Biochimica et Biophysica Acta, 732 (1983) 133–145
Elsevier

BBA 71723

BILAYER LIPID MEMBRANE PERMEATION AND RUPTURE DUE TO HOLE FORMATION

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(Received December 8th, 1982)
(Revised manuscript received April 6th, 1983)

Key words: Nucleation; Hole formation; Membrane permeation; (Bilayer)

A theory is developed for the permeation and rupture of bilayer lipid membranes due to fluctuation formation of holes (or pores) in them. The two monolayers of the bilayer lipid membrane are considered as mutually adsorbed on each other and the bilayer lipid membrane equilibrium is described by an adsorption isotherm in mean field approximation. The theory of nucleation is used for determination of the work for hole formation and the hole equilibrium size distribution as functions of the concentration C of monomer lipid in the solution. The bilayer lipid membrane permeation and rupture are analyzed from a unified point of view and expressions are derived for the dependence of the bilayer lipid membrane diffusion permeability coefficient and lifetime on C. The effect of foreign bodies (e.g., proteins) on the bilayer lipid membrane permeation and rupture is considered and a possible experimental application of the theory is discussed. The results obtained are directly applicable to dense monolayer films on liquid surfaces.

1. Introduction

It is generally agreed that passive transport through biomembranes and bilayer lipid membranes is in many cases controlled by existing small holes (or pores) in them [1,2]. Despite the fact that this problem has been dealt with in a number of papers, the physical reasons for the formation of holes in a bilayer lipid membrane are not yet entirely understood. An adopted point of view is, for instance, that the holes in a bilayer lipid membrane are of fluctuation character. Wiegel [3] has recently proposed a statistical model of a bilayer lipid membrane leading to fluctuation appearance of micropores, and Taupin et al. [4], Pastushenko et al. [5] and Chizmadzhev et al. [6] have considered the formation of holes on the basis of the fluctuation theory of nucleation.

Similar concepts for the existence of holes have also been used for describing the rupture of bilayer (Newtonian) black foam films [7–12], the evaporation of liquids through adsorbed monolayers [13–15], as well as the thermodynamic equilibrium of monolayers on liquid surfaces [16].

The present work is devoted to a unified analysis of the bilayer lipid membrane permeation and rupture when these are due to flucuation formation of holes (or pores) in the membrane. The aim is to obtain expressions for the dependence of the bilayer lipid membrane diffusion permeability coefficient and mean lifetime on the bulk concentration of dissolved monomer lipid which can be experimentally controlled. The analysis largely relies on a previous paper of ours [12] treating the rupture of bilayer black foam films.

2. The model

In accordance with the current bilayer lipid membrane models [1,2] we consider the membrane as composed of two lipid monolayers which are in contact with an ambient liquid (e.g., an aqueous

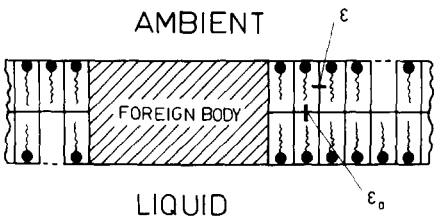

Fig. 1. Schematic representation of a bilayer lipid membrane (cross-section).

solution). Fig. 1 shows schematically a bilayer lipid membrane, the lipid polar heads (the circles) being oriented outwards. The hatched area represents a foreign "body" (e.g., a protein) in the membrane. Thus, although as a whole the membrane is modified by the presence of foreign bodies, on a smaller scale there exist regions in which it remains unmodified.

As in a previous paper for bilayer foam films [12], we now introduce the basic idea that the bilayer lipid membrane can be described by regarding its two monolayers as mutually adsorbed on each other. Physically, this approach is similar to that of Suezaki [17,18]. We also assume that the lipid molecules within each monolayer are packed in a two-dimensional lattice. In a homogeneous (or unmodified) region the number of lateral and vertical nearest neighbours of a given lipid molecule is z and z_o, respectively, and ϵ and ϵ_o (positive for attraction, negative for repulsion) are the lateral and the vertical bond energies of the molecule (Fig. 1). The system is held at a constant absolute temperature T and as T is greater than zero, vacancies of lipid molecules (shown as empty boxes in Fig. 1) exist inevitably in the two monolayers of the membrane, giving rise to the formation of clusters of vacancies, i.e., holes. At higher values of the vacancy density n_1 (cm^{-2}) larger holes form in the bilayer lipid membrane, making it permeable. At still higher n_1 values some of the holes become capable of irreversible lateral growth and cause the rupture of the membrane. Clearly, this process can be regarded as a two-dimensional analogue for instance of the formation and growth of voids from vacancies in solids [19,20] and can be analysed with the aid of the fluctuation theory of new phase formation [19,21].

3. Bilayer lipid membrane thermodynamic equilibrium

A bilayer lipid membrane can be in thermodynamic equilibrium only when the chemical potential μ_{BLM} of a lipid molecule in the membrane is the same as the chemical potential μ_s that the molecule would have if placed as a monomer in the bulk of the solution in contact with the membrane, i.e., when

$$\mu_{BLM} = \mu_s \tag{1}$$

In principle, the membrane can only be normally and/or laterally in contact with other phases. The 'normal' phase N (Fig. 2) is, in fact, the ambient liquid in Fig. 1 into which the bilayer lipid membrane is immersed. The 'lateral' phase L (also shown in Fig. 2) can be examplified by the meniscus with which the bilayer lipid membrane is in contact when obtained in a frame or tube (cf. Refs. 1 and 22). In either of these phases dissolved single lipid molecules of bulk concentration C (mol/l) and activity a must be present for the whole system to be in equilibrium. This is so, because μ_s in Eqn. 1 can be expressed as [23,24]

$$\mu_s = \mu_m + kT \ln(a/a_{CMC}) \tag{2}$$

or, approximately,

$$\mu_s = \mu_m + kT \ln(C/C_m). \tag{3}$$

Here k is the Boltzmann constant, a_{CMC} is the bulk activity of the monomer lipid dissolved in the solution at the critical micelle concentration (CMC) whose value is C_m (mol/l), and μ_m is the chemical potential of a lipid molecule in a micelle. At the critical micelle concentration μ_m equals the chemical potential of a dissolved monomer lipid, since

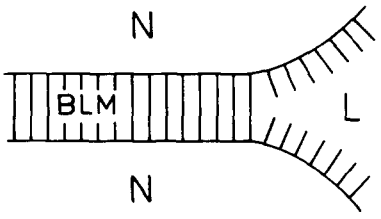

Fig. 2. Normal (N) and lateral (L) phases in contact with a bilayer lipid membrane (BLM).

then the micelles and the monomer lipid in the solution are in equilibrium.

It must be pointed out that C_m and a_{CMC} are quantities which should be regarded as having the more general meaning of saturation concentration and activity of the dissolved lipid monomer when the solution is saturated. This is so because at saturation the bulk (i.e., the undissolved) lipid phase may exist in the solution, for instance in the form of droplets or solid particles rather than micelles. The theory to follow is formulated in terms of C_m or a_{CMC}, but it should always be kept in mind that these have the above more general physical meaning. We note as well that, since for both the N- and L-phases μ_s is given by Eqn. 2 or 3, in the following a or C refer to either of these phases and all further results are equally applicable to both of them. Also, it must be emphasized that, although henceforth concentrations will be used instead of activities, all results obtained below remain valid for arbitrarily concentrated solutions if everywhere in them C is merely substituted with a.

The next step to describe the thermodynamic state of the bilayer lipid membrane with the help of Eqn. 1 is to express μ_{BLM} as a function of T and the density N_1 (cm^{-2}) of lipid molecules in one of the two membrane monolayers. We shall only do that for those bilayer lipid membrane regions which are homogeneous (i.e., free of foreign bodies), but even then finding μ_{BLM} is not a simple matter. Considering, however, each of the membrane monolayers as adsorbed on the other, in conformity with our lattice model of bilayer lipid membrane (Fig. 1), we can express μ_{BLM} by the following approximate relation [12,24].

$$\mu_{BLM} = \mu^* - z\epsilon\theta - z_o\epsilon_o\theta + kT\ln[\theta/(1-\theta)]. \quad (4)$$

Here $\theta = N_1/N_m$ is the coverage of the membrane monolayer by lipid molecules, $N_m = 1/A_o$ is the maximum density of lipid molecules in the monolayer, A_o (cm^2) is the effective area of a lipid molecule in a completely filled monolayer, and μ^* is a reference chemical potential. The second and the third terms on the right in Eqn. 4 account for the lateral and the vertical components, respectively, of the internal energy of a lipid molecule in the bilayer lipid membrane, and the fourth one is the configurational entropy contribution to μ_{BLM}. Physically, Eqn. 4 gives μ_{BLM} in the so-called mean field (or Bragg-Williams) approximation in the case of lattice gas monolayer adsorption [23].

Thus, combining Eqns. 1, 3 and 4, we find that in the scope of validity of the above expressions for μ_s and μ_{BLM} the bilayer lipid membrane thermodynamic state is characterized by the equation

$$C/C_o = [\theta/(1-\theta)]\exp(-w\theta/kT) \quad (5)$$

in which

$$w = z\epsilon + z_o\epsilon_o$$

$$C_o = C_m\exp[(\mu^* - \mu_m)/kT].$$

If E_{dis} is the activation energy for micelle dissolution, after Cases and Mutaftschiev [24], approximately, $\mu_m = \mu^* - E_{dis}$, so that C_o (mol/l) is related to the experimentally accessible C_m and E_{dis} by the expression

$$C_o = C_m\exp(E_{dis}/kT). \quad (6)$$

We can now easily determine the C, T-dependence of the density n_1 of vacancies that exist within each of the two bilayer lipid membrane monolayers. Since by definition $N_1 + n_1 = N_m$, Eqn. 5 transforms into

$$C/C_o = [(N_m - n_1)/n_1]\exp[-w(N_m - n_1)/N_m kT]. \quad (7)$$

In physical terms, this equation represents the adsorption isotherm (in mean field approximation) of the two-dimensional 'gas' of vacancies existing within each of the two membrane monolayers. The dependence of n_1 on C, calculated from Eqn. 7 with $w/kT = 9$, is shown in Fig. 3. As seen, the isotherm displays Van der Waals' loops which show the possibility of a first-order phase transition of the bilayer lipid membrane "gas" phase of vacancies into a 'condensed' phase of vacancies, i.e., a ruptured membrane. According to Eqn. 7, this two-dimensional phase transition is possible only when $w/kT > 4$. The value of 4 is due to the mean field approximation; the exact value is known [23] to be about twice as high.

Eqn. 7 can be used for determination of the bulk concentration C_e of dissolved monomer lipid at which the diluted and the condensed phases of vacancies are in thermodynamic equilibrium. With the aid of the Maxwell's rule for equality of the

hatched areas in Fig. 3 we find that $C = C_e$ at $n_1 = 0.5\ N_m$ and hence

$$C_e = C_o \exp(-w/2kT). \tag{8}$$

The above considerations show that at a given value of C a definite density n_1 of vacancies exists within each of the bilayer lipid membrane monolayers. In particular, at the equilibrium concentration C_e the phase equilibrium density $n_{1,e}$ sets up in the membrane (Fig. 3). This is the density at which the real diluted and the hypothetical condensed two-dimensional phases of vacancies would coexist if brought into contact with each other. As in practice $n_1 \ll N_m$, it follows from Eqns. 7 and 8 that to a good approximation n_1 and $n_{1,e}$ are given by the expressions

$$n_1 = N_m(C_o/C) \exp(-w/kT) \tag{9}$$

$$n_{1,e} = N_m \exp(-w/2kT). \tag{10}$$

Knowing n_1 and $n_{1,e}$, we can now determine the important quantity $\Delta\mu$ (erg per molecule) which is the thermodynamic driving force of the process of hole formation. As known [19,21], $\Delta\mu$ is defined by

$$\Delta\mu = kT \ln(n_1/n_{1,e}) \tag{11}$$

which owing to Eqns. 9 and 10 takes the form

$$\Delta\mu = kT \ln(C_e/C) \tag{12}$$

showing that $\Delta\mu$ can be experimentally controlled by varying the bulk concentration C of dissolved monomer lipid.

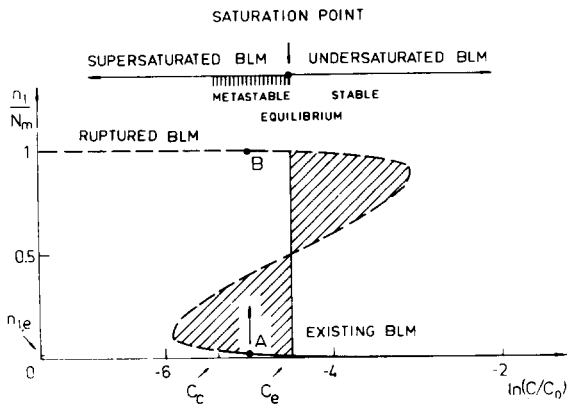

Fig. 3. Dependence of n_1 on C according to Eqn. 7 with $w/kT = 9$.

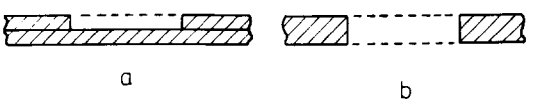

Fig. 4. Holes of monolayer (a) and bilayer (b) height (cross-section).

A point that must now be made is that in the above expressions the sign of $\Delta\mu$ characterizes the bilayer lipid membrane thermodynamic state. At $\Delta\mu < 0$ or $\Delta\mu = 0$ the membrane is undersaturated or saturated, respectively, and is in stable thermodynamic equilibrium with respect to the two-dimensional phase transition (Fig. 3). Then $C > C_e$ or $C = C_e$, respectively, and the existing vacancies cluster into holes which make the membrane permeable, but do not threaten its life because of their being incapable of spontaneous overgrowth. When $\Delta\mu > 0$, however, i.e., for $C < C_e$, the membrane is supersaturated and can only exist a certain finite time in metastable equilibrium (Fig. 3). The stable phase equilibrium is then restored via 'condensation' of the existing vacancies into holes which, when large enough to grow irreversibly, cause the rupture of the membrane. As indicated by the arrow in Fig. 3, this process would correspond to a transition from point A (diluted phase of vacancies, i.e. existing bilayer lipid membrane) to point B (condensed phase of vacancies, i.e. ruptured bilayer lipid membrane). The dashed portion of the isotherm in Fig. 3 represents physically unrealizable densities of vacancies in the membrane. The metastable region (Fig. 3) is limited between C_e and a critical concentration C_c which can only be determined on the basis of kinetic considerations (see Section 6).

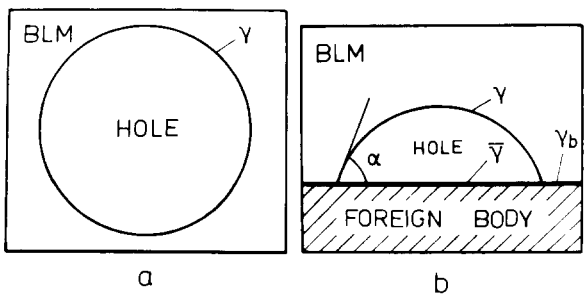

Fig. 5. Homogeneous (a) and heterogeneous (b) hole formation (top view).

4. Hole size distribution and work for hole formation

If bilayer lipid membrane permeation and/or rupture are due to existing holes in the membrane, the hole equilibrium size distribution n_i (cm^{-2}) should play an important role in their theoretical description. The quantity n_i is the density of holes constituted of i vacancies and statistical considerations [25] have shown it to be given by

$$n_i = N_o \exp(-\Delta G_i/kT) \tag{13}$$

provided $n_1 \ll N_o$. Here N_o (cm^{-2}) is the density of available lattice sites on which a vacancy can be formed, and ΔG_i is the free energy change associated with the formation of a hole of size i. Eqn. 13 shows that finding n_i actually reduces to finding ΔG_i, which can also be called work for hole formation.

As in the case of Newton black foam films [10,12], we shall now determine ΔG_i by using results known from the theory of nucleation [25]. In principle, holes of monolayer (Fig. 4a) and/or bilayer (Fig. 4b) height can form in a bilayer lipid membrane. Also, their formation can be homogeneous or heterogeneous in the sense that the hole is, respectively, either surrounded by lipid molecules only (Fig. 5a) or in contact with a foreign 'body', e.g., a protein (Fig. 5b). The latter seems quite possible, because the protein size is typically tens of times greater than that of a lipid vacancy.

In all cases of hole formation ΔG_i can be represented as ($i \geq 1$) [25]

$$\Delta G_i = -i\Delta\mu + P_i \tag{14}$$

where $\Delta\mu$ is defined by Eqn. 12 in which C_e is given by Eqn. 8 for monolayer holes and by the equation

$$C_e = C_o \exp(-z\epsilon/2kT) \tag{15}$$

for bilayer holes. The first term in Eqn. 14 is the energy gain (at $\Delta\mu > 0$) due to the clustering of i vacancies into a hole, and P_i is the energy expended on the creation of the two-dimensional hole/bilayer lipid membrane interface. For large enough holes P_i can, therefore, be approximated by the hole peripheral free energy which, as shown in Appendix A and Ref. 12, is of the form

$$P_i = (4\pi A_{ef})^{1/2} f^{1/2}(\alpha)\gamma i^{1/2}. \tag{16}$$

Here $A_{ef} = A_o$ or $A_{ef} = A_o/2$ is the effective area of a vacancy in a mono- or bilayer hole, respectively, γ (erg/cm) is the hole specific edge free energy, and α is the two-dimensional wetting angle (Fig. 5b), its relation with γ and other specific edge free energies being given by Eqn. 47.

The dependence of the numerical factor f on α is shown in Fig. 6. Analytically, it is of the form (see Appendix A)

$$f(\alpha) = (\alpha - \sin\alpha\cos\alpha)/\pi \tag{17}$$

so that with α varying from 0 (complete wetting) to π (no wetting) f changes from 0 to 1, respectively. No wetting corresponds energetically to homogeneous hole formation and, indeed, at $f = 1$ Eqn. 16 coincides with the known formulae for this case [12]. Half-wetting occurs at $\alpha = \pi/2$ and then $f = 0.5$. Thus, physically, f appears as a parameter characterizing the activity of the foreign bodies with respect to hole formation. This is so,

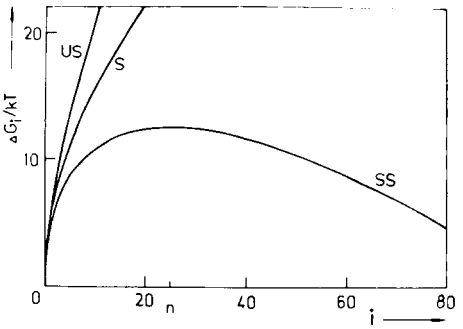

Fig. 7. Work for hole formation in dependence on the hole size for undersaturated (US), saturated (S) and supersaturated (SS) bilayer lipid membranes.

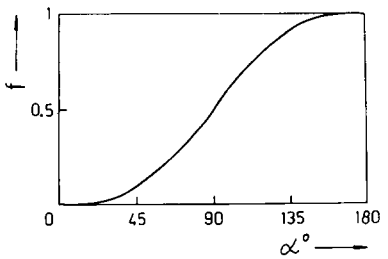

Fig. 6. The activity factor as a function of the wetting angle.

for in view of Eqns. 14 and 16 better wetting, i.e., lower f-values, result in lower work for hole formation. The holes in a bilayer lipid membrane will, therefore, be more easily formed in contact with those foreign bodies that have a lower activity factor f. If in the membrane only foreign bodies with $f \approx 1$ are present, they could hardly contribute to the background homogeneous hole formation.

We must now note that Eqn. 16 gives P_i in macroscopic (called also classical) approximation. Indeed, it is valid only for holes of large enough size i, because only then have these well-defined peripheries to which adequate size-independent γ and α values can be ascribed. The difficulty in dealing with the smallest holes can be overcome by using the microscopic (or atomistic) approach of Walton [21,26]. As shown by Sigsbee [25], for all $i \geqslant 1$

$$P_i = -ikT \ln(n_{1,e}/N_m) - E_i \tag{18}$$

where $E_i > 0$ is the energy gained on assembling i single vacancies into an i-sized hole. This is the general formula for P_i, but the problem with using it is that the i-dependence of E_i is not explicitly known. This dependence can only be found by model considerations involving the hole configuration and that is why Eqn. 18 applies to all cases of hole formation. In the heterogeneous case, for example, E_i should also include the binding energy of the hole to the active foreign body.

Fig. 7 gives an idea of the dependence of ΔG_i on i in a supersaturated (curve SS), saturated (curve S) and undersaturated (curve US) system. The curves are drawn according to Eqns. 14 and 16 with $b = 5$ and $s = 0.5$ (curve SS), $s = u = 0$ (curve S) and $u = 0.5$ (curve US). The dimensionless specific edge free energy b, supersaturation $s \geqslant 0$ and undersaturation $u \geqslant 0$ are defined by

$$b = (4\pi A_{ef} f)^{1/2} \gamma / kT \tag{19}$$

$$s = -u = \Delta\mu / kT = \ln(C_e / C) \tag{20}$$

As seen in Fig. 7, for an undersaturated or saturated bilayer lipid membrane ΔG_i is an increasing function of i. Large holes are, therefore, unlikely to form and the membrane is in stable thermodynamic equilibrium. For a supersaturated bilayer lipid membrane, however, ΔG_i reaches a maximum of value ΔG_n, known as nucleation work, at the so-called nucleus (or critical) size n. Because of ΔG_i decreasing for $i > n$, any hole larger than the nucleus is capable of spontaneous growth and the membrane can then exist only in temporary, metastable equilibrium. Using Eqns. 14 and 16 and the condition for maximum results in Ref. 12

$$n = b^2/4s^2, \tag{21}$$

$$\Delta G_n = kTb^2/4s = kTsn. \tag{22}$$

Going back to Eqn. 13, we see that in it only N_o remains to be determined. For homogeneous hole formation N_o is merely N_m, i.e.

$$N_o = N_m. \tag{23}$$

For heterogeneous hole formation due to equally active foreign bodies N_o can be approximated by

$$N_o = Ml/d \tag{24}$$

where d (cm) is the diameter of a lipid molecule, l (cm) is the average length of the foreign body periphery effective for hole formation, and M (cm^{-2}) is the density of foreign bodies in the membrane. Fig. 8 represents n_i in dependence of i calculated from Eqns. 13, 14 and 16 with $b = 5$ and $s = 0.5$ (curve SS), $s = u = 0$ (curve S) and $u = 0.5$ (curve US). As seen, when a bilayer lipid membrane is undersaturated or saturated, n_i decreases with increasing i and that is why the membrane is stable. For a supersaturated bilayer lipid membrane, however, stable equilibrium is impossible, because n_i increases with i when this is larger than the nucleus size n. In this case n_i is actually replaced by the steady-state hole size distribution which is again a decreasing function of i [21].

5. Permeability of a bilayer lipid membrane with holes

Let j ($cm^{-2} \cdot s^{-1}$) be the density of the flux of species that translocate across a bilayer lipid membrane and let S (cm^2) be the total membrane area. The total flux F of species flowing per second through the membrane can then be expressed as $F = pjS$ where the numerical factor $p \leqslant 1$ is defined by

$$p = S_a/S, \tag{25}$$

S_a (cm^2) being only that bilayer lipid membrane area which is accessible for permeation.

If permeant transfer across the bilayer lipid membrane is due to passive diffusion, j is known to be [1]

$$j = -D\Delta C_p/\Delta x$$

where D (cm^2/s) is the permeant diffusion coefficient, Δx (cm) is the bilayer lipid membrane thickness, and ΔC_p (mol/l) is the difference in permeant concentration on each side of the membrane. We thus arrive at the familiar expression [1]

$$F = -P_d S \Delta C_p$$

where P_d (cm/s), defined by

$$P_d = pD/\Delta x, \tag{26}$$

is the bilayer lipid membrane diffusion permeability coefficient. As seen, p plays an important role in the determination of P_d, since it accounts for the fact that only a part of the total bilayer lipid membrane area is actually permeating. It must be emphasized that, similarly, p will also appear as a numerical factor in the osmotic permeability coefficient [1] as well as in other bilayer lipid membrane characteristics proportional to S_a.

The problem of finding P_d from Eqn. 26 is thus reduced to finding p. Clearly, p can only be obtained with the aid of a concrete model for the physical nature of the bilayer lipid membrane area accessible for permeation. We consider here the case when the permeability of a bilayer lipid membrane is due to holes (or pores) existing in it. As already noted, this is not a new idea [1,2] and it implies that the lifetime of the permeating holes is sufficiently long. To find p, which may now be termed porosity factor, we denote by i_o the size (i.e. the number of vacancies) of the smallest permeable hole. When the hole periphery hinders the permeant passage through the hole i_o is an effective size smaller than the actual size of the smallest permeable hole. Since $i\,A_{ef}$ is the area of an i-sized hole, S_a is given by

$$S_a = \sum_{i \geq i_o} (iA_{ef}) n_i S$$

and using Eqn. 25, we thus find that

$$p = A_{ef} \sum_{i=i_o}^{\infty} i n_i \tag{27}$$

for an undersaturated or saturated bilayer lipid membrane and that

$$p = A_{ef} \sum_{i=i_o}^{n} i n_i \tag{28}$$

for a supersaturated bilayer lipid membrane. In the latter case the summation cannot go to infinity, because the sum is divergent (see curve SS in Fig. 8). When the nucleus hole is of not less than about 10 vacancies, cutting off the sum at the nucleus size n practically leads to the correct result obtained by using the corresponding steady-state hole size distribution.

Thus, finding p requires summing in_i in accordance with Eqns. 27 and 28. Analytically, this cannot be done exactly, since the work ΔG_i for hole formation from Eqn. 14 and, hence, n_i from Eqn. 13 are complicated functions of i. However, we can always rewrite both Eqns. 27 and 28 in the following simple form

$$p = A_{ef} g i_o n_{i_o}, \tag{29}$$

the numerical factor g being defined in Appendix B. Mathematically, Eqn. 29 simply means that the

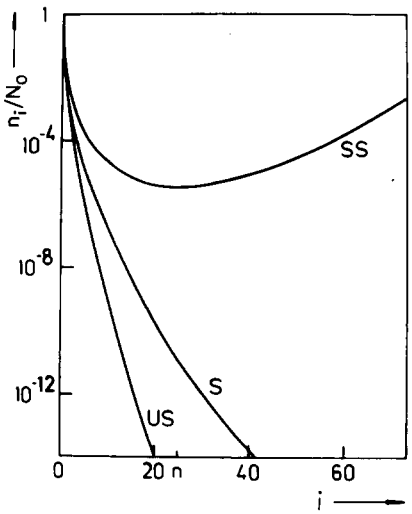

Fig. 8. Hole equilibrium size distribution for undersaturated (US), saturated (S) and supersaturated (SS) bilayer lipid membranes.

whole sum is only g-times greater than the first summand in it, and in view of the strong exponential decrease of n_i with i we can expect g to be close to unity. Indeed, the numerical results in Appendix B show that g is typically a number between 1 and 2.5.

Recalling Eqn. 13 transforms Eqn. 29 into the general formula

$$p = p_o \exp(-\Delta G_o/kT) \tag{30}$$

where $\Delta G_o = \Delta G_{i_o}$ is the work for formation of the smallest permeable hole, and p_o is given by $p_o = g i_o A_{ef} N_o$. In the case of bilayer homogeneous holes, for example,

$$p_o = g i_o/2, \tag{31}$$

as then $A_{ef} = A_o/2$ and $N_o = 1/A_o$. In view of Eqns. 14 and 20, Eqn. 30 becomes

$$p = p_o \exp(-ui_o - P_o/kT) = p_o (C_e/C)^{i_o} \exp(-P_o/kT). \tag{32}$$

Here $P_o = P_{i_o}$ is the peripheral free energy of the smallest permeable hole which, classically, is approximated by $bi_o^{1/2}\,kT$ for large enough i_o. For P_d from Eqn. 26 we thus find

$$P_d = (p_o D/\Delta x) \exp(-ui_o - P_o/kT)$$

$$= (p_o D/\Delta x)(C_e/C)^{i_o} \exp(-P_o/kT) \tag{33}$$

As seen, p and P_d decrease with increasing C; besides, the greater the size i_o of the smallest permeable hole, the stronger the decrease. This is so, because lower C leads to higher vacancy density and thereby to more and larger holes. If permeant diffusion is affected by the hole size, in Eqn. 33 D should be regarded as an effective quantity obtained by appropriate averaging of the actual $D(i)$-dependence.

In an attempt to illustrate the variation of the permeability coefficient with C, in Fig. 9 we show P_d from Eqn. 33 in dependence on u (or s). The calculation is done for bilayer holes by using $P_o/kT = bi_o^{1/2}$, with $i_o = 2$ and a number of b values (as indicated on the curves). The above value of i_o would appear if the smallest permeable hole in the membrane is a bilayer dimer. Experimental findings [1,2] and known values of the areas of lipid molecules and solvated permeant ions support such a possibility. The value of b is determined by γ and the activity factor f. Lower γ-values (i.e., smaller binding energies ϵ due to weaker lipid-lipid lateral attraction) and/or lower f-values (i.e., smaller wetting angles α resulting from stronger foreign body-vacancy attraction) lead to lower b-values and, accordingly, to higher bilayer lipid membrane permeability. In fig. 9 b is varied from 0.5 to 5, which seems to be in the range of practical interest. Indeed, with the order-of-magnitude value of 10^{-6} erg/cm for γ [27–29] and with $f = 1$ (homogeneous holes), $A_{ef} = A_o/2 = 20$ Å^2 (bilayer holes) and $T = 300$ K, from Eqn. 19 it follows that $b \approx 4$. Since according to Eqn. 31 $p_0 \approx 1$ when $i_o = 2$ and $g \approx 1$, Fig. 9 gives directly $P_d \Delta x/D$ (or p) for homogeneous bilayer lipid membrane permeation by bilayer holes, the dimer being the smallest permeable one.

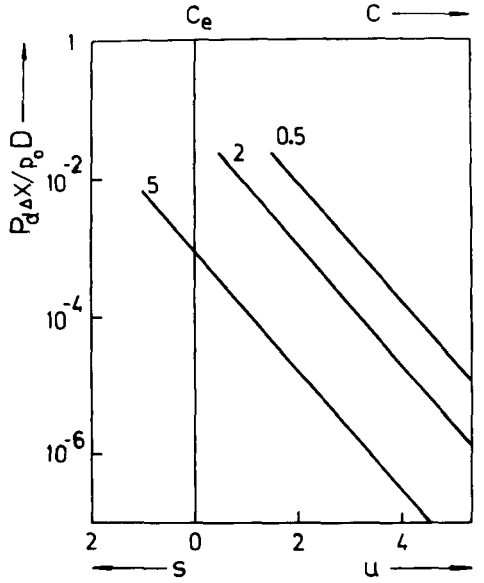

Fig. 9. Bilayer lipid membrane diffusion permeability coefficient as a function of u and s (or C) according to Eqn. 33 (b-values indicated).

Fig. 9 displays clearly the strong dependence of the membrane permeability (or porosity) on the monomer lipid concentration C. Increasing C makes the bilayer lipid membrane less and less porous until permeability is practically arrested. With decreasing C the membrane becomes increasingly permeable, but, if supersaturated, may rup-

ture by irreversible lateral growth of supernucleus holes. Only within a certain concentration range will the bilayer lipid membrane be both stable and sufficiently permeable.

6. Bilayer lipid membrane rupture determined by hole nucleation

As already pointed out, when a bilayer lipid membrane is supersaturated, it can only live a certain finite time in metastable equilibrium. To find the bilayer lipid membrane average lifetime τ (s) we shall directly use the results of Kashchiev and Exerowa [12] for rupture of bilayer foam films. It has been shown that in the case of hole stationary nucleation and fast enough lateral growth τ is of the form

$$\tau = 1/JS \tag{34}$$

where J ($cm^{-2} \cdot s^{-1}$), given by [19,21]

$$J = Z\omega N_o \exp(-\Delta G_n/kT), \tag{35}$$

is the stationary nucleation rate. In Eqn. 35 Z is a numerical factor from about 0.01 to 1, and ω (s^{-1}) is the frequency of vacancy joining to the nucleus. This quantity is expected to be proportional to n_1, i.e. to $1/C$, and can be obtained only be means of kinetic considerations concerning the concrete nucleation mechanism.

Combining Eqns. 34 and 35, we obtain the general formula

$$\tau = A \exp(\Delta G_n/kT) \tag{36}$$

where $A = 1/Z\omega N_o S$ with N_o given by Eqns. 23 and 24 for homogeneous and heterogeneous nucleation, respectively. Because of the relatively weak dependence of A on s (i.e. on C) this factor is often considered as s-independent [19,21]. In view of Eqns. 14 and 20, from Eqn. 36 we thus find that

$$\tau = A(C/C_e)^n \exp(P_n/kT). \tag{37}$$

This equation holds true down to $n = 1$, but it does not give the full dependence of τ on C, since for the nuclei of a few vacancies only the nucleus size n and peripheral free energy P_n are unknown functions of s. For large enough nuclei, however, ΔG_n is approximated classically by Eqn. 22, and Eqn. 36 results in an explicit $\tau(C)$-dependence of the form

$$\tau = A \exp(b^2/4s) = A \exp[b^2/4 \ln(C_e/C)] \tag{38}$$

Fig. 10 shows the strong dependence of τ from Eqn. 38 on s (i.e. on C) at various b values. The b values (indicated on the curves) are those already used in Fig. 9. As seen, increasing b (i.e., the hole specific peripheral energy γ and/or the activity factor f) makes the membrane live longer, as then the nucleation work ΔG_n increases, too. This means that a bilayer lipid membrane with no active foreign bodies in it is less vulnerable to rupture than when containing such bodies. Fig. 10 also shows that above a certain critical supersaturation s_c, i.e., below a corresponding critical monomer lipid concentration C_c, the membrane ruptures within less than τ_c seconds. In other words, only for $s \leqslant s_c$ (or $C \geqslant C_c$) will the membrane live long enough. In physical terms, s_c is the highest, and C_c the lowest value of s and C, respectively, up to which the bilayer lipid membrane metastability region extends on the s- or C-axis (Fig. 3). The value of s_c (or C_c) is determined from the definition equality [12]

$$\tau(s_c) = \tau_c$$

which, classically, combined with Eqn. 20 yields

$$s_c = \ln(C_e/C_c) = b^2/4 \ln(\tau_c/A) \tag{39}$$

What is important to note here is that the value of the time parameter τ_c is arbitrary. It may be seconds, hours or years – just as high as is the length

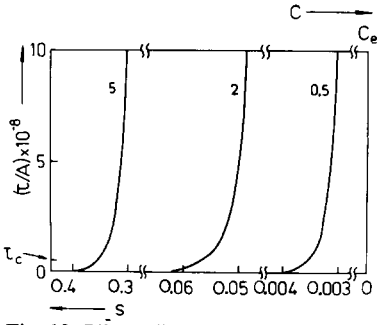

Fig. 10. Bilayer lipid membrane average lifetime as a function of s (or C) according to Eqn. 38 (b values indicated).

of the period during which a particular bilayer lipid membrane must not rupture. Postulating different lifetimes τ_c for the membrane will thus result in different values for s_c or C_c.

7. Bilayer lipid membrane permeability diagram

We shall now summarize the results for bilayer lipid membrane permeation and rupture in a picture which can be referred to as a bilayer lipid membrane permeability diagram. This diagram shows the region of b,C-values in which a bilayer lipid membrane is both permeable and not threatened by rupture.

Let p_1 be the minimum value of p below which the membrane is practically impermeable. Similarly, let p_2 be the maximum p-value above which the membrane is much too porous. Then, in classical approximation, setting p from Eqn. 32 equal to p_1 or p_2 results in

$$\ln(C/C_e) = (1/i_o)\ln(p_o/p_{1;2}) - b/i_o^{1/2} \tag{40}$$

This equation gives the b,C-values at which a bilayer lipid membrane ceases permeating, the permeability region being defined by $p_1 \leqslant p \leqslant p_2$.

In Fig. 11 we show a bilayer lipid membrane permeability diagram. It is obtained by graphically representing Eqns. 39 and 40 in a b,C-space with $i_o = 2$, $p_o = 1$, $p_1 = 10^{-5}$, $p_2 = 10^{-3}$ and $\tau_c/A = 10^8$ therein. The values of the above quantities are illustrative, but other values do not change the diagram qualitatively. As seen, given a sufficiently low value of b, the membrane can permeate only within a certain interval of concentration C of monomer lipid in the solution. Increasing C makes it eventually impermeable and decreasing C causes its nucleation rupture. For higher b-values however, e.g., with no active foreign bodies in it, the membrane remains either impermeable or ruptures with decreasing C.

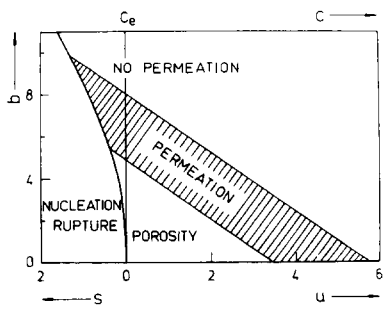

Fig. 11. Bilayer lipid membrane permeability diagram.

8. Possible experimental application

The results obtained above admit a direct experimental verification if the bilayer lipid membrane permeability coefficient and lifetime are studied in dependence on the experimentally controllable temperature and monomer lipid concentration (or activity). Recalling Eqns. 8 and 15 and using D in the known form $D = D'\exp(-U/kT)$ where D' (cm^2/s) is a frequency factor and U is the effective activation energy for permeant diffusion through a hole, from Eqn. 33 we find that P_d is the following function of C and T:

$$P_d = BC^{-i_o}\exp(-Q/kT) \tag{41}$$

Here $B = (p_o D'/\Delta x)C_o^{i_o}$ is a C,T-independent factor and Q, the activation energy for permeation, is given by $Q = i_o w/2 + P_o + U$ for monolayer holes, and by $Q = i_o z\epsilon/2 + P_o + U$ for bilayer holes. Classically, $P_o = (4\pi A_{ef} i_o f)^{1/2}\gamma$. In view of Eqn. 19, from Eqn. 38 we also find that in classical approximation

$$\tau = A\exp[K_1/(K_2 - \ln C)] \tag{42}$$

where the constants K_1 and K_2 are given by

$$K_1 = \pi A_{ef} f\gamma^2/(kT)^2 \tag{43}$$

$$K_2 = \ln C_e,$$

and A can be regarded as C-independent, but may be a function of T through the frequency ω of vacancy joining to the nucleus.

Eqn. 41 shows that $\ln P_d$ is a linear function of $\ln C$, the slope yielding the size i_o of the smallest permeable hole. As for τ, classically, according to Eqn. 42 a straight line should result when plotting $\ln\tau$ vs. $(K_2 - \ln C)^{-1}$. From the slope of the linear dependence the hole effective specific edge free energy $\gamma f^{1/2}$ can be determined through Eqn. 43. At high enough supersaturations the nucleus is of a few vacancies only and n and P_n may be constant with respect to C in the experimental C-interval. Then, atomistically, Eqn. 37 predicts a

linear dependence of $\ln \tau$ on $\ln C$ with a slope $n + 1$, since A is expected to be proportional to C (see Section 6).

Eqn. 41 is in qualitative agreement with existing experimental observations [1,2], as it predicts Arrhenius-type temperature dependence of P_d. It reveals that the activation energy Q for permeation includes not only the activation energy U for diffusion, but also the total peripheral energy P_o of the smallest permeable hole and the energies of lipid-lipid interaction. As to the C-dependences predicted by Eqns. 41 and 42, no comparison with experiment is possible at present because of the lack of experimental $P_d(C)$ and $\tau(C)$ data. We note, however, that in the case of rupture of bilayer black foam films Exerowa et al. [9,10] have found Eqn. 42 to describe well the observed $\tau(C)$-dependences. Qualitatively, Eqn. 42 is also in agreement with the experimental findings of Kruglyakov et al. [22,30] for the lifetime of bilayer hydrocarbon films. The observed strong dependence of the film lifetime on the total hydrocarbon concentration is like that shown in Fig. 10. However, as in these experiments the total concentration is in the range of the critical micelle concentration, the monomer hydrocarbon concentration C is not known and this precludes the use of Eqn. 42 for a quantitative analysis. Finally, it is worth noting that the theoretically predicted possibility for an increase in the permeability of a bilayer lipid membrane with foreign bodies in it is in conformity with the known experimental fact [1,2] that the presence of large molecules (e.g. proteins, antibiotics) in a bilayer lipid membrane may stimulate its permeability.

9. Conclusion

The two-dimensional lattice model of a bilayer lipid membrane proposed in the present work allows a simple description of the thermodynamic equilibrium of the membrane and the fluctuation formation of holes (or pores) in it. It turns out that, depending on the concentration of monomer lipid in the solution, a bilayer lipid membrane can exist in either stable or metastable equilibrium, a conclusion that has also been reached by Suezaki [17,18]. The appearance of holes is characterized by the work for their formation and, besides that, the greater the hole total peripheral energy, the greater this work. When a hole is formed in contact with a foreign body incorporated into the bilayer lipid membrane, the hole peripheral energy may be substantially reduced. As a result, it is possible that such foreign bodies are active with respect to the process of hole formation. The present analysis remains valid for arbitrarily concentrated solutions if everywhere in the formulae the various monomer lipid concentrations are replaced by the corresponding activities. It is worth noting as well that with $\Delta\mu$ defined by Eqns. 11 or 12 the results obtained are directly applicable to dense monolayer films on liquid surfaces.

If the hole is of large enough size, regardless of whether it is a mono- or bilayer one, its total peripheral energy can always be expressed with the aid of its specific edge free energy, γ. The value of γ, however, should be expected to differ depending on whether the hole periphery is hydrophilic or hydrophobic. Petrov and co-workers [27,28] and Israelachvili et al. [29] have proposed models for calculating γ in these two cases. As to the holes of the smallest size of a few vacancies, for them the macroscopic quantity γ can only be used as an approximation. In this case the correct quantity to work with is the atomistic hole peripheral energy P_i given by Eqn. 18. As already noted, however, using P_i is difficult because its general dependence on i is unknown.

Experimentally, the monomer lipid concentration can be varied appreciably only when it is lower than the critical micelle concentration. For this reason it is necessary to see whether C_e is below or above C_m. Combining Eqns. 6 and 8, we can express C_e via C_m in the following form

$$C_e = C_m \exp[(E_{dis} - w/2)/kT]. \quad (44)$$

This important relation shows that there is no general rule about the magnitude of C_e with respect to C_m. Physically, this is quite understandable. As $w/2$ plays the role of activation energy for bilayer lipid membrane dissolution, Eqn. 44 says that when the lipid molecule is more strongly bound in the bilayer lipid membrane than in the micelle, i.e., when $w/2 > E_{dis}$, $C_e < C_m$. In this case the bilayer lipid membrane exists in stable thermodynamic equilibrium in the range of con-

centrations satisfying $C_e \leqslant C < C_m$. Conversely, $C_e \geqslant C_m$ when $w/2 \leqslant E_{dis}$ and at any concentration C lower than C_m the bilayer lipid membrane is thermodynamically unstable: if existing, it can only be in metastable equilibrium. Eqn. 44 allows a firm conclusion about C_e to be made only when E_{dis} is negative, which, for instance, is experimentally found for surfactant micelles [31]. Then, as $w/2 > 0$, it follows that $C_e < C_m$. It must be noted that, in the case of formation of bilayer holes in the bilayer lipid membrane, C_e is an effective quantity determined by Eqn. 15 which can also be related to C_m. Using Eqns. 6 and 15 yields in this case

$$C_e = C_m \exp[(E_{dis} - z\epsilon/2)kT] \tag{45}$$

which, physically, is analogous to Eqn. 44. Eqns. 44 and 45 allow C_e in the formulae for u, s, P_d and τ to be expressed via C_m, E_{dis} and the energies of lipid-lipid interaction.

Considering P_d and τ from a unified point of view makes it possible to theoretically construct the bilayer lipid membrane permeability diagram (Fig. 11) on which the region of nucleation rupture is also seen. This diagram shows that given the membrane/solution system (i.e., a fixed value of b), permeation can only take place in a certain C-interval. It is worth noting as well that the bilayer lipid membrane permeability diagram can be represented in C,T-coordinates provided the values of the energy parameters in Eqns. 39 and 40 are known.

The formulae obtained for P_d and τ are simple enough to be easily put to experimental verification. As already discussed, studying the dependence of P_d on C would allow a direct determination of the size i_o of the smallest permeable hole, and finding P_d as a function of T would yield the activation energy Q for permeation which, for its part, is a combination of various energies. As to the dependence of τ on C obtaining it experimentally seems to be of interest, as analyzing it could give information about the lipid-lipid interaction and the mechanism of formation of holes in a bilayer lipid membrane.

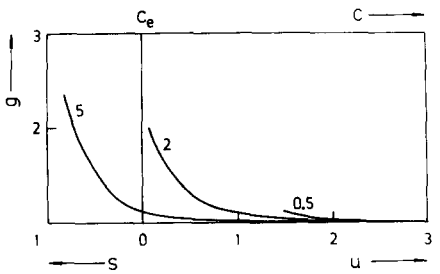

Fig. 12. Variation of g with u and s according to Eqns. 48 and 49 (b values indicated).

Appendix A. Finding ΔG_i for heterogeneous hole formation

Analogously to the known case [19,25] of formation of cap-shaped clusters on a foreign substrate, the work ΔG_i for heterogeneous formation of a segment-shaped hole (Fig. 5b) of i vacancies reads

$$\Delta G_i = -i\Delta\mu + L_c\gamma + L_r(\bar{\gamma} - \gamma_b). \tag{46}$$

Here L_c and L_r are the lengths of the curved and the rectilinear peripheries of the hole, and $\bar{\gamma}$ and γ_b are the specific edge free energies of the foreign body/hole and foreign body/membrane two-dimensional phase boundaries. respectively (Fig. 5b). The γ terms in Eqn. 46 represent the work done for creating the total hole periphery, and the γ values themselves are related by the two-dimensional Young formula

$$\gamma_b = \bar{\gamma} + \gamma\cos\alpha \tag{47}$$

in which α is the wetting angle. As elementary geometrical considerations yield

$$L_c = 2\alpha(A_{ef}i/\pi f)^{1/2}$$

$$L_r = 2\sin\alpha(A_{ef}i/\pi f)^{1/2}$$

using Eqn. 47, from Eqn. 46 we get Eqn. 14 with P_i and $f(\alpha)$ defined by Eqns. 16 and 17, respectively.

Appendix B. Analyzing g

The numerical factor g in Eqn. 29 is defined by

$$g = \sum_{i=i_o}^{\infty} in_i/i_o n_{i_o}$$

for a saturated or undersaturated bilayer lipid

membrane and by

$$g = \sum_{i=i_o}^{n} i n_i / i_o n_{i_o}$$

for a supersaturated bilayer lipid membrane ($n \gtrsim 10$). Classically, in view of Eqns. 13, 14, 16, 19 and 20, g reads

$$g = \sum_{i=i_o}^{\infty} (i/i_o) \exp\left[-u(i-i_o) - b\left(i^{1/2} - i_o^{1/2}\right)\right] \qquad (48)$$

and

$$g = \sum_{i=i_o}^{n} (i/i_o) \exp\left[s(i-i_o) - b\left(i^{1/2} - i_o^{1/2}\right)\right] \qquad (49)$$

for undersaturated and supersaturated bilayer lipid membranes, respectively. These equations apply to both mono- and bilayer holes, but in the latter case only $i = i_o$, $i_o + 2$, $i_o + 4$, etc. should be used in the summation, i_o being an even number. Also, given the b- value, Eqns. 48 and 49 hold provided $u \gtrsim 2 - b$ and $s \gtrsim b/6$, respectively, because only then are $n_1 \ll N_o$ in Eqn. 13 and $n \gtrsim 10$ in Eqn. 21.

Fig. 12 represents g from Eqns. 48 and 49 as a function of u (or s). The calculation is done for bilayer holes with $i_o = 2$ and $b = 0.5$, 2 and 5 (as indicated on the curves). As seen, g varies very slightly in the whole range of practically interesting u and b values and this justifies regarding it as a number between 1 and 2.5. Using for example $i_o = 6$ in Eqns. 48 and 49 results in a g value insignificantly different from that in Fig. 12.

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