Biochimica et Biophysica Acta, 735 (1983) 95–103
Elsevier

BBA 71863

THE EFFECTS OF BILAYER THICKNESS AND TENSION ON GRAMICIDIN SINGLE-CHANNEL LIFETIME

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(Received June 16th, 1983)

Key words: Gramicidin; Channel lifetime; Lipid bilayer; Membrane thickness

Measurements have been made of gramicidin single-channel lifetimes in monoacylglycerol bilayers chosen so that their thickness ranged from above to below the length of the gramicidin channel. Contact angles, electrical capacities and bulk-phase interfacial tensions have also been determined for these systems. The mean channel lifetime decreased with the hydrocarbon thickness of the membrane until the latter reached 2.2 nm, after which the lifetime was relatively constant. A theoretical model has been proposed which relates the mean channel lifetime (or dissociation constant) to both the thickness and the tension of the bilayers. The analysis of the present results and of those of previous studies has led to the idea that aggregates of water molecules may play an important rôle in the dissociation of the gramicidin channel.

Introduction

In lipid bilayer membranes, gramicidin A forms dimeric ion-conducting channels which appear to exist in equilibrium with monomeric polypeptide [1–5]. The factors which affect this equilibrium and its component rate processes have been investigated by several groups. The equilibrium constant for the reaction

$$G + G \underset{k_d}{\overset{k_r}{\rightleftharpoons}} G_2 \qquad (1)$$

has been shown to depend on the thickness and surface tension of the membrane [2,6]. It is presumed that when the membrane is thicker than the length of the channel, a deformation or dimpling must occur in order to accommodate the dimeric complex and that, in general, this requires that work be done against surface forces. The following quantitative relationship was derived:

$$\ln K = \ln K_0 - \frac{\pi r \sigma (h - h_0)}{kT} \qquad (2)$$

where h is the thickness of the hydrocarbon part of the membrane, h_0 is the length of the lipophilic part of the channel, σ is the Gibbs tension of the (double-sided) membrane, r is the radius of the equivalent 'square-well' deformation, k is the Boltzmann constant and T is the absolute temperature. K_0 is the equilibrium constant when $h = h_0$. Eqn. 2 was found to be obeyed to within experimental error over four orders of magnitude of K [6]. According to Eqn. 2, the channel stability should be maximal when the hydrocarbon thickness of the membrane is equal to the length of the lipophilic part of the dimer, but whether this is so has not been investigated.

Of the two rate constants, only the dissociation constant, k_d, has been examined in a wide range of systems. This is partly because there are technical difficulties in measuring k_r or K in many

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instances and partly because, since k_d is equal to the reciprocal of the mean channel lifetime, it is readily accessible. Despite the attention it has received, the factors which determine k_d have not been fully elucidated.

In the initial paper on this subject it was shown merely that the mean channel lifetime, and hence k_d, was different for membranes of different thickness and composition [1]. Within these measurements, k_d correlated with membrane thickness, but no attempt was made to account for this observation. In a subsequent, more detailed, study, it was noted that, while the correlation with thickness held quite widely, there were some clear exceptions [7]. Two further investigations, using a variety of lipid membranes, have revealed little or no correlation with thickness but a strong correlation with membrane tension [8,9]. One of these papers [9] proposed an empirical relationship between mean channel lifetime and tension, but in neither paper is there any discussion of mechanisms.

Since $K = k_r/k_d$, it is obvious from Eqn. 2 that either or both of the rate constants must depend in some way on the thickness-tension product, $\sigma(h - h_0)$. The tension is presumably important, since, in a membrane of thickness greater than the length of the channel, it would tend to pull against the hydrogen bonds which maintain the integrity of the helical structure and hence promote dissociation or, at least, loss of conductivity. The direction of pull of the tension very probably depends on the extent of membrane deformation caused by the conducting channel. Hence, the bilayer thickness should also be a factor in channel stability. Thus, both the bilayer tension and its thickness could appear in an expression for the dissociation rate constant. Such an expression would also contain a frequency factor, however, suggesting that membrane 'fluidity' might have some influence on the rate processes.

In this paper, an attempt has been made to demonstrate that membrane thickness is of some importance. This has been done by studying the mean channel lifetime in lipid bilayers which have thicknesses both larger and smaller than the length of the channel. In order to interpret the results, the tensions of the bilayers have also been accurately measured. This is not a straightforward procedure, since, in such thin bilayers, the tension is significantly less than in the equilibrium single bulk interfaces. A technique recently developed for this purpose has therefore been employed [10]. Finally, a theoretical analysis of the dependence of k_d on thickness and tension is described which suggests that the rate-limiting step in the dissociation may not be as previously supposed.

Methods and Materials

Methods

Gramicidin single-channel recordings. Black films were formed by the pipette method [11] from freshly prepared solutions of monoacylglycerols in hydrocarbons. The experimental cell consisted of a vertical poly(tetrafluorethylene) (PTFE) cylindrical vessel, embodying a 1 mm hole across which the membranes were formed, mounted in an outer chamber of fused quartz. The whole apparatus could thus be subjected to the rigorous cleaning with solvents and strong acids required for successful single-channel work. The electrical circuitry and procedure used to record single-channel events was essentially as described previously [12]. The applied potential was ± 50 mV and the temperature 23 ± 1°C.

Unlike the studies using monoeicosenoin and monoolein, where one experiment (from cleaning to taking the last channel event) was typically performed in 1.5 days, membranes formed from the shorter-chain-length monoacylglycerols, monopalmitolein and monomyristolein, initially produced unacceptable background noise levels. This made resolution of the single channel recordings impossible until 24 h after assembly. The noise seemed to be associated with a time-dependent wetting of the poly(tetrafluorethylene) surrounding the hole by the membrane-forming solution. The lipid concentration chosen for the experiments depended in part upon the difficulties introduced by this effect. High concentrations of monoacylglycerol (approx. 280 mM) were required to overcome the noise problem. Leaving a membrane apparatus assembled for a day or two did not result in any change in the measured channel lifetime for monopalmitolein-decane films where background noise was not a problem. It is therefore expected that no unacceptable change, such as oxidation or hydrolysis of the lipid or hydrolysis

of gramicidin, occurred in the solvent-free systems.

Bilayer thickness and tension. The thickness of the hydrocarbon region of the bilayers was calculated from the electrical capacity at 500 Hz, as measured by an a.c. bridge technique [13]. The bilayer tension, σ, was calculated from the expression

$$\sigma = 2\gamma\cos\theta \quad (3)$$

where γ is the tension of the interface between the bulk aqueous and film-forming solutions with which the bilayer is in equilibrium, and θ is the contact angle [14]. The tension, γ, was measured for the aqueous and lipid solutions appropriate to the various systems in which single-channel lifetimes were examined. All the tension measurements were made in a water bath maintained at 24.5°C. The densities of the monoacylglycerol-squalene solutions were taken as equal to that of pure squalene except for the monomyristolein system where the high concentration of the lipid made this approximation unacceptable. In this instance, the density was estimated on a volume fraction basis and indicated that the density difference between the lipid and aqueous phases decreased by approx. 4%. The contact angle, θ, was measured for a lens of bulk lipid solution in a horizontal bilayer. The lens was formed by the injection of a known volume of lipid from a micropipette, as described elsewhere [10]. It was found necessary in some instances to pre-equilibrate aqueous and lipid solutions in order to obtain contact angles which were independent of time.

Materials

Monoacylglycerols were obtained from Nu Chek Prep, MN, and were used without further purification. Grade I squalene was from the Sigma Chemical Company (St. Louis, MO), and the *n*-alkanes were from Koch-Light Laboratories, Colnbrook, U.K. They were passed through an alumina column to remove polar impurities. Gramicidin was supplied by Koch-Light Laboratories and consisted of a 72:9:19 mix of gramicidins A, B and C; it was used without further purification dissolved in ethanol (Analar grade) to a nominal concentration of 10^{-9} M. Potassium chloride and caesium chloride were of AR grade and were roasted overnight at 700°C. The water was doubly distilled.

TABLE I

GRAMICIDIN MEAN CHANNEL LIFETIMES IN MONOACYLGLYCEROL-SQUALENE BILAYERS OF DIFFERENT THICKNESS AND TENSION

For the channel lifetimes the applied potential was ±50 mV and the temperature 23±1°C. For the tension, contact angle and capacitance data, the temperature was 24.5°C. Number of channels measured is in parentheses.

Monoacylglycerol/ aqueous phase	Mean channel lifetime, (s±S.E.)	Bulk interface tension ($mN \cdot m^{-1}$)	Contact angle, (degrees)	Capacitance per unit area ($\mu F \cdot cm^{-2}$)	Half bilayer tension, $\sigma/2$ ($mN \cdot m^{-1}$)	Hydrocarbon region thickness, h (nm)
Monoeicosenoin (7.8 mM)/0.5 M KCl	0.7± 0.1 (141)	2.68±0.01	23.60±0.47	0.683	2.46±0.01	2.85
Monoolein (133 mM)/ 0.5 M KCl	37 ± 2.5 (185)	1.98±0.02	28.44±0.48	0.791	1.74±0.02	2.45
Monoolein (66 mM)+ monopalmitolein (73 mM)/1.0 M CsCl	119 ± 9 (284)	1.68±0.02	29.65±0.42	0.848	1.46±0.02	2.30
Monopalmitolein (145 mM)/0.5 M KCl	286 ±22 (233)	1.35±0.01	30.22±0.18	0.897	1.17±0.01	2.17
Monopalmitolein (209 mM)+monomyristolein (104 mM)/1.0 M CsCl	211 ±47 (71)	1.09±0.02	31.78±0.42	0.952	0.93±0.02	2.06
Monomyristolein (300 mM)/1.0 M CsCl	228 ±16 (165)	0.47±0.04	40.80±0.97	1.061	0.36±0.03	1.84

Results

Mean channel lifetimes, bulk interfacial tensions, contact angles and capacities per unit area for some monoacylglycerol-squalene bilayers are shown in Table I. In the final two columns are given the half-bilayer tension ($\sigma/2$) and the estimates of the thickness (h) of the hydrocarbon region of the membrane. The mean channel lifetime, τ, is the average of all the analysable events. Channels of duration less than 0.1 s were difficult to measure accurately in the apparatus used and so the value for monoeicosenoin bilayers must be regarded as an upper estimate. CsCl (1.0 M) was used in three instances in preference to 0.5 M KCl to improve resolution. The lifetime of the channels in the monoolein + monopalmitolein bilayers was not affected by this substitution (results not shown). Fig. 1 shows the cumulative frequency distribution of channel lifetimes in monoolein-squalene and monopalmitolein-sequalene bilayers. The exponential fit to the data is reasonably good in both instances, suggesting that, as in thicker membranes [1,4], the channel closure is a first-order process.

Mean channel lifetimes for gramicidin in a number of other systems have also been measured. The monoolein-*n*-alkane bilayers initially examined in Ref. 1 have been re-examined in 0.5 M KCl and the series has been extended to include *n*-octane and *n*-dodecane. Also studied were bilayers made from monoolein (28 mM) in squalene/*n*-decane mixtures of *n*-decane mole fractions 0.1, 0.4, 0.6 and 0.8. The aqueous solution was 0.5 M NaCl and the numbers of events analysed in each instance was over 270. For both the above sets of measurements, the temperature was as in Table I, 23 ± 1°C. The results have been plotted as the dissociation rate constant, k_d, in Fig. 3.

Discussion

The gramicidin mean channel lifetimes for the systems described in Table I vary by at least a factor of 300. When plotted against the hydrocarbon thickness of the membrane, the mean lifetime increases as the thickness decreases down to 2.2 nm (Fig. 2). Below this value, the upward trend in τ ceases and there appears to be a slight decrease, though this is probably not significant. A plot of τ against the half-bilayer tension ($\sigma/2$) has a form similar to that of the plot against thickness (Fig. 2).

The failure of the monotonic relationships be-

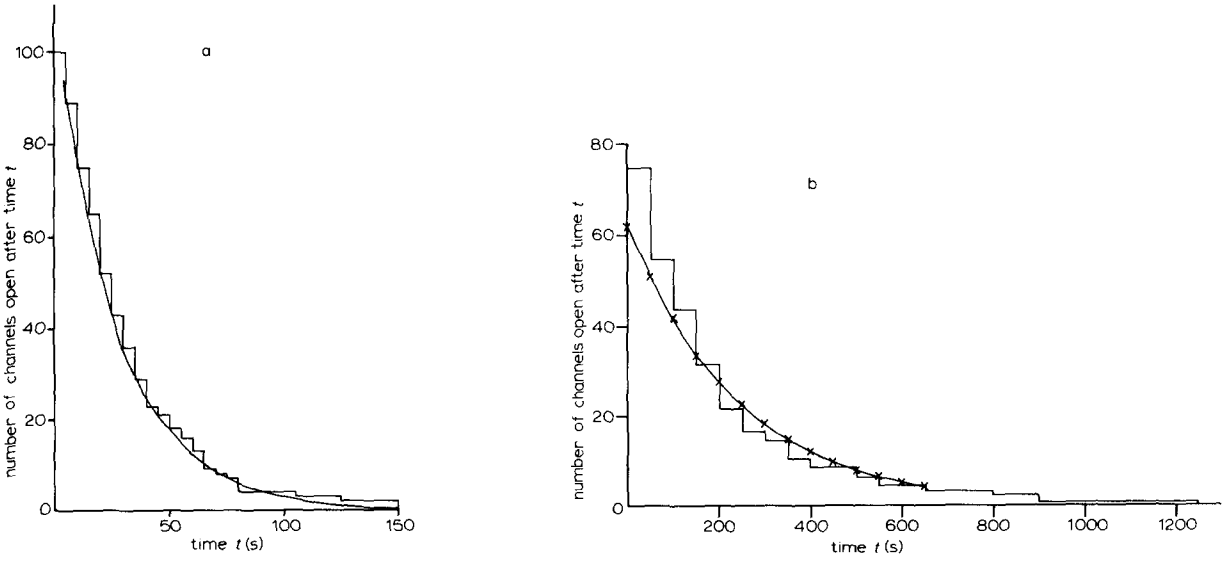

Fig. 1. Cumulative frequency distributions of single channel lifetimes for gramicidin in (a) monoolein-squalene and (b) monopalmitolein-squalene bilayers. The histogram shows the experimental data and the smooth curve shows the fit of that data to an equation of the form $y = a\,exp(-\lambda t)$ where y is the number of channels open after t s. The coefficient of determination, r^2, is 0.99 and 0.98 in graphs (a) and (b), respectively.

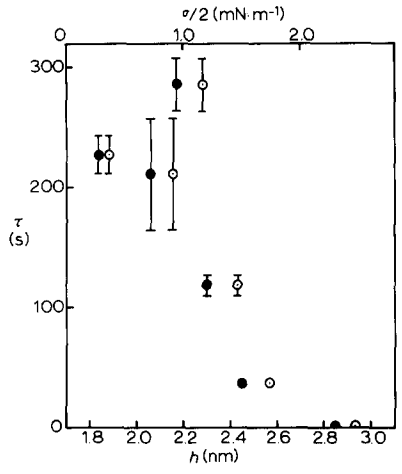

Fig. 2. Mean channel lifetime (τ) as a function of thickness (h) of the hydrocarbon region of monoacylglycerol-squalene bilayers (●) and the half-bilayer tension ($\sigma/2$) (⊙). The error bars represent the S.E. of the means of groups of approx. 20 channel lifetimes from each experiment. The total number of channels measured in each instance is given in Table I.

tween the mean channel lifetime and the thickness and tension, respectively, for bilayers where $h \approx 2.2$ nm raises the question of the significance of this thickness. The total length of the gramicidin channel was originally estimated to be 2.5–3.0 nm [15]. A subsequent study by X-ray techniques of gramicidin channels containing two cations (the relevant situation for the present systems [12]) indicated a maximum length of 2.6 nm [16]. The length of the lipophilic exterior of the channel is likely therefore to be somewhat less than 2.6 nm. In view of this, it seems reasonable to assume that the change in the behaviour of the mean channel lifetime at $h \approx 2.2$ nm arises because the hydrocarbon thickness of the bilayer becomes smaller than the lipophilic length of the channel. The relative constancy of the mean channel lifetime in membranes of thickness less than the lipophilic length of the channel might be accounted for if, in these circumstances, the channel were tilted and no longer perpendicular to the plane of the bilayer though with its ends still flush with the surfaces.

The obvious conclusion from the foregoing discussion is that the membrane thickness plays some role in determining the mean channel lifetimes. The evidence that the membrane surface tension is important is, however, also strong, and possibly stronger. The remainder of this paper will be devoted to describing a simple model for the dissociation of the gramicidin channel in which both the bilayer thickness and the tension are significant.

It is assumed that the reaction which causes loss of conduction is unimolecular and that its rate constant k_d is given by a form of the transition state theory of reaction kinetics [17], i.e.:

$$k_d = \nu \exp\left(-\frac{\Delta G^*}{kT}\right) \tag{4}$$

where ν is a frequency factor and ΔG^* is the free energy of activation. ΔG^* is considered to have two major components: one, ΔG_0^* which is the activation free energy in membranes of thickness equal to the channel length, and the other, ΔG_h^*, which constitutes a correction to ΔG_0^* when the membrane thickness is greater than the channel length. Eqn. 4 can thus be rewritten:

$$k_d = \nu \exp\left(-\frac{\Delta G_0^*}{kT}\right)\exp\left(-\frac{\Delta G_h^*}{kT}\right) \tag{5}$$

In membranes of thickness h, when h is greater than the length, h_0, of the channel, it is supposed that the closure of the conducting structure is aided by the stress of the membrane interfacial tension resolved along the axis of the channel. The precise reason that conductivity is lost is not fully understood. The most likely mechanism at present is the dissociation of the head-to-head linked dimer [15,18–20] although other views have been advanced [21]. In any event, it seems reasonable to suppose that the transition state complex is formed by an axial movement apart of the two molecules forming the channel. If this distance is denoted z, and if the interfacial tension of the membrane is constant, then

$$\Delta G_h^* = zl(\sigma/2)\cos\theta \tag{6}$$

where l is the length of the perimeter of the channel and θ is the angle between the membrane surface at the contact line and the channel axis. The perimeter length, l, is a constant (the value of which is discussed below) for all systems studied here and θ is envisaged as some function of (h –

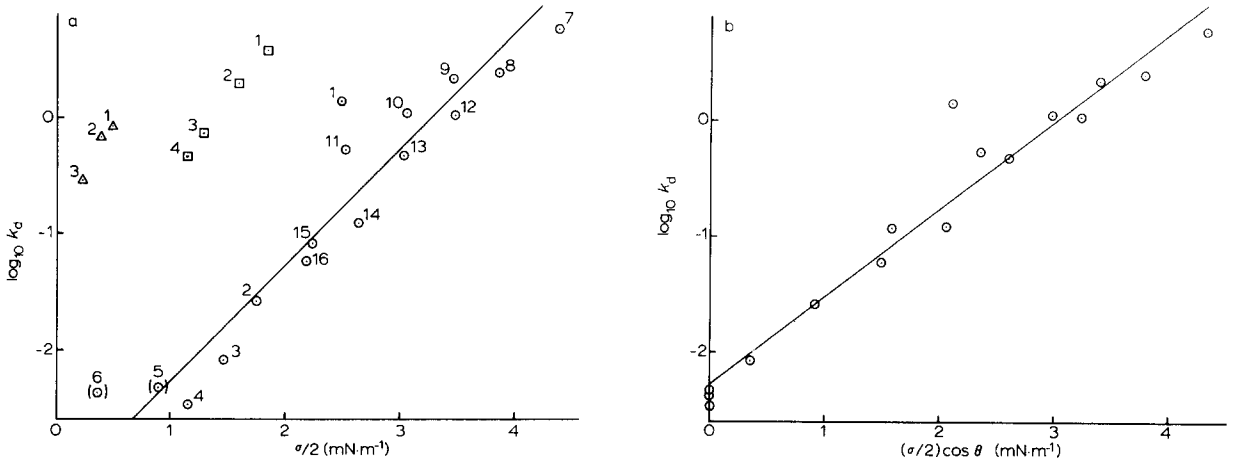

Fig. 3. The dependence of $\log_{10} k_d$ where k_d is the rate constant for the loss of conduction in the gramicidin channel, on (a) the half-bilayer tension ($\sigma/2$) and (b) $(\sigma/2)\cos\theta$ (see Eqn. 7). (a) Points (⊙) 1–6 are, in descending order, for the systems described in Table I; 7–11 are for monoolein + *n*-octane, *n*-decane, *n*-dodecane, *n*-tetradecane and *n*-hexadecane (the tensions for these systems are taken from Ref. 14); 12–16 are for monoolein bilayers formed from mixtures of squalene and *n*-decane, the mole fractions of the latter being 0.8, 0.6, 0.4, 0.1 and zero, respectively (the tensions for these systems are from Ref. 10). The monoacylglycerol and electrolyte concentrations for the tension measurements in points 7–16 are not precisely the same as for the channel lifetime determinations but from the known dependences of the tensions on these quantities the maximum discrepancy is likely to be not greater than 0.1 mN·m^{-1}. Points (△ and □) are from Neher and Eibl [8], △ (1–3) a phosphatidylcholine analogue in *n*-octane, *n*-decane and *n*-dodecane, respectively; □ (1–4), *N*,*N*-dimethylethanolamine analogue in *n*-octane, *n*-decane, *n*-dodecane and *n*-tetradecane, respectively. A regression line through the monoacylglycerol points is shown ($r^2 = 0.863$). The points in parentheses are for bilayers in which $h < 2.2$ nm and have been omitted from the calculation of the line. (b) $\text{Log}_{10} k_d$ (as in a) vs. $(\sigma/2)\cos\theta$ for the monoacylglycerol systems of Fig. 3a. $\text{Cos}\theta$ was calculated as described in the text. For membranes in which $h \leq 2.2$ nm, $\cos\theta$, and hence $(\sigma/2)\cos\theta$, have been assumed to be zero. The regression line is shown ($r^2 = 0.947$).

h_0); e.g., when the membrane thickness is equal to the channel length ($h - h_0 = 0$) $\theta = 90°$, $\Delta G_h^* = 0$ and $k_d = k_{d,0}$ but, as $(h - h_0)$ increases, θ decreases and the effective tension, $(\sigma/2)\cos\theta$, becomes progressively larger. Eqns. 5 and 6 can be combined and written in a logarithmic form as

$$\ln k_d = \ln k_{d,0} + \frac{zl(\sigma/2)\cos\theta}{kT} \qquad (7)$$

As the angle θ cannot be measured, a first attempt to test Eqn. 7 was made by putting $\theta = 0$. A plot of $\ln k_d$ against $\sigma/2$ is shown in Fig. 3a. For systems in which $h \geqslant h_0$ the result is fairly linear, although there is some scatter. The assumption of $\theta = 0$ is essentially that used in an earlier paper [6] where the deformation of the lipid in a thick membrane was approximated by a square-well profile. If the radius, r, of this square well is taken to be 0.8 nm [6], $l(= 2\pi r)$ becomes approx. 5 nm and, from the slope of the plot, $z \approx 1.8$ nm.

The introduction of a finite θ on the lines discussed above should have little effect on the points for the thicker membranes, but, as the membrane thickness approaches the length of the channel, the points will be moved along the tension axis towards zero. Any quantitative treatment of θ is bound to be somewhat unsatisfactory, if only because it is necessary to use macroscopic concepts in a system of molecular dimensions. It is also unfortunate that the problem is not trivial mathematically. Thus in the axially symmetric menisci assumed to be formed in the thicker membranes the form of the surface contours can only be obtained by solving the appropriate form of the Laplace equation (see, for example, Ref. 22). Attention to detail at this level is, however, scarcely justified by the arbitrary nature of some of the other assumptions and, for present purposes, a simple empirical approach has been used to demonstrate the effect of the contact angle.

The contour of the lipid surface in any plane

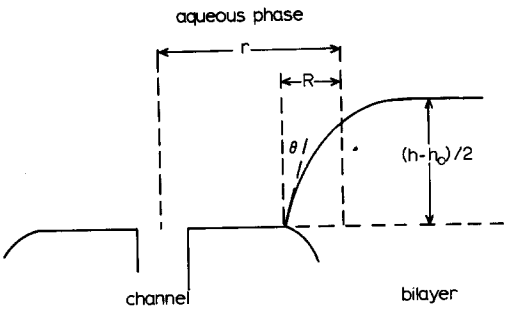

Fig. 4. A schematic illustration of the end of the gramicidin channel in a lipid bilayer, showing also the assumed contact angle, θ. r is the radius of the equivalent square well in the lipid (see Ref. 6) and R is defined by Eqn. 8.

containing the axis of the channel (Fig. 4) has been assumed to be represented by the equation

$$y = \tfrac{1}{2}(h - h_0)(1 - e^{-x/R}) \tag{8}$$

where y is distance parallel to the channel axis and x is distance parallel to the plane of the bilayer. The origin is taken to be the three-phase contact line at the end of the channel. The value of the radius parameter, R, has been chosen to be consistent with the previous estimate of approx. 0.8 nm for the radius, r, of the equivalent square well in the lipid [6]. If the channel radius is taken as 0.2 nm, then $R \lesssim 0.6$ nm. Cosθ can be deduced from the slope of Eqn. 8 at the origin and is

$$\cos\theta = \frac{h - h_0}{\left[(h - h_0)^2 + 4R^2\right]^{1/2}} \tag{9}$$

A plot of ln k_d against $(\sigma/2)\cos\theta$, for $h_0 = 2.2$ nm and $R = 0.2$ nm, is shown in Fig. 3b. The scatter in the points is somewhat reduced and the slope of the line is, as expected, smaller. The value of z calculated from the slope is, however, much the same (approx. 1.8 nm) as for $\theta = 0$ in Fig. 3a. This follows from the assumption that the distance of the origin of Eqn. 8 from the channel axis is $(r - R)$ (Fig. 4). Thus, if r is considered as fixed by earlier studies [6], the introduction of a finite R necessarily reduces l from its former value of $2\pi r$ to $2\pi(r - R)$. The angle θ has been estimated in various other ways and with a number of different assumptions, but the scatter in the points, the linearity of the plot and the value of z are not greatly affected.

The Arrhenius activation energy for the loss of single channel conductance in monoolein-decane bilayers has been given as no greater than 79.4 kJ $\cdot$ mol^{-1} [1]. If the enthalpy of activation is calculated from this (see, for example, Ref. 17), it can be deduced from Eqn. [4] by putting $\nu = kT/h$ that the entropy of activation, ΔS^*, is approx. 23 J $\cdot$ mol^{-1} $\cdot$ K^{-1}. This value is very similar to that found in other unimolecular reactions [17].

Before considering the significance of the above analysis it is of interest to compare the results with those of earlier investigations. At first sight, the data of Rudnev et al. [9] should be comparable, particularly as these authors found the plot of ln k_d (they actually plotted ln τ) against membrane tension to be linear. Unfortunately, the membrane tensions reported by these authors show serious discrepancies with those in this and previous papers from this laboratory and with the results of Crilly and Earnshaw [23] who, in turn, claim agreement with White (see Ref. 23). Owing, presumably, to these discrepancies, the value of z calculated from the data of Ref. 9 is 1.08 nm, compared with the present 1.8 nm. The results of Neher and Eibl [8] are for phospholipid analogues. Where appropriate, their data have been plotted in Fig. 3a. These suggest that changes in the nature of the lipid polar group lead to a displacement of the ln k_d vs. $\sigma/2$ plot, but not necessarily to a significant change in its slope. In other words, the parameter z remains roughly the same. From the Arrhenius activation energy for channel dissociation in phosphatidylethanolamine analogue membranes [8], ΔS^* is found to be approx. 27 J $\cdot$ mol^{-1} $\cdot$ K^{-1}, again a reasonable value.

The above analysis suggests that for membranes formed from a given type of lipid (e.g. monoacylglycerol, phosphatidylcholine, etc.) the surface tension and, to a lesser degree, the bilayer thickness are the most important factors determining the dissociation rate constant. There is no indication that fluidity is significant, although, without some quantitative measure of this property, it obviously cannot be completely eliminated from consideration. The surprising feature of the analysis is the value of the parameter z (approx. 1.8 nm), which is much larger than would be expected for

the rupture of hydrogen bonds between the heads of the two polypeptide molecules in the middle of the bilayer. Smaller values of z would result if l or σ were assumed to be larger. However, l can scarcely be larger for purely geometrical reasons and the value of the tension σ which has been used was found to account satisfactorily for the effects of thickness and tension on the equilibrium constant for the monomer-dimer reaction [6]. There is thus no obvious argument for increasing either quantity, although it must be remembered that the application of the thermodynamic quantity σ to a 'small' assembly might incur significant errors. If $z \approx 1.8$ nm, it is necessary to consider the possibility of a transition state in which the two gramicidin molecules are displaced in some way relative to each other by this distance. It is conceivable that there is some partially unwound form of the two helices which satisfies this requirement. There is, however, another interesting possibility. When the hydrogen bonds which link the molecules break and the molecules move apart, it may be that the space between them is transiently filled with water molecules. These molecules could accumulate by movement down the central channel and would be likely to do so partly to fill the incipient hole, and partly because the ends of the gramicidin molecules would be strongly hydrophilic. If a meniscus of water were to form in this way between the two gramicidin molecules, this aqueous bridge could be stretched to a considerable distance before it ruptured. Such a mechanism would clearly help to account for the large value of z. The activation energy for such a process would also not necessarily be inconsistent with that observed. It can readily be calculated that the work done in forming the lipid/water interface of the bridge would be either larger or smaller than the experimental activation energy according to which extreme values of the possible interfacial areas and tensions are assumed. Finally, it is very likely that the activation energy would depend on the nature of the lipid in the membrane. This is certainly true for the two systems monoolein-decane and dioleylphosphatidylcholine-decane so far examined [6,24], although of course such a difference could easily have arisen for other reasons.

Perhaps the most unsatisfactory feature of the model is that it predicts a transition state which is somewhat longer than the thickness of several of the membranes used. This does not present a serious conceptual difficulty because, as mentioned above, the channel can presumably tilt out of the perpendicular to the bilayer surfaces without, apparently, large changes in k_d. It does, however, complicate the quantitative assessment of the contact angle, θ.

Acknowledgements

J.R.E. acknowledges support from the Medical Research Council and was the Oliver Gatty Student of Cambridge University. D.N. is an Oppenheimer Research Fellow of Cambridge University. J.P.D. was supported by a N.I.H. Fellowship.

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