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The influence of gangliosides on the hydrophilic pore edge line tension and monolayer fusion of lipid membranes

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It is shown that inclusion of gangliosides into planar phospholipid bilayers leads to an essential suppression of their monolayer fusion. In contrast to phospholipid lysoforms, the gangliosides do not cause an associative decrease of the specific energy of the hydrophilic pore edge determined in the experiments on the electrical breakdown of membranes. This phenomenon depends on the effective size of the polar headgroup of the gangliosides. It is supposed that the effect of gangliosides on the parameters mentioned is based on the fact that they have large hydrated polar headgroups which create steric hindrances, which prevent (i) the membranes to come into contact during monolayer fusion; (ii) the gangliosides to take part in hydrophilic pore formation.

Introduction

In previous studies we have shown that the elastic bending energy of membrane monolayers, in structural defects like hydrophilic pores at electrical breakdown and stalk-bridges at monolayer fusion, is determined by the effective form of the bilayer molecules [1] (Fig. 1). At the formation of a hydrophilic pore and a stalk, the monolayers are bent in opposite directions. This results in an inverse dependence of elastic energies of these defects on the effective form of the lipid molecules. The pore edge line tension can be characterized experimentally by the specific energy of a hydrophilic pore edge. And the stalk energy can be characterized by the waiting time of monolayer fusion [1]. We have previously shown that the bilayers tension and the pore edge energy decrease linearly, and the expectation time increases exponentially as the content of lysophosphatidylcholine in phosphatidylethanolamine grows. In other words, lysophosphatidylcholine (a cone-shaped molecule) destabilizes bilayers, stimulates the hydrophilic pore formation, suppresses monolayer fusion and increases the stalk energy. The qualitative correlation between the parameters mentioned was also observed for bilayers from other phospholipids.

In this paper we have investigated the connection between the waiting time of monolayer fusion, the pore edge line tension and the tension of bilayers formed from mixtures of phospholipids with brain gangliosides and cerebroside. The gangliosides have large hydrated oligosaccharide polar headgroups. Dispersed in water, they form micelles like phospholipid lysoforms and detergents (see, for example, Ref. 2). Proceeding from some properties of gangliosides, it should be expected that their inclusion into phospholipid membrane, by analogy with lysophosphatidylcholine, must decrease the pore edge line tension and the membrane tension and increase the waiting time of monolayer fusion. However, the study of the parameters mentioned for

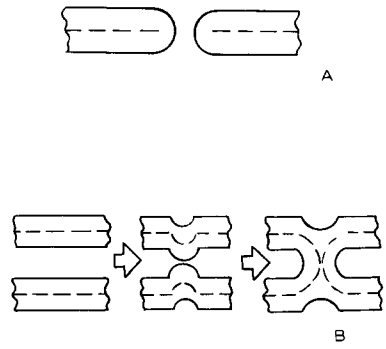

Fig. 1. Structural defects in a lipid bilayer. (A) A hydrophilic pore produced as a result of electrical breakdown. (B) Formation of a stalk-bridge between the contacting monolayers of two membranes. The dashed line is the boundary between the membrane monolayers.

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ganglioside-containing bilayers revealed the absence of a distinct correlation between them.

Materials and Methods

Human brain gangliosides were extracted by the method described in Ref. 3. We have used chromatographically pure egg phosphatidylethanolamine (PE) and phosphatidylcholine (PC), cerebroside and cardiolipin, which were solved in distilled *n*-decane or hexadecane. The measurements were carried out in an unbuffered KCl (chemically pure) solution (0.2 M), pH 6.1 ± 0.1.

The membranes were formed in the usual manner, on holes of diameter 1 mm [4]. The waiting time of monolayer fusion, τ_{mf}, was determined by measuring the time interval between the formation of a planar-parallel contact between the bilayers and the formation of a contact bilayer as a result of monolayer fusion. This parameter was registered visually and according to the change in the shape of the capacitive signal [1].

The pore edge line tension, γ, was determined by the dependence of the bilayer average life time on the voltage applied [1].

The membrane tension, σ, was determined by hydrostatic inflation of bilayers, as described in Ref. 5.

The capacity of bilayers, C_M, was determined by measuring the capacitive current, when a voltage of ±20 mV as a linear sweep wave form was applied from a generator at a rate of 100 V/s [6].

The measurement of the diameter of membranes and the visual control of their interaction were carried out using two binocular microscopes. In measuring the electrical characteristics of the bilayers, there were used Ag-AgCl electrodes connected with a Г6-28 generator (U.S.S.R.) and an operational amplifier, Keithley-427 (U.S.A.), to the output of which a storage oscilloscope, C8-13 (U.S.S.R.), was connected. The cell was thermostated at 30 °C.

Results

The hydrophobic part of ganglioside molecules consists of ceramide. The polar headgroups contain 2–4 saccharine radicals normally connected with 1–4 *N*-acetylneuraminic acid radicals carrying a negative charge (Fig. 2) [7]. As compared to usual phospholipids, the oligosaccharide polar headgroups of gangliosides are much larger and protrude from the bilayer surface by about 2 nm [8] into the solution surrounding the membrane. Cerebroside (Fig. 2), in contrast to gangliosides, does not contain *N*-acetylneuraminic acid, but consists of ceramide and galactose and practically does not swell in water [9]. In Fig. 2 the gangliosides and cerebroside are presented in succession of decreasing effective size of polar headgroups.

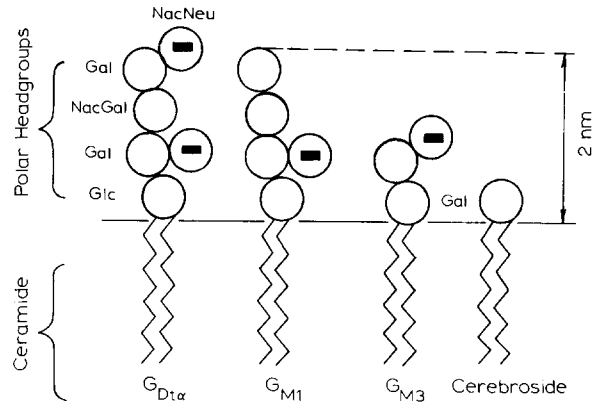

Fig. 2. Schematic diagram of $G_{D1\alpha}$, G_{M1}, G_{M3} and cerebroside molecules. The circles with a horizontal dash show the negative charged residues of *N*-acetylneuraminic acid (NAcNeu), and the circles labeled by Gal, Glu, NAcGal denote the galactose, glucose and *N*-acetylgalactosamine residues, respectively.

Fig. 3 shows the experimental dependence of the average life time of membranes, τ_l, on voltage U for bilayers from mixtures of PE with cerebroside and the gangliosides G_{M3}, G_{M1}, G_{D1a}. It also shows the theoretical curves 1 and 2 plotted according to the following formula [10]:

$$\tau_l = A \exp\left\{ \frac{\pi\gamma^2}{kT\left[\sigma + C_M((\epsilon_s/\epsilon_M)-1)\frac{U^2}{2}\right]} \right\} \quad (1)$$

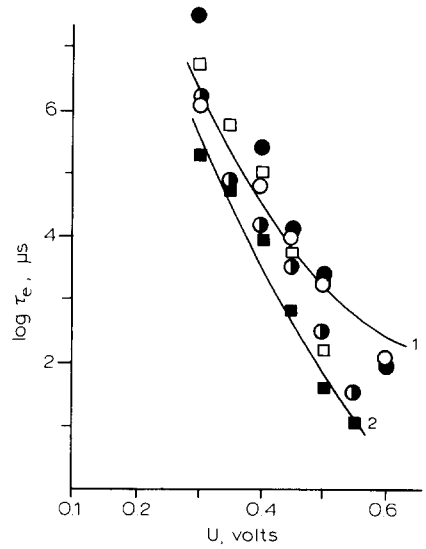

Fig. 3. The logarithm of the average life time of bilayers formed from mixtures of PE with gangliosides and cerebroside in decane as a function of the voltage applied. ○, PE membranes in decane; ●, PE/G_{D1a}; ◐, PE/G_{M1}; □, PE/G_{M3}; ■, PE/cerebroside. The percentage of gangliosides and cerebroside in membranes is 18 mol%. Curves 1 and 2 are the theoretical curves plotted according to Eqn. 1 for bilayers from PE ($\gamma = 1.5 \cdot 10^{-11}$N, $\sigma = 4.8$ mN/m, $C_m = 4.6$ mF/m^2) and PE/cerebroside ($\gamma = 2.2 \cdot 10^{-11}$N, $\sigma = 10$ mN/m, $C_m = 4.5$ mF/m^2), respectively. The standard errors of $\log\tau_l$ do not exceed the size of the symbols.

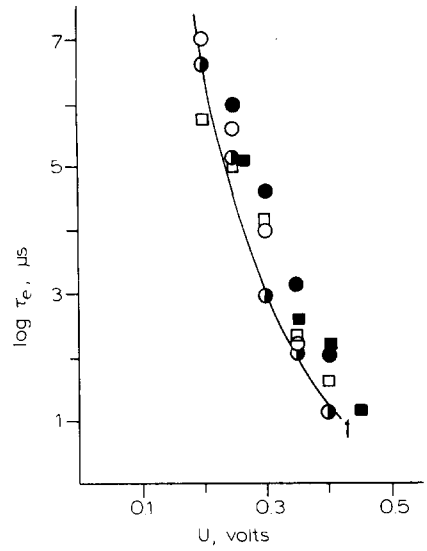

Fig. 4. The logarithm of the average life time of bilayers from mixtures of PC with gangliosides and cerebroside as a function of the voltage applied. ○, PC membranes in decane; ●, PC/G_{D1a}, ○, PC/G_{M1}; □, PC/G_{M3}, ■, PC/cerebroside. The percentage of gangliosides and cerebroside in membranes is 15 mol%. Curve 1 is the theoretical curve plotted according to Eqn. 1 for bilayers from PC/G_{M1} ($\gamma = 1 \cdot 10^{-11}$N, $\sigma = 0.9$ mN/m, $C_m = 3.6$ mF/m^2). The standard errors of $\log\tau_l$ do not exceed the size of the symbols.

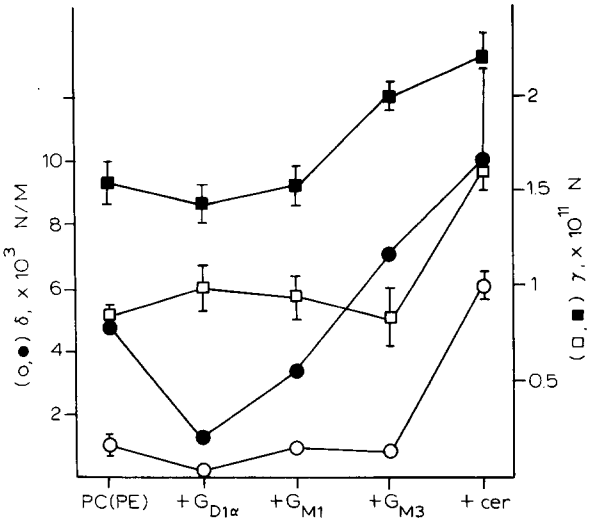

Fig. 5. The tension σ (●, ○) and the pore edge line tension γ (■, □) as functions of the effective size of ganglioside polar head groups. ● and ■, for bilayers from mixtures of PE with G_{D1a}, G_{M1}, G_{M3} and cerebroside, respectively; ○ and □, for bilayers formed from mixtures of PC with gangliosides.

where A is a pre-exponential factor, ϵ_s and ϵ_M are the dielectric permeability of the water solution and the membrane, respectively. The other parameters have their usual denotations. By measuring the specific capacity C_M and tension σ of the corresponding bilayers, one can determine that combination of the parameters γ and A, which provides the best fit of the theoretical curves to the experimental data [1] (see Table I).

Fig. 4 shows analogous dependences which characterize the electromechanical stability of bilayers formed from mixtures of PC with the gangliosides mentioned and the theoretical curve 1 for the mixture PC/G_{M1}.

The values of γ for membranes of different composition determined from the dependences shown in Figs. 3 and 4, are presented in Table I. There are also given the values of the specific capacity, tension and the waiting time of monolayer fusion of bilayers. It is obvious that in this case there is no correlation observed previously [1] between these parameters. Thus, G_{D1a} sharply de-

TABLE I

The specific capacity, the tension, the hydrophylic pore edge line tension and the waiting time of monolayer fusion of membranes formed from mixtures of PE and PC with gangliosides and cerebroside

Experimental conditions: 0.2 M KCl, pH 6.0, $t = 30$ °C. n.d., not determined

Lipid composition	Specific capacity (10^{-3} F/m^2)	Tension (10^{-3} N/m)	Line tension (10^{-11} N)	Waiting time of monolayer fusion τ_{mf} (min)
PE [a]	4.6±0.1	4.8 ±0.1	1.5 ±0.1	0
PE/G_{D1a} [b]	4.6±0.1	1.3 ±0.2	1.4 ±0.1	>15
PE/G_{M1}	4.3±0.1	3.3 ±0.4	1.5 ±0.1	0.4 (7.5 [c])
PE/G_{M3}	4.1±0.1	7.0 ±0.2	2.02±0.07	0
PE/cerebroside	4.5±0.2	10 ±3	2.2 ±0.1	0
PC	3.2±0.2	1.0 ±0.3	0.86±0.04	0
PC/G_{D1a}	3.8±0.2	0.23±0.06	1.0 ±0.1	15
PC/G_{M1}	3.6±0.3	0.9 ±0.2	1.0 ±0.1	n.d.
PC/G_{M3}	3.4±0.2	0.8 ±0.1	0.8 ±0.2	n.d.
PC/cerebroside	4.0±0.3	6.0 ±0.4	1.6 ±0.1	n.d.

[a] Bilayers were obtained from solutions of lipid mixtures in *n*-decane.
[b] Percentge of ganglioside in the mixtures with PE was 18 mol% and with PC, 15 mol%.
[c] The value of τ_{mf} for bilayers formed from lipid mixture in hexadecane.

TABLE II

The effect of the ionic strength and the Ca^{2+} concentration on the waiting time of monolayer fusion of the membranes formed from PE/G_{D1a} in decane (18 mol%)

Ionic strength	Waiting time of monolayer fusion (min)
0.2 M KCl	>15
0.2 M KCl + 9 mM Ca^{2+}	>15
0.2 M KCl + 15 mM Ca^{2+}	1
1 M KCl	>15
1 M KCl + 9 mM Ca^{2+}	>15
1 M KCl + 15 mM Ca^{2+}	2

creases σ and increases τ_{mf}, whereas γ varies weakly. σ also decreases in case of the PE/G_{M1} mixture, but γ and τ_{mf} do not practically change. For a more descriptive illustration of the dependence of γ and σ on the type of gangliosides, we have plotted a diagram (Fig. 5). The initial phospholipids (PE, PC) and their mixtures with gangliosides are plotted on the x-axis in the succession of decreasing effective size of their polar headgroups. γ and σ of the corresponding membranes are plotted on the y-axis. The value of γ changes weakly within the experimental errors in the following order: PE, PE/G_{D1a} PE/G_{M1}, and increases essentially for PE/G_{M3} and PE/cerebroside. An analogous order is observed for PC with the only difference that γ increases essentially only for the membranes from PC/cerebroside.

It is notable, that the increase in the ionic strength of KCl solution from 0.2 to 1 M does not lead to any significant decrease of τ_{mf} (Table II). Therefore, in the case with gangliosides the delay in the monolayer fusion cannot be caused by the electrostatic repulsion of charged bilayers. In the control experiments the value of τ_{mf} is close to zero for bilayers from PE/cardiolipin (18 mol%) in a 0.2 M KCl solution. Adding of Ca^{2+} ions into a 0.2 or 1 M KCl solution stimulates the monolayer fusion only at concentrations of ≥ 9 mM for PC/G_{D1a} and ≥ 15 mM for PE/G_{D1a}.

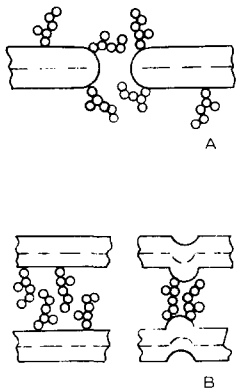

Fig. 6. Schematic diagram of a hydrophylic pore in bilayer (a) and contacting membranes (b) in the presence of gangliosides (the sugar residues of polar headgroups are labeled by circles). (A) gangliosides do not take part in hydrophilic pore formation, as a result of which γ of phospholipid membranes remains unchanged. (B) gangliosides either increase the equilibrium distance between the bilayers or prevent the local convergence of membranes, thus hindering the stalk formation (cf. Fig. 1).

Discussion

Investigation of the parameters γ, σ and τ_{mf} of phospholipid bilayers in the presence of different gangliosides and cerebroside has shown that there is no strong correlation between them. For phospholipid bilayers the stronger the tension σ, the higher the value of the pore linear tension γ, whereas the waiting time of monolayer fusion τ_{mf} is shorter [1]. The absence of correlation between the parameters given can be explained by the fact that the gangliosides have large hydrated oligosaccharide headgroups. Thus, the sharp decrease of σ at practically unchanged γ of bilayers formed from PE/G_{D1a} (Fig. 5, Table I) is, in our opinion, due to the fact that the size of the unhydrated polar headgroup of G_{D1a} (approx. 2 nm) is comparable to the critical radius of the pore, r^*, at which, according to the electrical breakdown theory [10], the membranes are irreversibly ruptured, r^* is 1.8 nm for bilayers from PE/G_{D1a} when the potential difference on the membrane is 0.3 V, and it decreases to 0.4 nm at 0.6 V. It is natural to assume that as a result of steric hindrances the ganglioside membranes do not enter the hydrophylic pores, which, consequently, totally consist of the phospholipid molecules of the initial bilayer (Fig. 6a). In this case γ should not change despite the essential change in σ. As the effective size of the ganglioside headgroups decreases, γ increases essentially starting from G_{M3} or cerebroside (see Fig. 5). Apparently, when reaching the critical size, the polar headgroups of the corresponding gangliosides can take part in hydrophilic pore formation and change the pore edge energy γ.

The strong suppression of monolayer fusion of bilayers formed from decane solutions of PC/G_{D1a} and PE/G_{D1a} is, in our opinion, due to the large hydrated headgroup of G_{D1a}. As follows from Table I, the ganglioside G_{M1} having a smaller polar headgroup, caused only an unessential increase in τ_{mf} for decane-containing membranes. At the same time, τ_{mf} for bilayers formed from the hexadecane solution of PE/G_{M1} (18 mol%), which contains practically no microlenses, sharply increased as compared to the control sample (see Table I). We have chosen decane as lipid solvent because of the low stability of bilayers formed from PC/ganglioside mixtures in hexadecane.

Suppression of 'adhesion' of two spherical phosphatidylcholine bilayers in the presence of 10–15 mol% brain gangliosides (total fraction) was earlier described in Ref. 11. According to the authors, the rate of mem-

brane adhesion increases at a lower ganglioside concentration (lower than 10 mol%). But the statement of spherical bilayer adhesion without fusion contradicts the data of the work mentioned as well as other data [13,15] on the increase in the specific capacity in the region of contacting by 2–2.8-times as compared to a single bilayer. In our opinion, this must be interpreted as monolayer fusion (also see Refs. 12 and 16). According to our data, the rate of increasing of the area of the contact bilayer formed by monolayer fusion, is proportional to the membrane tension, which describes well the effect of gangliosides and Ca^{2+} on the 'rate of adhesion' in Ref. 11.

There is no clearly defined mechanism of inhibition of monolayer fusion by gangliosides. One of the possible versions is an efficient increase in the equilibrium distance between the contacting membranes due to highly hydrated polar headgroups of gangliosides (Fig. 6B). It is known that the ganglioside polar headgroups bind about 60 water molecules at hydration [17]. Besides, there are data (see Ref. 18), according to which the gangliosides stabilize the hydrated state of phospholipid membranes. Another possible mechanism is, that there might be steric hindrances on the way to drawing bilayers together, which is necessary to overcome the hydration barrier and the stalk formation [13,14] (Fig. 6B). Under the last assumption, to provide monolayer fusion there must be a lipid domain large enough for a local contact and stalk formation. The characteristic size of such a domain can be estimated from the minimum content of the ganglioside G_{D1a} in a bilayer formed from PE, which affects τ_{mf}: it is about 10 mol% which corresponds to a phospholipid domain with a diameter of approx. 3 nm (the diameter of the zero-radius stalk is approx. 4 nm [13,14]). Stimulation of monolayer fusion in the presence of Ca^{2+} (approx. 10 mM) can be explained by lateral phase segregation of PE or PC and G_{D1a} in a bilayer under the action of that cation, i.e., by separation of phospholipid domains [19,20].

We hope that a more detailed investigation of monolayer fusion of ganglioside-containing bilayers will enable us to understand the effect of gangliosides on the waiting time of monolayer fusion.

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