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ENERGY OF FORMATION OF BIMOLECULAR LIPID MEMBRANES

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SUMMARY

A method for measuring the energy of formation, W, of artificial, bimolecular lipid membranes, in aqueous solutions, is described. The method is based on the determination of the radius of curvature of the membrane as a function of the pressure difference across it. This is accomplished by the use of capacitance measurements on bowed membranes. The method allows W to be determined as a function of the film extension. The energy of formation of the membranes under a variety of ionic solutions was found to be 3.4 erg·cm^{-2}.

INTRODUCTION

Recent experiments with artificial, bimolecular lipid membranes have shown that these systems have properties common to the naturally occurring cellular membranes. These properties include a thickness of approx. 60 Å, a refractive index of approx. 1.6 (ref. 1), an isotopically measured permeability coefficient for water of approx. 4 μsec^{-1} (ref. 2) and a frequency dependence of the dielectric constant on the electrolyte concentration[3].

Further, artificial membranes containing lecithin and cholesterol have, in common with many naturally occurring membranes, a low frequency capacitance of around 0.6 μF·cm^{-2} (ref. 3). If, as seems likely, the protein part of natural membranes is in series with the lipid[4], then the low-frequency capacitance of these membranes is essentially that of the lipid[5]. The latter for many systems is primarily phospholipid and cholesterol (see *e.g.* ref. 6). Since capacitance may be regarded as an index of molecular packing and polarizability it seems reasonable to suppose that many natural and artificial membranes are similar in this regard, so that energy relations deduced for one system should have some relevance to the other.

An important step towards obtaining the energies of binding in the artificial bilayers lies in an accurate determination of their energy of formation, here defined as the isothermal work required to increase both interfaces of the membrane by one unit of area. Measurements made to date (ref. 7; T. E. THOMPSON, personal communication) relied on measuring the pressure difference ΔP needed to bow the membrane to an hemisphere, the latter being detected visually. However, the low light reflectance of the films leads to inaccuracies in determining when the films are hemispherical.

The measurement of the energy of formation as a function of membrane area by this method is difficult and to our knowledge has not been attempted. Nevertheless

this information is important, both for determining whether film extension involves a corresponding increase in intermolecular spacing, and for determining a suitable criterion by which to compare the film tensions deduced for natural and artificial membranes.

The present work describes a very sensitive method for determining the energy of formation of these membranes as a function of surface extension.

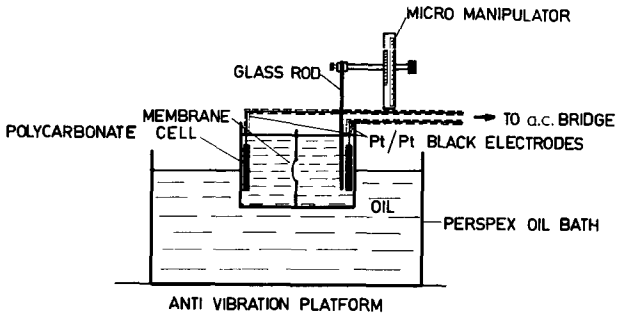


Fig. 1. A diagram of the experimental apparatus used in the energy of formation measurements. The membranes were formed over a 7-mm diameter hole in the septum dividing the polycarbonate cell into two compartments. The membrane was bowed by lowering a glass rod into one compartment with a micromanipulator. The area of the bowed film was deduced using capacitance measurements (see text).

The method relies on measuring the pressure difference as a function of the radius of curvature for a bowed membrane. Determination of the radius of curvature of the 'black' membrane is accomplished to a fairly high degree of accuracy through the use of measurements of the low-frequency capacitance of the bowed film.

EXPERIMENTAL METHODS

Cell and associated apparatus

A diagram of the experimental apparatus is shown in Fig. 1. Each compartment of the polycarbonate cell was filled with about 20 ml of electrolyte solution.

The membranes were formed over a 7-mm hole in the septum which divides the cell into two compartments. The cell was mounted in an oil bath which was rigidly attached to a concrete block. The latter itself was mounted on an anti-vibration platform. This arrangement was found necessary to reduce vibrations to a tolerable level. The oil bath was made from 'perspex' so that the membranes could be viewed under reflected light.

Formation of the membrane

The technique for generating the membranes was essentially that of HANAI, HAYDON AND TAYLOR[3] with the exception that, in the present work, the membranes were much larger in area. The membranes were generated from a solution containing egg phosphatidyl choline, cholesterol, *n*-tetradecane and *n*-heptane dissolved in a chloroform–methanol (3:2, v/v) solvent.

The measurement of the energy of formation

The method for obtaining the energy of formation W relied on measuring the

radius of curvature R of the bilayer membrane as a function of the pressure difference ΔP across it. It is readily shown that

$$\Delta P = \frac{2W}{R} \tag{1}$$

Because of the low light reflectivity (approx. 10^{-6}) of the bilayer membranes, it is most difficult to estimate R, and hence ΔP, by the conventional optical techniques. In the present work these parameters were estimated as follows.

A rod of uniform diameter was lowered into one compartment of the cell with a micromanipulator. This produced a pressure difference across the membrane given by

$$\Delta P = \rho g \left[\frac{a\Delta x}{(S_1 - a)} - v\left(\frac{1}{(S_1 - a)} + \frac{1}{S_2}\right)\right] \tag{2}$$

where a is the cross sectional area of the rod; Δx is the change in length of the submerged portion of the rod; S_1 is the cross sectional area of the compartment into which the rod is lowered; S_2 is the cross sectional area of the other compartment; ρ is the density of the aqueous electrolyte; g is the acceleration due to gravity; v is the volume of liquid in the other cell compartment which is displaced by the bowed membrane.

Assuming that the membrane is homogeneous in its properties, it will have the same radius of curvature everywhere which is given by

$$R = \frac{A}{2\pi(A/\pi - r^2)^{\frac{1}{2}}} \tag{3}$$

Hence,

$$v = \frac{\pi}{6}\left(\frac{A}{\pi} - r^2\right)^{\frac{1}{2}}\left(\frac{A}{\pi} + 2r^2\right) \tag{4}$$

where A is the area of the membrane and r is the radius of the membrane when flat. The latter is almost equal to the radius of the hole on which the film is generated.

Using Eqns. 1, 2, 3 and 4, the energy of formation is given by,

$$W = \frac{\rho g A}{4\pi(A/\pi - r^2)^{\frac{1}{2}}}\left[\frac{a\Delta x}{(S_1 - a)} - \frac{\pi}{6}\left(\frac{A}{\pi} - r^2\right)^{\frac{1}{2}}\left(\frac{A}{\pi} + 2r\right)\left(\frac{1}{S_1 - a} + \frac{1}{S_2}\right)\right] \tag{5}$$

Thus W can be calculated from measurements of A as a function of Δx. The membrane area was determined by the method of HANAI, HAYDON AND TAYLOR[8] in which area is obtained from measurements of its capacitance, *i.e.*,

$$A = \frac{C}{C_{\min}}\pi r^2 \tag{6}$$

At each position of the rod, the conductance and capacitance of the circuit were measured by an a.c. bridge. The reproducibility of the measurements was then checked by repeating the run. The membrane was then broken and the measurements repeated. From these measurements the membrane capacitance could be calculated (see APPENDIX).

Where possible the electrical measurements were made at frequencies below the dispersion frequencies for the system (see *e.g.* ref. 3). However, because of the decrease

in dispersion frequency with decreasing concentration of electrolyte, this was not possible for the experiments in which the concentration of salt was < 10 mequiv/l.

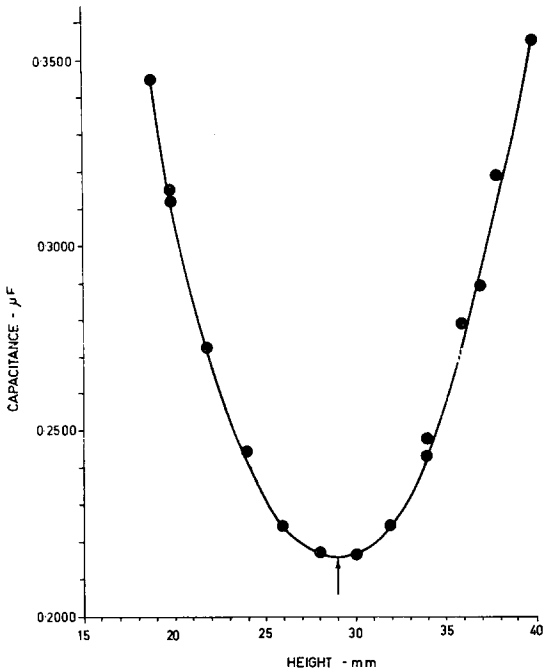


Fig. 2. The membrane capacitance plotted as a function of the height of the rod. The minimum on this curve corresponds to the capacitance of the membrane when it is flat.

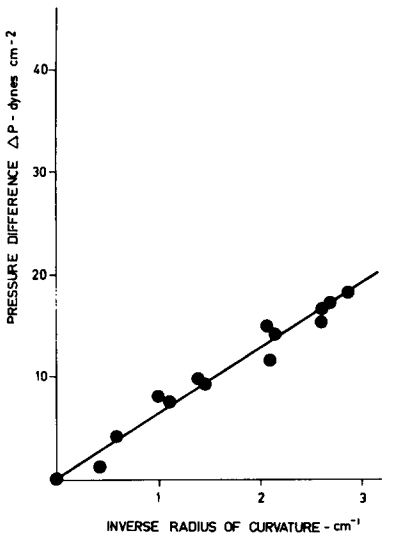


Fig. 3. The calculated inverse radius of curvature ($1/R$) plotted as a function of the pressure difference across the membrane.

RESULTS

Fig. 2 shows a plot of the measured value of membrane capacitance as a function of the position of the rod (relative to some fixed position) when the external solution was 2 M NaCl at 20°. Similar curves were obtained for all the systems examined, namely NaCl, KCl, LiCl and $CaCl_2$ at various concentrations in the range 1 mequiv/l to 2 equiv/l and in the temperature range 6–40°.

Substitution of the known capacitance per unit area of membrane, as deduced when the film is flat, and the measured value of the film capacitance into Eqn. 6 above allows the determination of the area of the membrane at different rod heights. This enables the determination of v and R in Eqns. 3 and 4 and hence ΔP (Eqn. 2).

Fig. 3 shows a plot of ΔP against the inverse radius of curvature when the outside solution was 2 M NaCl at 20°. It is seen from Eqn. 2 that the slope of this curve equals $2W$. For all the systems investigated, the plot of ΔP against $1/R$ was linear at high pressure differences (*i.e.* $\Delta P > 10$ dyne·cm^{-2}). Occasionally the plot was curved at low pressure differences. This was attributed to an uncertainty in estimating when the membrane is exactly flat.

Most systems examined showed some variation in the energy of formation from

membrane to membrane. Within these variations it was found that the energy of formation was independent both of the nature and concentration of the electrolyte and also of temperature, and had a value of 3.4 ± 0.6 erg·cm^{-2}.

DISCUSSION

The energy of formation for a lipid bilayer film relates not only to the interfacial free energies but also to the interaction energy between the two bulk aqueous solutions that it separates. This latter energy (per unit area), E, is given by

$$E = -\frac{H}{12\pi\delta^2}$$

where H is the HAMAKER–VAN DER WAALS constant and δ is the thickness of the membrane. For two water layers separated by air H has a value of $6 \cdot 10^{-13}$ erg (ref. 9), and it seems unlikely that the value of H in the present case will differ by more than an order of magnitude from this (*e.g.* see ref. 10). Using this value for H and a membrane thickness of 50 Å the interaction energy between two water layers separated by a lipid bilayer is around 0.06 erg·cm^{-2}. This value is much smaller than the value found for W, suggesting that there is not much error involved in ignoring it and assuming that

$$W = 2\gamma$$

where γ is the free energy of the molecules at the membrane-water interface.

On this basis, the interfacial free energy (per unit area) has a value of about 1.7 erg·cm^{-2}. This is considerably higher than the 0.1 erg·cm^{-2} figure reported for a number of cellular membranes[4]. However the present results are comparable with the value of 2 erg·cm^{-2} reported for the interfacial energy of a carbon tetrachloride solution of phospholipids in contact with an aqueous solution[11].

There are essentially two methods by which a bimolecular film may undergo extension. Firstly the intermolecular spacing may increase and secondly additional molecules may be introduced into the membrane from a reservoir (the torus) of such molecules.

The present results show that the energy of formation is independent of the film extension. This result is consistent with the assumption on which the calculations were based, namely that the membrane capacitance is proportional to its area. Since the result would not be expected if the assumption were invalid it is concluded that it is most likely correct. It appears, therefore, that the increase in film area is accomplished by the withdrawal of additional molecules from the torus.

It is uncertain whether an increase in the area of surface of cellular membranes involves an increase in intermolecular spacing. This complicates an objective comparison of the different values found for γ in the two systems. However the method of membrane extension for the artificial membranes described above is consistent with there being more work involved in increasing the intermolecular spacing than in withdrawing additional molecules from the torus. It is deduced from this that extension of the surfaces of artificial membranes in the same manner as that associated with natural membranes involves at least 3.4 erg·cm^{-2} of membrane.

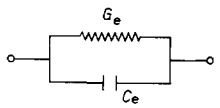
It is interesting to note that cellular membranes contain proteins which lead to very low interfacial tensions when adsorbed at an oil–water interface. It seems likely

that the small value of the interfacial free energy for natural membranes relative to the artificial membranes arises from the presence of the protein in the former systems.

APPENDIX

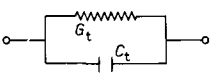
The bridge measurements yielded the equivalent parallel combination of conductance and capacitance of the entire circuit, including the electrodes, both with and without a membrane present.

When there is no membrane, the equivalent circuit given by the bridge measurements is

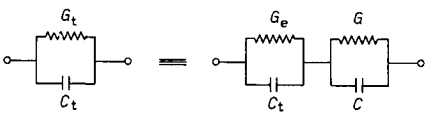


where both G_e and C_e are frequency dependent.

When there is a membrane present the equivalent circuit is



In the present work it was found that the stray capacitance and conductance shunting the membrane was negligible. The membrane capacitance and conductance can then be obtained from the following equivalent circuits:



where C and G refer to the membrane capacitance and conductance and are given by:

$$G = \frac{G_e G_t(G_e - G_t) + \omega^2(C_e^2 G_t - C_t^2 G_e)}{(G_e - G_t)^2 + \omega^2(C_e - C_t)^2}$$

$$C = \frac{G_e^2 C_t - C_t^2 C_e + \omega^2 C_t C_e(C_e - C_t)}{(G_e - G_t)^2 + \omega^2(C_e - C_t)^2}$$

G_e, C_e, G_t and C_t were measured at each position of the rod and hence C and G could be calculated as a function of the depth of the rod.

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