from measurements of P, D, a

* A discussion of this assumption and the derivation of Eqn. 1 will be presented elsewhere.



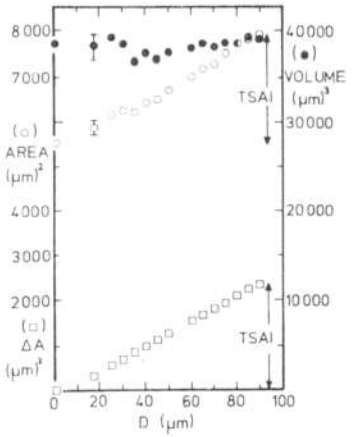


Fig. 1. The elastimeter of Mitchison and Swann [1] comprises a manometer which produces a negative pressure P in a glass micropipette which abuts the protoplast and produces a small area of increased curvature in the membrane. With a small pipette, small changes in area (<0.1%) may be imposed and the resting tension estimated. With a larger pipette (5—10 μm) the stress-strain relation may be measured for large changes in area. The diameter of the pipette is three orders of magnitude larger than the thickness attributed to plant plasma membranes, so curvature energies are expected to be negligible. The membrane behaves as a two-dimensional fluid in this experiment: the geometry shown is accurate to optical resolution. We have confirmed our calibration of the device by measuring the surface tension of the air-water interface as 68 ± 5 mN · m^{-1}.

Fig. 2. The accuracy of Eqn. 1 (which is equivalent to the validity of the assumption of constant volume) is assessed by using a large pipette to make large deformations in the plasma membrane area. D and d (see Fig. 1) are measured microscopically and from geometry

$$A = \begin{cases} \pi(D^2 + 2a^2 + d^2) & D < a \\ \pi(2Da + a^2 + d^2) & D \geqslant a \end{cases} \qquad V = \begin{cases} \frac{\pi}{6}[d(d^2 + 3a^2) + D(D^2 + 3a^2)] & D < a \\ \frac{\pi}{6}[d(d^2 + 3a^2) + 2a^2(3D - a)] & D \geqslant a \end{cases}$$

The area, A, (open circles) and volume, V, (solid circles) thus calculated and ΔA calculated from Eqn. 1 (squares) are plotted against D. The vertical bars (shown on only one datum for clarity) represent the error incurred by the inability to resolve distances to better than 300 nm. It is clear from this typical result that the volume is conserved and that Eqn. 1 is accurate to the accuracy of the experiment. The membrane lysed at D = 95 μm so the tolerable surface area increment (TSAI) is as indicated.

and R. For populations of protoplasts isolated and maintained in 0.53 osM sorbitol, the resting tension (γ_r) is very small (mean = 100 μN · m^{-1}) although there is a wide variation and values from zero to 1 mN · m^{-1} have been measured. When these protoplasts are subsequently equilibrated in either hypertonic (0.70 osM) or hypotonic (0.41 osM) sorbitol solutions, the range of γ_r observed remains the same. After these manipulations the protoplasts are spherical and the apparent membrane area increases or decreases by 15%. The spherical shape and the ability to sustain a tension suggests that the contraction or expansion is associated with an effective areal contraction or expansion of the plasma membrane.

The small change in γ_r with large osmotic perturbations of protoplast size is not consistent with a purely intrinsic contraction since this would require a very low value of the area elastic modulus (k_A), of the order 100 μN · m^{-1}. Though a near-zero value of k_A is possible in principle for a pure, one-component bilayer at its transition temperature, the presence of different lipid species

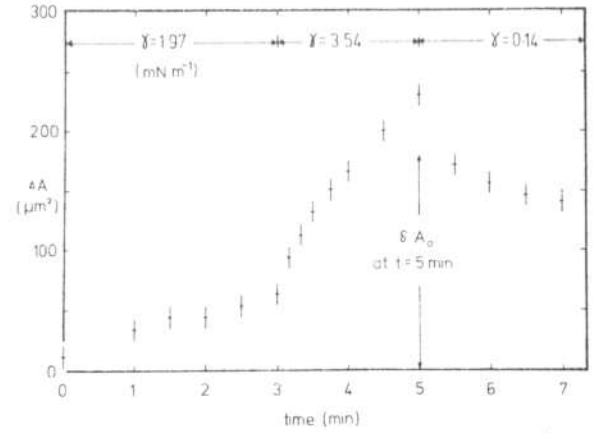


Fig. 3. In this plot of $\Delta A(t)$, a tension of 1.97 mM · m^{-1} is applied at $t = 0$ and a small (elastic) deformation is produced almost immediately. The area increases with time, and the rate of increase is larger when a larger tension (3.54 mM · m^{-1}) is applied at $t = 3$ min. When the tension is relaxed to a value near zero (0.14 mN · m^{-1}) at $t = 5$ min, the elastic component of ΔA is contracted leaving an apparently plastic change δA_0 of about 150 μm^2. $A_0(t)$, the untensioned area, is the area which would be occupied by the material in the membrane at time t were it not subject to a tension. Thus defined, change in A_0 must be due to the exchange of material between the plane of the membrane and the reservoir.

or proteins removes the phase transition anomalies and produces much larger k_A s [9,10] *.

Since only a small part of the change in membrane area may be achieved by intrinsic contraction or expansion of membrane area (and proportional thickening or thinning), we propose the existence of a reservoir into or from which membrane material is transferred during large contractions or expansions. The reservoir may include a subduction of material into internal or external vesicles, or sub-microscopic buckling or folding. Such folds could be stabilized by membrane-membrane attraction in order to sustain a finite tension, however folding has not been observed in electron microscope studies of osmotically-contracted protoplasts [11].

A large imposed tension produces a change with time in the untensioned area, $A_0(t)$ (Fig. 3). If the tension is rapidly relaxed to γ_r, the change in A_0 is measured as a change in A after the excursion. A_0 increases with time under large γ and decreases under small γ; further, the tension required to maintain a given ΔA relaxes over several minutes towards γ_r (data not shown).

Rapid increases in tension of order 1 mN · m^{-1} followed quickly by equal decreases produce small area changes (of order 1%) which are largely reversible (Fig. 4). Rapid contractions are presumed predominantly elastic and so are used to estimate k_A from the equation $\gamma = k_A(A - A_0)/A$. In 28 experiments k_A was calculated during rapid relaxation of the tension, yielding $k_A = 230$ mN · m^{-1} with standard deviation 50 mN · m^{-1}. Though care was taken to avoid intrusion of the tonoplast during these experiments, the implicit assumption

* We note, however, that the ability of membranes to withstand tensions of several mN · m^{-1} is important in the study of phase transitions since for some lipids a 1 mN · m^{-1} change in tension is expected to have as large an effect on lipid order as a temperature change of 1°C [9].

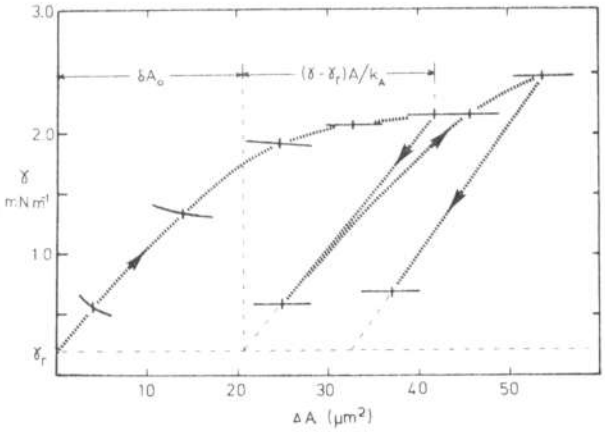


Fig. 4. A stress-strain plot for a sequence of slow expansion, rapid contraction, moderately rapid expansion and rapid contraction: the points are taken at 20-s intervals. We presume the contractions to be predominantly elastic since γ is low during contraction and thus $\delta A_0(t)$ should be small (Fig. 3), and thus calculate k_A. The first expansion may therefore be divided into its elastic and time-dependent components as shown. The second expansion is about twice as fast as the first and produces a correspondingly smaller δA_0. The error bars representing optical resolution are not horizontal since at small D, errors in D affect measurement of γ as well as ΔA. We observe that since resolution errors are more systematic than random, the probable error in k_A is smaller than the bars suggest.

that the cytoplasm is homogeneous and of low viscosity renders this an overestimate.

The stress-strain relation of the plasma membrane of isolated protoplasts may therefore be described in terms of two paradigms, an elastic law and a surface energy law. Over short periods of time (seconds), the amount of material in the plane of the membrane is conserved and deformations follow a simple elastic relation. Over long periods of time (minutes), the tension reverts to its resting value, independent of the induced change in area, and thus the deformations follow a surface energy law.

In a pertinent analogy, a lipid bilayer in equilibrium with an organic solution of lipid (a reservoir) follows an elastic law at high audio frequencies [12] but a surface energy law over longer time scales (seconds or minutes) [13,14]. If the membrane-reservoir system be at equilibrium, the resting tension may be related to the differences in free energy per molecule between the reservoir and the unstretched membrane [13]. A resting tension of 100 $\mu N \cdot m^{-1}$ implies a difference of only 0.006 times the thermal energy (kT) per lipid molecule, however to a vesicle of more than a thousand lipid molecules this could be a substantial energy of vesiculation. Thus imposed tensions of order 1 $mM \cdot m^{-1}$ would be expected to change an equilibrium between membrane and reservoir.

The measured values of k_A and the tension required to lyse the membrane indicate that an intrinsic expansion of more than about 2% will cause membrane lysis. The ability of a protoplast to survive greater membrane expansions than this (as occur during osmotic rehydration or the thawing of extracellular ice) will depend on the amount of material readily available in the reservoir and the rate at which it may be incorporated. An understanding of the stress-strain relation and the reservoir concept therefore provides a molecular description of

the tolerable surface area increment observed in Refs. 7 and 8. This application, and a more detailed account of these experiments, will be presented elsewhere.

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