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Stalk mechanism of vesicle fusion

Intermixing of aqueous contents

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Abstract. A mechanism for rupture of a separating bilayer, resulting from vesicle monolayer fusion is investigated theoretically. The stalk mechanism of monolayer fusion, assuming the formation and expansion of a stalk between two interacting membranes is considered. The stalk evolution leads to formation of a separating bilayer and mechanical tension appearance in the system. This tension results in rupture of the separating bilayer and hydrophilic pore formation. Competition between the mechanical tension and hydrophilic pore energy defines the criteria of contacting bilayer rupture. The tension increases with an increase of the absolute value of the negative spontaneous curvature of the outer membrane monolayer, K_s^o. The pore edge energy decreases with an increase of the positive spontaneous curvature of the inner membrane monolayer, K_s^i. The relations of spontaneous curvatures of outer and inner monolayers, leading to separating bilayer rupture, is calculated. It is demonstrated that his process is possible, provided spontaneous curvatures of membrane monolayers have opposite signs: $K_s^o < 0$, $K_s^i > 0$. Experimental data concerning the fusion process are analysed.

Key words: Vesicle fusion, stalk mechanism, stalk evolution, monolayer fusion, trilaminar structure, pore formation

Introduction

Membrane fusion attracts the attention of many researchers owing to the participation of this phenomenon in numerous biological processes (Evered and Whelan 1984). A great number of works deal with the search for conditions necessary for the fusion of cells and model membranes: lipid vesicles, planar bilayer lipid membranes etc (Pinto da Silva and Nogueira 1977; Papahadjopoulos et al. 1979; Rand 1981; Duzgunes 1985; Chernomordik et al. 1985). In recent years attempts have been made to study the detailed molecular mechanism of membrane fusion (Chernomordik et al. 1985; Markin and Kozlov 1984; Israelachvili 1985; Ohki 1984; Cullis et al. 1985; Lucy and Ahkong 1986; Raundino 1986; Siegel 1986). Possible fusion mechanisms have been classified according to the nature of the contact between membranes that precedes fusion. Mechanisms suggesting a wide-area contact were named adhesion mechanisms (Markin and Kozlov 1984; Kozlov and Markin 1984). Such a mechanism may be represented by the adhesion-condensation mechanism, involving a phase transition of lipid molecules in the membrane contact zone (Kozlov and Markin 1984). A membrane fusion mechanism that starts after a local point contact has been established between the membranes is called a stalk mechanism (Markin et al. 1984; Chernomordik et al. 1985). The stalk mechanism suggests that the formation of narrow bridges (stalks) connecting the membranes (Gingell and Ginsberg 1978), is an intermediate stage in the fusion process (Fig. 1 a, b). If a stalk extends between the external monolayers of the interacting membranes only, it is named a monolayer stalk. An expansion of such a bridge leads to a trilaminar structure, a separating bilayer bordering on the two bilayers along its perimeter (Fig. 1 b), being formed. The formation of the trilaminar structure is often referred to as monolayer fusion to emphasize that this process does not ensure mixing of inner volumes of cells and vesicles. The fusion process is completed by destruction of the separating bilayer.

The stalk hypothesis has been developed and substantiated experimentally by Chernomordik et al. (1985). It has been proven quantitatively that in the system of two bilayer lipid membranes the fusion process should involve an intermediate stage of monolayer fusion triggered by the stalk mechanism (Chernomordik et al. 1985; Melikyan et al. 1983).

The conditions necessary for stalk and trilaminar structure formation have been analyzed in previous

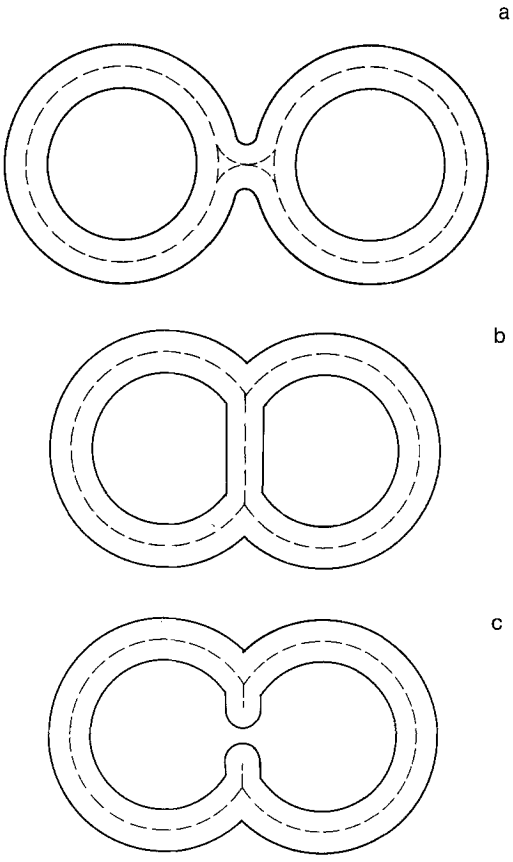


Fig. 1 a–c. Stalk mechanism of membrane fusion. **a** Zero-radius stalk formation, **b** stalk expansion and contact bilayer formation, **c** formation of a pore in a separating bilayer

papers (Markin et al. 1984; Chernomordik et al. 1985). To form the stalk, the membranes must overcome the hydration repulsion which prevents their approach. This process is achieved by local bending of bilayers under the action of thermal fluctuations. The local approach of membranes results in a loss of stability in the contact area and formation of a monolayer stalk. Further evolution of the stalk consists of its expansion and the formation of a trilaminar structure (Markin et al. 1984; Chernomordik et al. 1985). The probability of stalk formation and the conditions encouraging its expansion are determined by the degree of membrane hydration and by the elastic curvature energy of opposed membrane monolayers (Chernomordik et al. 1986; Leikin et al. 1987). The latter is closely related to the curvature of monolayers in the unstrained state. Such spontaneous curvature reflects the effective shape of lipid molecules determined both by an actual structure of each separate molecule and by specific interactions in a monolayer (Petrov and Bivas 1984).

The studies mentioned above do not analyze the final stage of the fusion process: the rupture of the separating bilayer. This paper probes this question using the concepts of structure reorganization, elastic curvature and spontaneous curvature used in the preceding research. We obtain the relationship between spontaneous curvatures of outer and inner vesicle monolayers at which the expansion of the monolayer stalk leads to the rupture of the separating bilayer, i.e. to the completion of the fusion process.

The model

Let us consider the fusion of two vesicles (Fig. 1). A lipid bilayer making up the vesicle membrane consists of two monolayers free to slide laterally over each other. However, transversely they constitute a bilayer couple i.e. the bilayer can not laminate into two separate monolayers. The membrane monolayer in contact with the external solution is called the outer one and the second monolayer bordering the inner volume of the vesicle is called the inner one. Let us assume that in the course of membrane fusion the two stages occur in succession. First the outer monolayers of the vesicles fuse, i.e. monolayer fusion takes place. Secondly, the inner monolayers fuse, this results in mixing of vesicle volumes, and the aqueous contents intermix.

Both stages are related to structural reorganization of the lipid monolayers. When monolayer fusion occurs, the reorganisation of outer monolayers leads to the emergence of a monolayer stalk, a bridge between the membranes (Markin et al. 1984), and then to the formation of trilaminar structure (Fig. 1 b). At this point the aqueous contents of the vesicles appear to be separated by a single separating bilayer, made up of the inner monolayers of the interacting membranes. The structural reorganization, occurring at the second stage of the process and involving inner monolayers of the vesicle membranes, leads to the formation of an inverted pore in the separating bilayer which constitutes a hole, the edge of which is made up of polar heads of lipid molecules (Fig. 1 b).

The two structures emerging in the process of fusion have much in common. In fact, the formation of each of them is related to the bending of the membrane monolayer. The stalk wall is formed by curved outer monolayers, whereas the edge of the inverted pore consists of curved inner monolayers. However, a substantial difference between these structures is that the bending occurs in opposite directions. To form the stalk, the outer monolayer should be bent in such a way that polar heads of lipid molecules contract and the hydrophobic tails extend. Such bending we will consider to be a negative one, since it occurs within a bulge in the monolayer having the direction opposite to that of the external normal to the monolayer plane. Within the inverted pore the inner monolayer is bent

in the positive direction, the lipid molecule polar heads extend and the hydrophobic tails contract.

The process of stalk and inverted pore formation is determined by the changes in the system elastic of bending. Let us consider the energy of a system of two fusing vesicles. We assume that the vesicles have the same parameters and relate all the following equations to one of them. The major contribution to the change in the system energy, caused by the stalk and pore formation, comes from the elastic curvature energy and extension-compressive energy of the monolayers.

Elastic curvature energy per unit of surface of a monolayer is given by (Helfrich 1973)

$$W_c = \frac{B_m}{2}(K_m + K_p - 2K_s)^2, \tag{1}$$

where B_m is the elastic curvature modulus (bending rigidity) of a monolayer, which is approximately equal to 10^{-20} J; K_m and K_p are the principal (meridional and parallel) curvatures of a monolayer, K_s is the spontaneous curvature constituting monolayer curvature in hypothetical unstrained state. The value of K_s is determined by the effective shape of lipid molecules making up the monolayer. When the lipid molecules have a narrow polar head and wide hydrophobic tail, the monolayer tends to bend in the negative direction (with respect to the external normal) and its spontaneous curvature is negative $K_s < 0$. In contrast, when the lipid molecules are narrow-tailed and wide-headed, spontaneous curvature of the monolayer is positive, $K_s > 0$. In the first case, $K_s < 0$, the lipid molecule has the effective form of inverted cone; in the second case $K_s > 0$ it has the form of a cone. If the areas of a polar head and hydrophobic tail are the same spontaneous curvature of the monolayer is equal to zero, $K_s = 0$, and the molecules have the effective shape of a cylinder.

The stalk energy

Let us consider the stalk formed by fused outer monolayers of two interacting vesicles having spontaneous curvature K_s^o.

The stalk wall is formed by the curved monolayers (Fig. 1 a). To find the elastic curvature energy of the stalk wall, we assume that the neutral surface, dividing the monolayer into two equal parts, is formed by the rotation of a semicircle with diameter $2r$ corresponding to the height of the stalk. Let h denote the thickness of the monolayer, $\delta = h/2$, and the internal radius of the stalk R corresponds to the radius of a trilaminar structure formed by its surface. With these assumptions the elastic curvature energy of the stalk membrane have been calculated (Markin et al. 1984) to be

$$W_s = \pi B_m \left\{ \frac{2}{r} \frac{(r+\delta+R)^2}{\sqrt{(R+\delta)(2r+\delta+R)}} \operatorname{arctg} \sqrt{\frac{2r+\delta+R}{R+\delta}} + K_s^o [2\pi(r+\delta+R) - 8r] - 4 \right\}, \tag{2}$$

where it has been suggested that $W = 0$ for a planar monolayer.

Later we will focus our attention on the case where the stalk is formed between the apposed membranes separated by an equilibrium distance d_w, determined by the hydration repulsion and Van-der-Waals attraction. Taking into account that $2r = d_w + h$ and $d_w \simeq h \simeq 3$ nm (Gruen et al. 1984; Rand 1981), we assume $r = h$ so that Eq. (2) is reduced to the following form

$$W_s = W_{0s} + 2\pi\gamma_s R, \tag{3}$$

where

$$W_{0s} = \pi B_m (0.62 + 1.42 K_s^o h) \tag{4}$$

is the energy of the stalk of zero radius and

$$\gamma_s = \frac{\pi B_m}{h}(K_s^o h + 0.25) \tag{5}$$

is the linear tension of a monolayer stalk.

It follows from Eqs. (3) and (5) that there are different ways for the evolution (formation) of bridge. If $\gamma_s > 0$ an increase in stalk radius is energetically unfavourable. In contrast, when the linear tension is negative, $\gamma_s < 0$, the stalk tends to expand because it results in a decrease in its elastic energy. The linear tension of the stalk is determined by spontaneous curvature of the monolayer constituting its wall. The lower is K_s^o, the less is the value of γ_s. This is connected with the fact that the stalk wall has negative meridional curvature comparable in its value with the thickness of a bilayer. Such a surface is favourable for the placement of lipid molecules with narrow polar heads and wide hydrophobic tails. In other words, the more negative the monolayer spontaneous curvature is, the less curvature energy is accumulated in the membrane of the monolayer stalk. It follows from Eq. (5) that the extension of the stalk ($\gamma_s < 0$) is favourable once

$$K_s^o h < -0.25, \tag{6}$$

i.e. the radius of spontaneous curvature is less than four monolayer thicknesses.

Energy of pore edge

The calculation for elastic curvature energy of the monolayer constituting the pore edge is similar to the

calculation for the stalk energy. It leads to the expression (Markin and Kozlov 1985)

$$W_p = W_{0p} + 2\pi\gamma_p R, \tag{7}$$

where

$$W_{0p} = \pi B_m [0.84 - 2.28 K_s^i h], \tag{8}$$

and the linear tension of the pore edge is equal to

$$\gamma_p = \frac{\pi B_m}{h}[0.5 - K_s^i h]. \tag{9}$$

Here R is the pore radius; K_s^o is the spontaneous curvature of the inner monolayer of the vesicle membrane.

It follows from Eq. (9) that the linear tension of the pore is reduced as the inner monolayer spontaneous curvature grows. The dependence of γ_p on K_s^o is opposed to the corresponding dependence for the stalk linear tension. This reflects the fact that the bending of the monolayer of the stalk wall and that of the pore edge occurs in opposite directions. Therefore, unlike the stalk formation, the location in the pore edge of lipid molecules having wide polar heads and narrow hydrophobic tails, i.e. molecules with positive spontaneous curvature, is favourable.

Monolayer extension – compression

We will assume that expansion of the stalk is energetically favourable. Let us consider the case when there is no pore in the separating bilayer in the course of the expansion (Fig. 1 b). Stalk expansion leads to an increase in the effective area of the outer monolayer of the vesicle at the expense of the area of the "hole" in this monolayer which emerges as a result of the stalk formation. Owing to the fact that outer and inner monolayers are connected transversely and are free to slip laterally, the expansion of the stalk results in stretching of the inner monolayer and in compression of the outer monolayer. At this point the relationship $A_o + a_t = A_i$ is valid, where A_o and A_i are the areas of the outer and inner monolayers respectively, and a_t is the area of the "hole" in the outer monolayer, i.e. the area of separating bilayer between the vesicles. Extension, as well as compression, of monolayers leads to accumulation of elastic energy. Its value for the inner and outer monolayers is given by

$$W_{e0} = \Gamma\frac{(\Delta A_c)^2}{\tilde{A}}; \quad W_{e0} = \Gamma\frac{(\Delta A_i)^2}{\tilde{A}}, \tag{10}$$

where Γ is the lateral elasticity modulus of a monolayer ($\sim 5 \cdot 10^{-2}$ N/m; Kwok and Evans 1981); ΔA_o and ΔA_i denote deviation of areas of the outer and inner monolayers from equilibrium value. The equilibrium area A, assumed to be nearly the same for both monolayers, corresponds to their undeformed state.

The extension of the inner monolayers leads to stretching of the contact bilayer separating the inner volumes of the vesicles. It may result in the formation of a pore in the separating bilayer that completes the process of fusion. Our task is to identify the conditions under which stalk extension results in pore formation.

Rupture of the separating bilayer

Let us consider two similar vesicles separated by an a_t-area separating bilayer formed as a result of the stalk expansion. We assume that there is an a_p-area pore in the contact bilayer and that both the pore and the bilayer are circular. We suppose also that a_t and a_p are substantially less than the equilibrium area of the vesicle monolayers A. The elastic energy of this system per vesicle including the curvature energy of the stalk wall and pore edge as well as the extension-compressive energy of the monolayers, is given by

$$W = \Gamma\frac{(\Delta A_o)^2}{\tilde{A}} + \Gamma\frac{(\Delta A_i)^2}{\tilde{A}} + 2\sqrt{\pi}\gamma_s\sqrt{a_t} + 2\sqrt{\pi}\gamma_p\sqrt{a_p}. \tag{11}$$

Equation (11) is valid, provided that $a_p < a_t$. The relationship between the vesicle monolayers leads to an additional condition connecting the variations in the areas of outer (ΔA_o) and inner (ΔA_i) monolayers with the areas a_t and a_p (Fig. 1)

$$\Delta A_o + a_t = \Delta A_i + a_p. \tag{12}$$

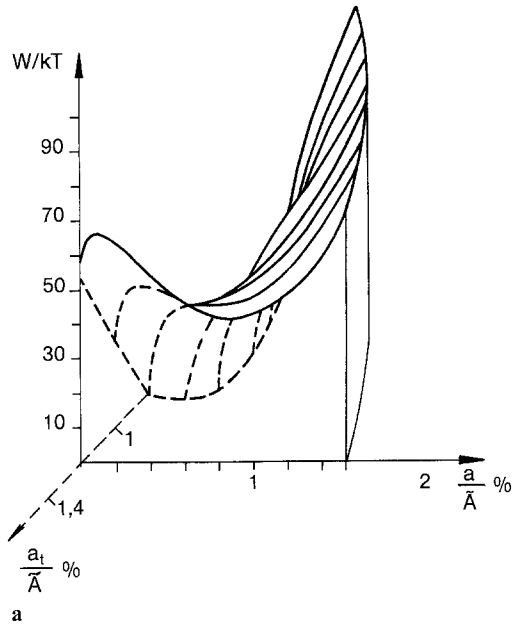
The equilibrium state of the system corresponds to a minimum of its elastic energy. Taking into account Eq. (12), we obtain that for given values a_t and a_p the minimal energy is equal to

$$W = \frac{\Gamma}{2}\frac{(a_t - a_p)^2}{\tilde{A}} + 2\sqrt{\pi}\gamma_s\sqrt{a_t} + 2\sqrt{\pi}\gamma_p\sqrt{a_p}. \tag{13}$$

An analysis of Eq. (13) indicated that the form of dependence of the system elastic energy W on the areas a_t and a_p is determined by the relation between linear tension of the stalk γ_s and the pore γ.

Consider the case when the linear tension of the stalk is negative, $\gamma_s < 0$. The graphs of the dependence of W on $a_t/\tilde{A}$ and a_p/A are shown in Fig. 2 b ($|\gamma_s| > \gamma_p$) and Fig. 2 a ($|\gamma_s| < \gamma_p$). From Fig. 2 one can see that when the pore is absent the dependence of the system energy on the contact bilayer area $W(a_t)$ has a minimum. The minimum corresponds to

$$a_t^* = 2^{4/3}\pi\frac{(\gamma_s^2 R_0^4)^{1/3}}{\Gamma^{2/3}}, \tag{14}$$



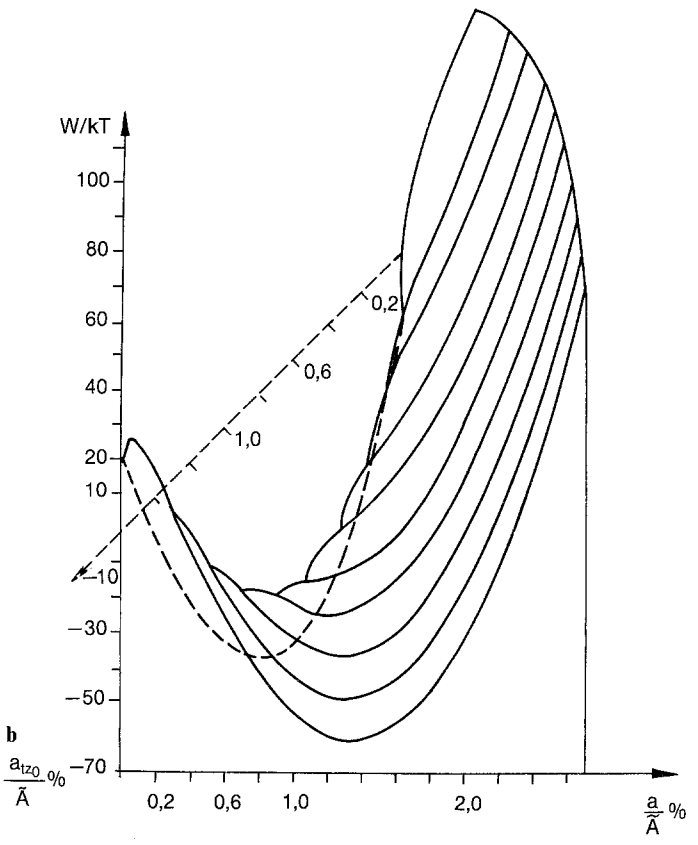


Fig. 2a and b. Dependence of elastic energy W on the area of the separating bilayer a_t and on the area of the inverted pore a_p. Different values of linear tensions γ_s and γ_p are investigated: **a** $|\gamma_s| < \gamma_p$, $\gamma_s = -1.6 \cdot 10^{-12}$ N, $\gamma_p = 3 \cdot 10^{-12}$ N; **b** $|\gamma_s| > \gamma_p$, $\gamma_s = -3 \cdot 10^{-12}$ N, $\gamma_p = 1.6 \cdot 10^{-12}$ N

where R_0 is the initial radius of the vesicle, $R_0 = \sqrt{\tilde{A}/4\pi}$. Area a_t^* corresponds to the equilibrium contact bilayer formed as a result of the stalk expansion. In this state the energy of the system is equal to

$$W^* = -\frac{3\pi}{2^{1/3}}\left(\frac{\gamma_s^4 R_0^2}{\Gamma}\right)^{1/3}. \tag{15}$$

We assume that rupturing the separating bilayer is possible when the formation of a pore in it may decrease the system energy below the value of W^*. The analysis of Eq. (13) indicates that if the absolute value of the linear tension of the stalk is lower than of a pore $|\gamma_s| < \gamma_p$, the formation and extension of the pore leads to an increase in the elastic energy (Fig. 2a). This means that the separating bilayer is stable. In the case when $|\gamma_2| > \gamma_p$ an increase in the pore area, accompanied by an increase of the separating bilayer radius, leads to a drop in the system energy. This conclusion is illustrated in Fig. 2b. The geometrical surface indicating a dependence of the energy W on areas a_t and a_p has a "canyon". The points on the bottom of this "canyon" correspond to the minimum values of energy with fixed values of a_t and variable values of a_p. Let us examine "the trajectory" of the system, marked by arrows in Fig. 2b, starting at the point $a_p = 0$, $a_t = a_t^*$ and going into the canyon and further down along its bottom. If $|\gamma_s| < \gamma_p$, the movement along the trajectory corresponds to a monotonous increase in energy (Fig. 2a), and the system energy always exceeds W^*. If $|\gamma_s| > \gamma_p$, the movement along the trajectory is accompanied by non-monotonous changes in the system energy. When the values of a_t, a_p are high enough the energy infinitely decreases. This means that the formation of a large pore is energetically favourable.

Thus

$$|\gamma_s| > \gamma_p \tag{16}$$

is the condition of the separating bilayer destabilization. It can be expressed in terms of spontaneous curvature of outer K_s^o and inner K_s^i vesicle monolayers. From Eqs. (5) and (9) we obtain

$$-K_s^o h > 0.75 + K_s^i h. \tag{17}$$

However, as mentioned above, in this case the pore expansion is accompanied by non-monotonous changes in the system energy, i.e. the system should overcome an energy barrier. The top of the barrier is the highest point along the "trajectory" described above, which corresponds to the saddle point of the function $W(a_t, a_p)$. It follows from Eq. (13) that the function $W(a_t, a_p)$ has a saddle point at

$$a_t = a_t^1 = a_t^*\left(1 - \frac{\gamma_p^2}{\gamma_s^2}\right)^{-2/3};$$

$$a_p = a_p^1 = a_t^* \frac{\gamma_p^2}{\gamma_s^2}\left(1 - \frac{\gamma_p^2}{\gamma_s^2}\right)^{-2/3}.$$

Substituting this expression and Eq. (14) into Eq. (13) we find that the height of the energy barrier is equal to

$$\Delta W = W(a_t^1, a_p^1) - W(a_t^*, 0)$$
$$= \frac{3\pi}{2^{1/3}}\left(\frac{R_0^2\gamma_s^4}{\Gamma}\right)^{1/3}\left[1-\left(1-\frac{\gamma_p^2}{\gamma_s^2}\right)^{2/3}\right]. \quad (18)$$

The analysis of Eq. (18) shows that the barrier ΔW goes down as γ_p^2/γ_s^2 decreases. Let us find the condition, which allows the system to overcome the energy barrier within the time τ comparable with the duration of the experiment. According to the theory of absolute rates of reactions, we express the time required to overcoming the energy barrier as

$$\tau = \frac{1}{\mu a_t}\exp\left(\frac{\Delta W}{kT}\right), \quad (19)$$

where μ is the characteristic frequency of fluctuations of the membrane monolayer, which can be estimated as $\mu \simeq 4 \cdot 10^{29}\,\mathrm{s}^{-1}\,\mathrm{m}^{-2}$ (Weaver and Mintzer 1981). It follows from Eqs. (18) and (19) that the condition for overcoming the barrier is

$$\frac{3\pi}{2^{1/3}}\left(\frac{R_0^2\gamma_s^4}{\Gamma}\right)^{1/3}\left[1-\left(1-\frac{\gamma_p^2}{\gamma_s^2}\right)^{2/3}\right] < \ln(\tau\mu a_t). \quad (20)$$

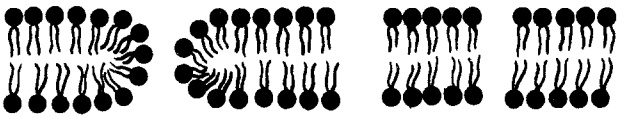
Here the value of a_t depends on the values of linear tension γ_s and γ_p. However, this dependence may by disregarded since a_t is in the logarithm's argument. The evaluation indicates that a_t constitutes approximately a half per cent of the initial area of the vesicle monolayer $a_t/\tilde{A} \simeq 5 \cdot 10^{-3}$. Assuming that the characteristic time for an experiment is $\tau \simeq 1$ min, it is possible to show that condition (20) produces a negligible correction to condition (16). Therefore we consider inequalities (16) and (17) to be the criterion for rupture of the separating bilayer.

Discussion

Having the theory developed in this paper one can give a total description of the stalk mechanism of membrane fusion. Now it is also possible to determine the conditions for realization of this process.

The fusion process includes the following principal steps:

1. The nucleation of a monolayer stalk, which connects the outer monolayers of interacting membranes;
2. The expansion of a stalk and formation of a separating bilayer between the inner volumes of vesicles (cells);
3. The formation and evolution of an inverted pore in the separating bilayer which leads to the completion of the fusion process.



a b

Fig. 3a and b. Molecular structure of pore in lipid bilayer; **a** inverted pore, **b** hydrophobic pore

It is possible to imagine two kinds of pore in a lipid bilayer: inverted and hydrophobic ones (Glaser et al. 1988).

The edge of an inverted pore is formed by a lipid monolayer (Fig. 3a). In contrast, in the edge region of a hydrophobic pore, the contact between aqueous solution and hydrophobic lipid tails takes place (Fig. 3b). The calculation shows that the bending elastic energy of a lipid monolayer in the edge of an inverted pore is less than the energy of a hydrophobic one. That is why we may postulate in the present investigation that all pores are inverted and characterised by elastic energy of monolayer bending.

The realisation of the stalk mechanism of membrane fusion implies that the basic conditions of this process are satisfied: the outer monolayers of interacting membranes have negative spontaneous curvature ($K_s^o < 0$), and inner monolayers have positive spontaneous curvature ($K_s^i > 0$).

These conditions for realisation of membrane fusion are due to the geometrical features of membrane structures, which arise during this process. The two main structures – stalk and inverted pore – require the bending of membrane monolayers in opposite directions. The curvature of the stalk wall is negative (Fig. 1a). Therefore the elastic energy of outer lipid monolayers, which form the stalk, is minimal if their spontaneous curvature is also negative. The curvature of the monolayer at the edge of the inverted pore is positive (Fig. 1b). So the change of bending energy of the lipid monolayer during the nucleation and evolution of the inverted pore in the separating bilayer is minimal if the spontaneous curvature of the inner monolayer is positive. The conditions for stalk expansion (Eq. (6)) and rupture of the separating bilayer (Eq. (17)) are shown in Fig. 4. The values of the spontaneous curvature placed below the curve in the figure correspond to the cases when the growth and the rupture of the separating bilayer, completing the fusion process, take place. The points above the curve do not meet the conditions required.

Let us now compare the conclusions of the theory developed with available experimental data.

Ca^{2+} stimulated membrane fusion. On the basis of the theory developed in this paper one can explain the

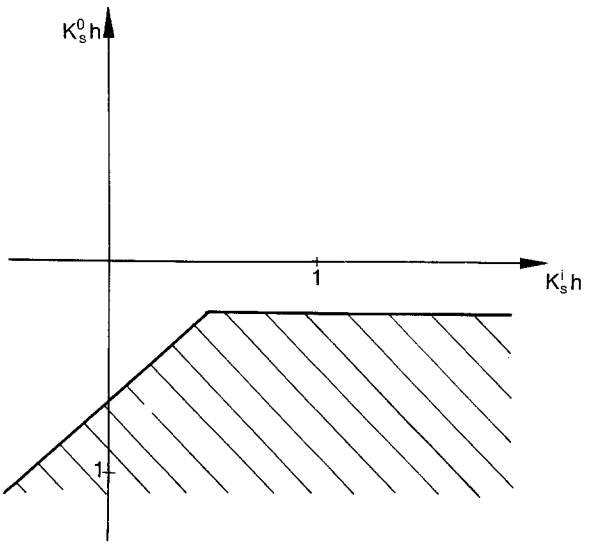


Fig. 4. The correlation between the spontaneous curvatures of the outer (K_s^o) and inner (K_s^i) monolayers, determining the possibility of membrane fusion. Values of the spontaneous curvatures placed below the curves correspond to the cases when the growth and the rupture of the separating bilayer take place. The points above the curve do not meet the criterion for fusion

fusion mechanism of phosphatidylserine (PS) liposomes in the presence of Ca^{2+} ions. Experimentally this process was investigated by Papahadjopoulos et al. (1979). The main idea of the explanation is that Ca^{2+} ions adsorbed at the outer monolayer of PS membranes neutralise the negative charge of the lipid heads. As a result, Ca^{2+} adsorption confers negative spontaneous curvature on the outer monolayer (this effect is also due to the more complicated electrostatic effects described below). At the same time Ca^{2+} ions cannot penetrate into the inner monolayer of PS liposomes. The negative charge which is not compensated by Ca^{2+} ions gives positive spontaneous curvature to the inner monolayer. As a result the interaction between Ca^{2+} ions and vesicles leads to opposite spontaneous curvature of membrane monolayers. This circumstance is favourable for the realization of fusion.

The Ca^{2+} ion adsorption can drastically change the bending stiffness of the monolayer. This point must be taken into account during the calculation of stalk linear tension γ_s according to Eq. (5).

Let us now consider more exactly the change of monolayer spontaneous curvature due to the action of Ca^{2+} ions. We assume that initially the calcium ions are absent in the system and consider spontaneous curvature of the vesicle monolayers.

The value of spontaneous curvature of the PS monolayer is not known. To estimate it we assume that the spontaneous curvature of a neutral PS monolayer (existing, for example, at low pH) is equal to zero. Starting with this assumption we find spontaneous curvature of a monolayer consisting of PS molecules bearing negative charge.

The value of spontaneous curvature of such a monolayer is expressed as follows (Lerche et al. 1986)

$$K_s = \frac{e_0^2 (h + \varkappa^{-1})}{8 a_0^2 B_m \varepsilon \varepsilon_0 \varkappa}, \tag{21}$$

where h is the thickness of a monolayer, B_m is the curvature elasticity modulus of monolayers, $\varkappa$ is the inverse Debye length in electrolyte solution, a_0 is the area per lipid molecule in medium section, of the monolayer, ε is the dielectric permittivity of the media, ε_0 is the dielectric constant. Taking $e_0 = 1.6 \cdot 10^{-19}$ Q, $h = 2$ nm, $\varkappa^{-1} = 1$ nm, $a_0 = 0.6$ nm^2, $\varepsilon = 80$, $\varepsilon_0 = 8.9 \cdot 10^{-12}$ F/m, $B_m = 10^{-20}$ J and using Eq. (21) we find that the spontaneous curvature of the monolayer has a substantial positive value, $K_s h \simeq +1$.

After addition of Ca^{2+} to the system, adsorption of the ions on external surfaces of the vesicles leads to neutralization of the total negative charge of their outer monolayers. This results in compensation of electrostatic repulsion between the polar heads, and the positive spontaneous curvature related to it is removed. Assume that each Ca^{2+} ion is adsorbed on a polar head of a lipid molecule. Than a half of the lipid molecules of the monolayer change their charge to the opposite one, since the cation charge is twice as great as that of a polar head. This brings the monolayer total charge to zero and a lattice of alternating charges of a chess-board type may be formed on its surface (Markin and Kozlov 1983). Attraction between the charge occurs in such a lattice. As a result the area of the monolayer in the region of polar heads tends to contract and spontaneous curvature of the monolayer becomes negative. Its value may be estimated using the expression for the attraction energy in the lattice of alternating charges (Markin and Kozlov 1983) as

$$K_s^o = 10^{-2} \frac{e_0^2 h}{\varepsilon \varepsilon_0 B_m a_0^{3/2}}. \tag{22}$$

Using Eq. (22), we obtain that $K_s^o h \simeq -0.3$.

Note that adsorption of Ca^{2+} leads to strong dehydration of PS monolayers (see, for example, Gruen et al. 1984). This dehydration should lead to a significant change in spontaneous curvature of the monolayer. Unfortunately there are no models which allow us to estimate this effect quantitatively. However, the change in spontaneous curvature induced by dehydration occurs in the same direction as the change caused by electrical effects (towards negative values). Therefore we can argue that spontaneous curvature of the outer monolayer become even more negative than the value estimated above.

Estimations similar to the one considered above suggest that this mechanism is equally feasible in other

well studied experimental systems. This refers to vesicles of mixed composition the membranes of which are composed of unsaturated phosphatidylethanolamine (PE) and PS; PE and cardiolipine in the presence of calcium etc., and equally to liposomes, the outer monolayers of which are treated with phospholipases C and D (Shragin et al. 1985).

"Non-bilayer" lipids – induced fusion. The direction of stalk evolution and the probability of stalk formation are determined by spontaneous curvature of monolayers making up the membranes. The formation of a monolayer stalk and an increase in its diameter are initiated by negative spontaneous curvature of the outer monolayers reflecting the presence of so-called "non-bilayer" lipids which under these conditions (temperature, pH, Ca^{2+} concentration) have a tendency to form a hexagonal (H_{11}) phase. In contrast, the transition from monolayer fusion to complete fusion is encouraged by positive spontaneous curvature of the inner monolayer. As has been shown above the positive spontaneous curvature may be induced, for example, by electrostatic interaction between charged polar heads of acid lipids which, unlike the molecules of the outer monolayers, do not interact with the Ca^{2+} ions. Thus, according to the theory developed, short-lived or relatively stable bridges connecting the outer monolayers of the vesicles may be formed in the suspension, depending on the relations between the signs and values of spontaneous curvatures of the outer and inner monolayers. The results, obtained by comparative study of kinetics of membrane and water marker redistribution in the course of liposome interaction under various conditions, allow us to suggest that all the situations discussed above may be observed in experiments. Apart from complete fusion (the mixing of both membrane and water markers) (Wilschut and Hoekstra 1986; Duzgunes et al. 1985) a fusion of membranes (the mixing of membrane markers) without coalescence of their aqueous contents (intermixing of water markers) was reported (Bentz et al. 1985; Bondeson and Sundler 1985; Ellens et al. 1985, 1986). It is important that under the conditions ensuring complete fusion, the rate of intermixing for membrane markers was greater than for the water ones in agreement with the hypothesis under consideration (Wilschut and Hoekstra 1986; Duzgunes et al. 1985).

In papers devoted to liposome fusion the important role played by "non-bilayer" lipids in this phenomenon has been widely discussed (Cullies et al. 1985; Markin et al. 1984; Siegel 1986). Since the presence of such lipids should be favourable for monolayer fusion of liposomes within the framework of our hypothesis, the corresponding experimental facts equally corroborate the theory. On the other hand if liposomes are initially formed solely of non-bilayer lipid, both monolayers of their membranes have negative spontaneous curvatures and it may be expected that the monolayer fusion will not be completed. This is precisely the situation observed by Bentz et al. (1985) and Ellens et al. (1986) for phosphatidylethanolamine liposomes.

As far as we know the influence of spontaneous curvature of membrane inner monolayers on the probability of fusion has never been discussed in the literature. From the viewpoint of the stalk hypothesis it may be expected that the introduction of Ca^{2+} ions into the inner volumes of liposomes of acid lipids should reduce the probability of fusion.

Influence of tension on membrane fusion. In conclusion let us point out two further common features of fusion phenomena that might be explained within the framework of the conceptions considered. It has been observed that swelling of liposomes in a hypotonic medium promotes their fusion (Miller and Racker 1976; Lucy and Ahkong 1986). This effect is easily explained by the fact that the osmotic swelling of liposome membranes increases tension of the separating bilayer and consequently increases the probability of its destruction, i.e. initiates complete fusion. The mechanical tension of a separating bilayer depends on the vesicle radius. The calculation based on Eqs. (10) and (14) leads to the following formula for tension

$$\sigma = \frac{1}{2^{2/3}} \left(\frac{\Gamma \gamma_s^2}{R_0^2} \right)^{1/3}. \tag{23}$$

One can see, that σ increases as the vesicle radius (R_0) decreases. As a result, the disruption of a separating bilayer is more frequent for small vesicles. This actually has been observed experimentally (Nir et al. 1983).

Thus, the stalk hypothesis explains a great number of experimental results. However only further research can provide an answer to the question on the mechanism (or mechanisms) of fusion in different experimental systems.

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