

Attachment of β_2 -glycoprotein I to negatively charged liposomes may prevent the release of daughter vesicles from the parent membrane

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Abstract The temperature-induced budding of POPC- β_2 -GPI molecules attached to the negatively charged cardiolipin-cholesterol, POPC-POPS-cholesterol and POPC-membrane surface may explain the observed attraction of POPG-cholesterol giant lipid vesicles in the presence of β_2 -glycoprotein I (β_2 -GPI) in the outer solution was studied experimentally and theoretically. The observed budding transition of vesicles was continuous which can be explained by taking into account the orientational ordering and direct interactions between oriented lipids. The attachment of positively charged β_2 -GPI to the negatively charged outer surface of POPC-cardiolipin-cholesterol, POPC-POPS-cholesterol and POPC-POPG-cholesterol giant vesicles caused coalescence of the spheroidal membranes that are involved in the budding process (Miao et al. 1994; Sens and Turner 2004; Laradji and Kumar 2004; Božič et al. 2006; Iglič et al. 2007a,b; Sens and Goj 2007). Theoretically, the protein-mediated attraction between the membrane of a bud and the parent membrane was described as an interaction between two electric double layers. It was shown that the specific spatial distribution of charge within

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The budding of the bilayer membrane is a process, vitally important for cells (Greenwald 2006; McMahon and Gallop 2005). Accordingly, it is of interest to understand mechanisms that are involved in the budding process (Miao et al. 1994; Sens and Turner 2004; Laradji and Kumar 2004; Božič et al. 2006; Iglič et al. 2007a,b; Sens and Goj 2007). An important role of membrane budding has been found in immune response and pathologic conditions (Farsad and De Camilli 2003; Greenwald 2006). It was shown that membrane skeleton-detached, laterally mobile membrane lipids and integral membrane protein components or their small complexes (membrane nanodomains) may sort in highly curved spherical or tubular regions of cell membranes depending on their intrinsic shape and/or direct interactions between them (Farsad and De Camilli 2003; Huttner and Zimmerberg 2001; Holopainen et al. 2000; Hägerstrand et al. 2006; Iglič et al. 2007a). Clustering of membrane components or membrane nanodomains in highly curved membrane regions therefore plays an important role in generation and stabilization of spherical and tubular membrane protrusions (Farsad and De Camilli 2003; Huttner and Zimmerberg 2001; Iglič et al. 2007b).

To understand the basic physical properties of membrane budding, the budding process has been studied in a simple system of bilayer membrane vesicles composed of a single phospholipid species (Sackmann 1994; Lipowsky 1991; Miao et al. 1994) where changes of the temperature

may cause changes of the vesicle shapes and may in certain conditions induce formation of buds of the membrane lipid vesicles was induced by increase of temperature and bilayer (Käs and Sackmann 1991).

Certain proteins that are present in human plasma and membrane (Fig. 1). In general, the binding of proteins to membrane surface were found to be driven by electrostatic forces and by penetration of protein hydrophobic protrusions in structures such as microvesicles and lipoproteins (Beveridge et al. 2005; Distler 2005). It was shown that β_2 -glycoprotein I (β_2 -GPI) can attach to the negatively charged lipid bilayer surface (Asherson et al. 1989; Roubey et al. 2000) and can induce adhesion between negatively charged giant phospholipid vesicles containing cardiolipin (Ambroz et al. 2006; Urbanija et al. 2007). β_2 -GPI is also able to induce the agglutination of mitochondria (Schoups 1979) whose membrane contains negatively charged cardiolipin and was found to bind to negatively charged (Khalil et al. 2006) low-density lipoprotein (LDL) nanoparticles (Polz and Kostner 1979), which contain the majority of cardiolipin in human plasma (Deguchi et al. 2000).

The majority of physiologic functions of β_2 -GPI are associated with its binding to negatively charged lipids, such as phosphatidylserine and cardiolipin, demonstrated in the in vitro studies (Hagihara et al. 1995; Wang et al. 2002). The electrostatic interactions of β_2 -GPI with negatively charged membrane phospholipids are considered crucial for its physiological and pathogenic roles. It was suggested that β_2 -GPI may have an important function in blood coagulation, in the clearance of phosphatidylserine- and cardiolipin-containing negatively charged apoptotic bodies from the circulation (Brighton et al. 1996; Balasubramanian and Schroit 1998) and in the membrane budding process (Urbanija et al. 2007). As β_2 -GPI is a major autoantigen for the production of antiphospholipid antibodies, it may play an important role in many diseases including antiphospholipid syndrome (Bouma et al. 1999; Miyakis et al. 2004). Two subgroups of antiphospholipid antibodies represent the laboratory criteria for antiphospholipid syndrome: β_2 -GPI dependent anticardiolipin antibodies (usually designed as anticardiolipin antibodies) and antibodies against β_2 -GPI (Miyakis et al. 2006).

Our study was focused on the influence of β_2 -GPI membrane binding on the budding transition of negatively charged 1-palmitoyl-2-oleoyl-sn-glycero-phosphatidylcholine-cardiolipin-cholesterol (POPC-cardiolipin-cholesterol), POPC-1-palmitoyl-2-oleoyl-sn-glycero-phosphatidylserine-cholesterol (POPC-POPS-cholesterol) and POPC-1-palmitoyl-2-oleoyl-sn-glycero-phosphatidyl-glycerol-cholesterol (POPC-POPG-cholesterol) giant vesicles. Using the negatively charged giant lipid vesicles we try to mimic the negatively charged surface of cells such as apoptotic cells and/or particles that have negatively charged lipids (phosphatidylserine or cardiolipin) in their outer membrane lipid layer.

In this work, the budding of negatively charged giant membrane lipid vesicles was induced by increase of temperature and by binding of β_2 -GPI to the outer lipid layer of the vesicle membrane (Fig. 1). In general, the binding of proteins to membrane surface may be driven by electrostatic forces and by penetration of protein hydrophobic protrusions in the membrane bilayer (Farsad and De Camargo 2003; Masuda et al. 2006). Positively charged proteins can bind to the negatively charged outer surface of cells, lipid vesicles or lipoproteins, where the electrostatic attractive forces may play a decisive role. In the case of β_2 -GPI it was shown that the character of membrane-GPI interactions (binding) is at least partially of an electrostatic nature, i.e. it is governed by an electrostatic attraction between the positively charged fifth domain of β_2 -GPI and negatively charged lipid headgroups (Willems et al. 1996; Gamsjaeger et al. 2005) (Fig. 1). Strong attractive interaction of highly positively charged fifth domain of β_2 -GPI with the membrane surface is possible only if the membrane surface is negatively charged (Willems et al. 1996; Gamsjaeger et al. 2005). The strength of membrane- β_2 -GPI interactions increases with increasing fraction of negatively charged lipids in the membrane, while it is suppressed by increased ionic strength in the solution (Gamsjaeger et al. 2005). The hydrophobic protrusion of membrane-attached β_2 -GPI which is embedded in the outer membrane layer (Fig. 1) increases the strength of β_2 -GPI membrane attachment (binding) (Bouma et al. 1999).

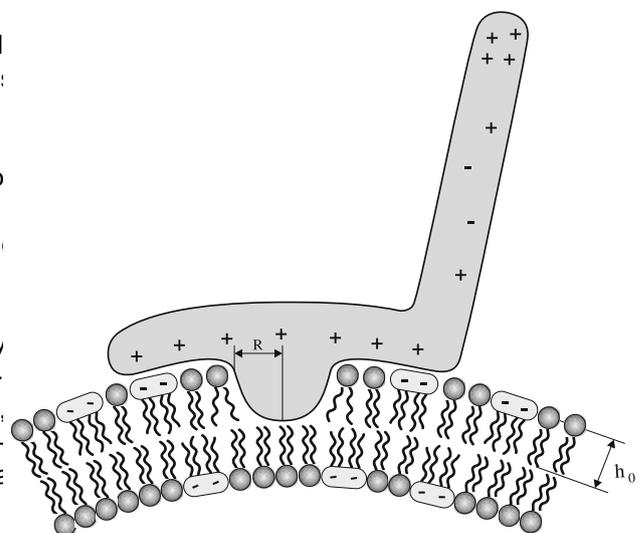


Fig. 1 Schematic figure of a positively charged β_2 -GPI molecule attached to the outer surface of a negatively charged bilayer membrane of giant lipid vesicles containing negatively charged molecules of cardiolipin (bearing two negative unit charges each). The hydrophobic protrusion of the membrane attached β_2 -GPI which is embedded in the outer lipid layer is also shown

Fig. 2 Budding of POPC–cardiolipin–cholesterol vesicle induced by increasing the temperature in the presence of β_2 -GPI in the outer solution: the first stage

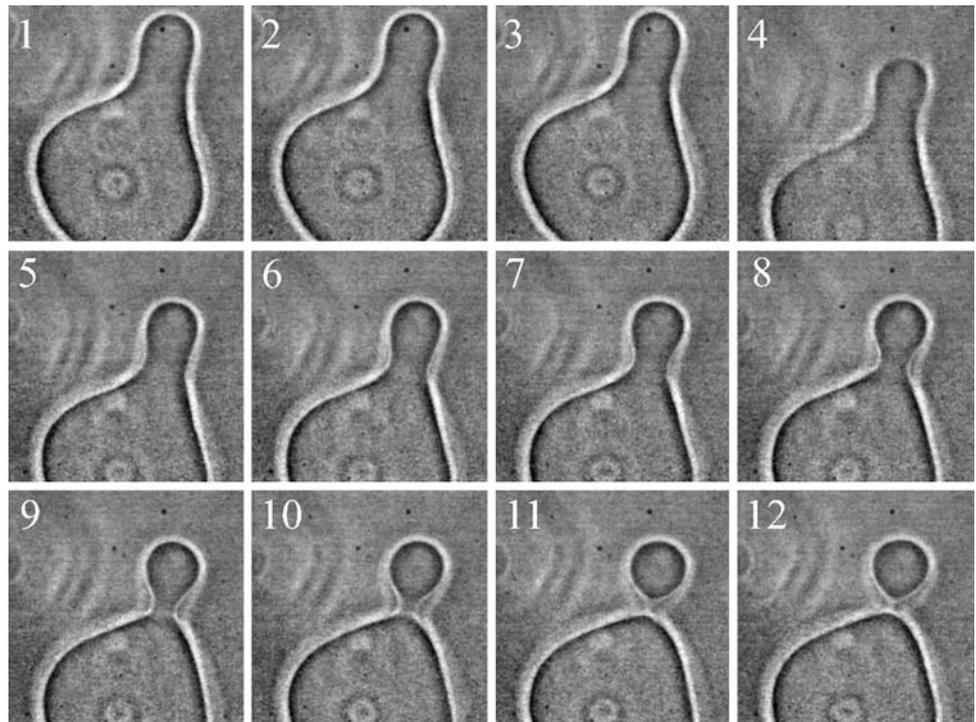
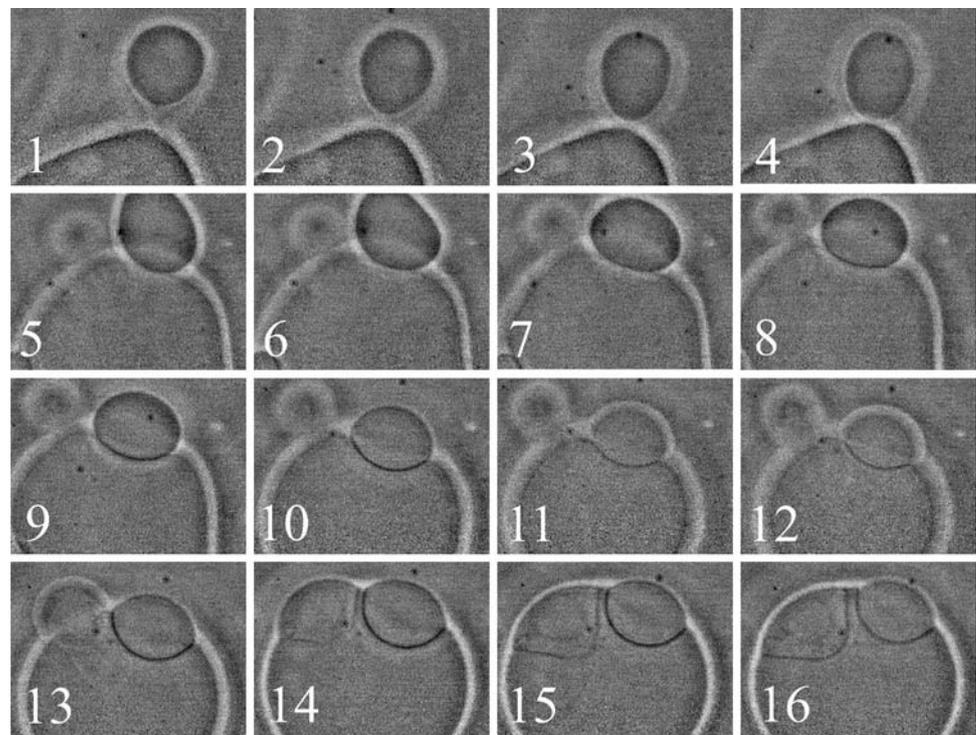


Fig. 3 Budding of POPC–cardiolipin–cholesterol vesicle induced by increasing the temperature in the presence of β_2 -GPI in the solution: the second stage. Adhesion of the daughter vesicle to the outer surface of the mother membrane can be observed



Theoretical discussion

Budding transition

In the observed budding transition of POPC–cardiolipin–cholesterol vesicles the initial pear-like shape continuously (smoothly) transforms into a limiting shape composed of a larger spheroidal parent vesicle and a smaller spheroidal daughter vesicle which are connected by a thin neck

In general, similar previously observed temperature-induced budding of liposomes was continuous or

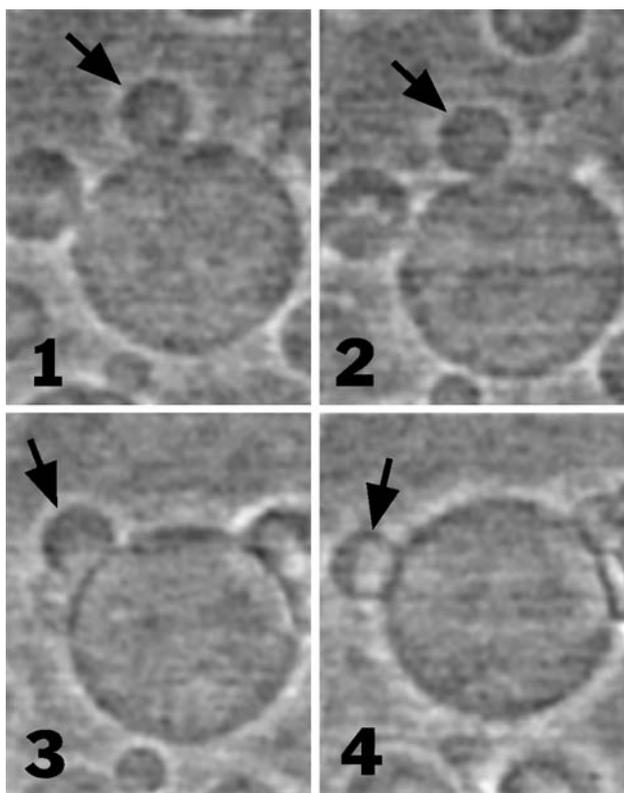


Fig. 4 Budding of POPC–POPS–cholesterol vesicle induced by increasing the temperature in the presence of β_2 -GPI in the solution: the second stage. The black arrows show the daughter vesicle which is in the nal phase adhered to the mother membrane

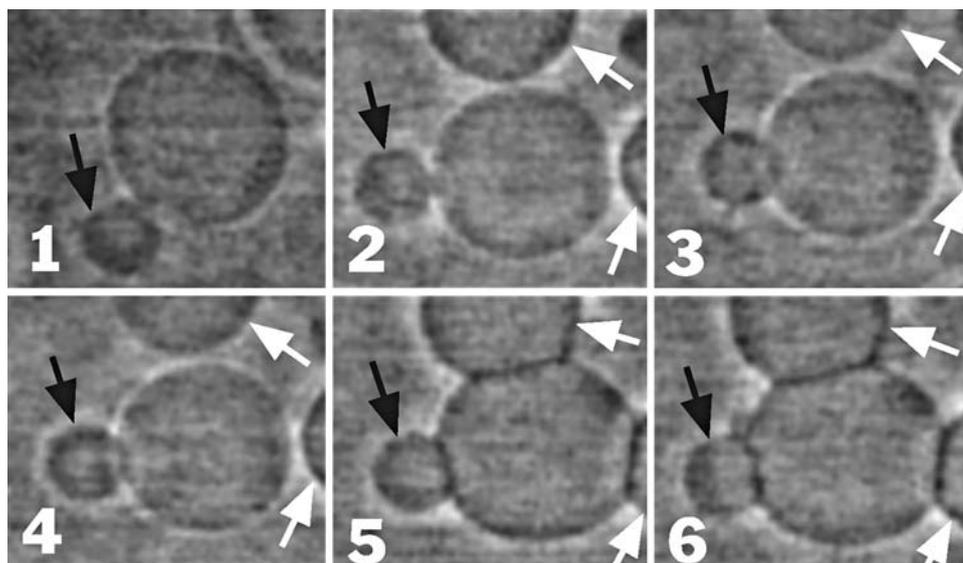
limiting shapes (Ka and Sackmann 1991). The area-difference-elasticity (ADE) model, based on the minimization of the local and non-local bending energy (Evans and Skalak 1980 Stokke et al. 1986 Helfrich 1974), cannot explain the above-mentioned continuous shape transitions and/or discontinuity in the vicinity of limiting shapes (Miao et al 1994). Namely, according to predictions of the ADE model the shape transition from the pear-like shape to the limiting shape composed of larger spheroidal parent vesicle and smaller spheroidal daughter vesicle is always discontinuous (Fig. 6a).

In accordance with the results of some previous studies (Kralj-Iglic et al. 2002, 2006) in this work the continuous transition and a discontinuity in the vicinity of the limiting shape are explained by taking into account the orientational ordering and direct interactions between oriented lipids. The intrinsic properties of membrane lipids and interactions between them are thereby assumed to influence the macroscopic features such as the equilibrium shape of the vesicle and/or the budding process of the membrane (Kralj-Iglic et al. 2006).

We assume that a single lipid molecule, due to its structure and local interactions, energetically prefers a local geometry that is described by the two intrinsic principal curvatures C_{1m} and C_{2m} (Kralj-Iglic et al. 2006). The intrinsic principal curvatures are in general not identical (Fig. 6). If they are identical ($C_{1m} = C_{2m}$), the in-plane orientation of the lipid molecule is immaterial. Such lipid molecule is called isotropic. If $C_{1m} \neq C_{2m}$ the lipid molecule is called anisotropic (Fig. 6). The orientation of such lipid molecule is important for its energy. It was assumed that the lipid molecule will spend on the average more time in the orientation that is energetically more favourable than in any other orientation (Kralj-Iglic et al. 2006).

discontinuous (Ka and Sackmann 1991). It was observed that vesicles formed of charged lipids can undergo continuous transitions to an outside budded state via the pear shape, while in pure vesicles made from POPC or DMPC the budding seems to be discontinuous in the vicinity of

Fig. 5 Budding of POPC–POPG–cholesterol vesicle induced by increasing the temperature in the presence of β_2 -GPI in the solution: the second stage. The black arrows show the daughter vesicle which is in the nal phase adhered to the mother membrane, while white arrows show the two neighbouring vesicles which are also adhered to the observed vesicle



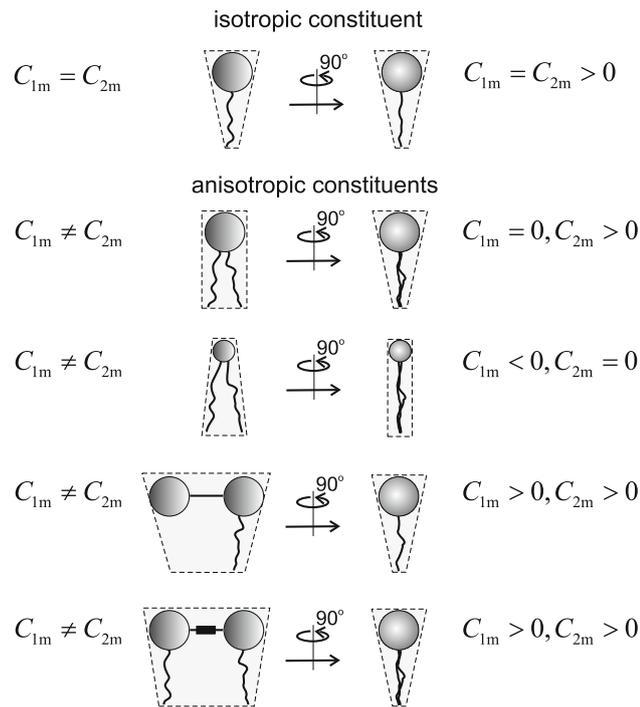


Fig. 6 Schematic representation of different intrinsic shapes of some isotropic and anisotropic membrane constituents. Front and side views are shown

Taking into account the orientational ordering and direct interactions between oriented lipids the expression for the free energy of a closed bilayer lipid vesicle can be written in the form (Kralj-Iglic et al. 2006):

$$F = \frac{k_c}{2} \int (2H)^2 dA + 2k_r A (\langle H \rangle - H_0)^2 - 2m_0 kT \int \ln \cosh \left(\frac{(\xi + \xi^*) D_m D}{2kT} (1 + \tilde{k}/kT) \right) dA. \quad (1)$$

The first term in Eq. 1 yields the local bending energy of a closed lipid bilayer vesicle (Helfrich 1973). The second term accounts for the nonlocal isotropic bending elasticity of the lipid bilayer membrane (Evans and Skalak 1980; Miao et al. 1994) while the third term accounts for the orientational ordering of lipid molecules which includes also the direct interaction contribution. Here $k_c = (3\xi + \xi^*) m_0 / 4$ is the local bending constant, $H_0 = (C_1 + C_2)/2$ is the mean curvature of the membrane, $\langle H \rangle = \frac{1}{A} \int H dA$ is average mean curvature, A is the membrane area, $D = (C_1 - C_2)/2$ is curvature deviator, C_1 and C_2 are two principal curvatures, C_{1m} and C_{2m} are intrinsic principal curvatures describing the intrinsic shape of the lipid molecules (see Fig. 6), $H_m = (C_{1m} + C_{2m})/2$ is intrinsic mean curvature, $D_m = (C_{1m} - C_{2m})/2$ is intrinsic curvature deviator, ξ and ξ^* are interaction constants, m_0 is the average area density of the lipid

molecules, k_r is the nonlocal bending constant and H_0 determines the average mean curvature of the membrane under lowest possible stress. The constant \tilde{k} describes the direct interactions between locally oriented lipid molecules (Kralj-Iglic et al. 2006). The value $\tilde{k} = 0$ corresponds to the case when direct interactions are not taken into account.

The binding of β_2 -GPI to the outer lipid monolayer results in an increase of its area which is the consequence of the incorporation of the hydrophobic part of the membrane bound β_2 -GPI in the outer lipid monolayer (see Fig. 1). Consequently, the binding of β_2 -GPI facilitates the observed budding of giant lipid vesicles induced by increase of the temperature.

For thin and not too strongly curved lipid bilayers the average mean curvature $\langle H \rangle$ is proportional to the area difference between the two membrane monolayers ($\langle H \rangle = \Delta A / 2A\delta$, where δ is the distance between the two monolayer neutral surfaces). Therefore, we can write the spontaneous average mean curvature as a function of the total number of membrane β_2 -GPI molecules bound in the outer layer (M) as

$$H_0 = \bar{H}_0 + M\pi R^2 / 2A\delta, \quad (2)$$

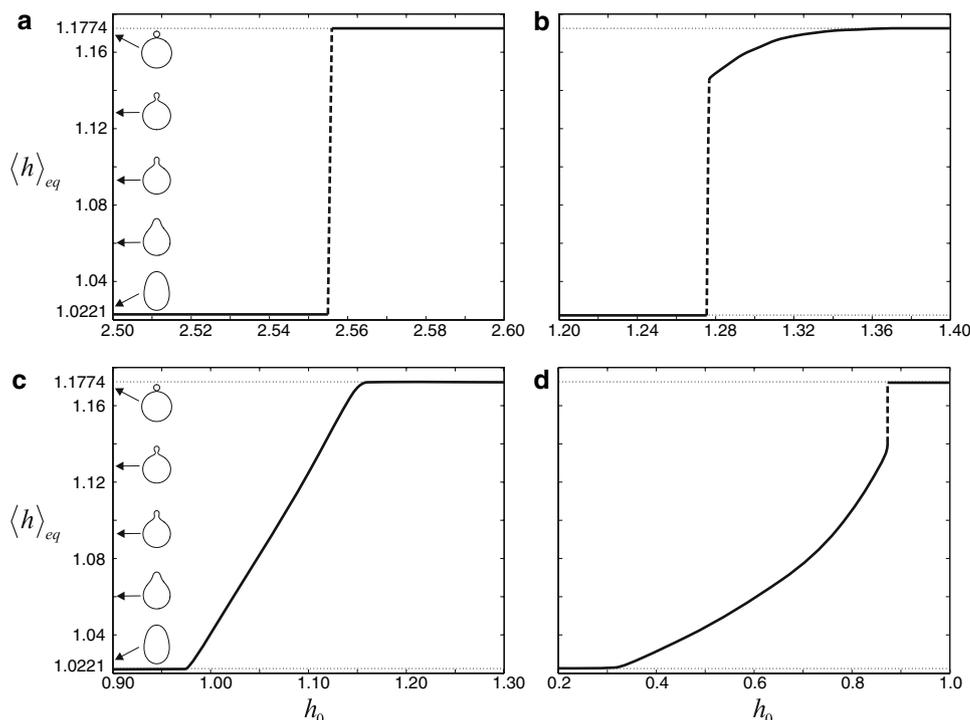
where R is the average radius of the hydrophobic protrusion of the attached β_2 -GPI which is embedded in the outer lipid layer (Fig. 1). The value of \bar{H}_0 increases with increasing temperature (Kraus and Sackmann 1991).

The equilibrium configuration of the closed axisymmetric bilayer vesicles and the corresponding distribution of the quadrupolar ordering was determined by minimizing the membrane free energy under relevant geometrical constraints as described in detail elsewhere (Iglic et al. 1999; Bozic et al. 2006; Iglic and Kralj-Iglic 2006). We require that the normalized membrane area $A/4\pi R_s^2 = 1$ and normalized enclosed volume $V/4\pi R_s^3$ are fixed. For the sake of simplicity we considered only the pear-shaped axisymmetric vesicle shapes. The corresponding shape equation was solved as described in Kralj-Iglic et al. (2006).

The equilibrium vesicle shapes as a function of normalized spontaneous average mean curvature $R_s H_0$ was calculated for given relative cell volume. Figure 7 thus shows the equilibrium value of normalized average mean curvature $\langle h \rangle = R_s \langle H \rangle$ as a function of h_0 . Case A corresponds to area-difference-elasticity (ADE) model, where the free energy consists of the local and nonlocal isotropic bending energy terms only (first two terms in Eq. 1), while in cases B–D we took into account also quadrupolar ordering of lipid molecules and the direct interactions between them ($\tilde{k}/kT \neq 0$).

Within so-called area-difference-elasticity (ADE) model the absolute minimum of the membrane free energy may be

Fig. 7 Calculated normalized average mean curvature corresponding to the minimum of the membrane energy ($\langle h \rangle_{eq} = R_s \langle H \rangle_{eq}$) as a function of h_0 for pear-shaped axisymmetric vesicle shapes with a relative volume $v = 0.95$ and $k_t/k_c = 2$ (Hwang and Waugh 1997). **a** Corresponding dependency of $\langle h \rangle_{eq}$ within ADE model where the orientational ordering of interacting lipid molecules is not taken into account. Calculated dependencies **b** and **c** take into account the orientational ordering of lipids and direct interactions between oriented lipids, where $\xi + \xi^* = D_m/4kTR_s = 1.5 \times 10^{-4}$, $4kTR_s^2/(3\xi + \xi^*) = 7 \times 10^6$ (Kralj-Iglic et al. 2006) and $\bar{k}/kT = 0.67$ (**b**), 0.8 (**c**) and 1.0 (**d**). Figure also shows some characteristic vesicle shapes corresponding to different values of $\langle h \rangle_{eq}$



by an appropriate choice of the parameters k_t/k_c and H_0 (Miao et al. 1994) where the transition is always discontinuous (Fig. 7a). However, a considerably higher value of k_t/k_c than the experimentally estimated one (Hwang and Waugh 1997) is needed to obtain this effect (Miao et al. 1994) or alternatively the values of H_0 should be larger than any of the sequence of pear shapes (Fig. 7). This gives a significant increase of the membrane free energy and of the lateral tension within the membrane. It seems unlikely that the vesicle would favour high lateral membrane tension within the membrane as it may develop processes to relax it, as for example transient pore formation (Raphael and Waugh 1996; Holopainen et al. 2002).

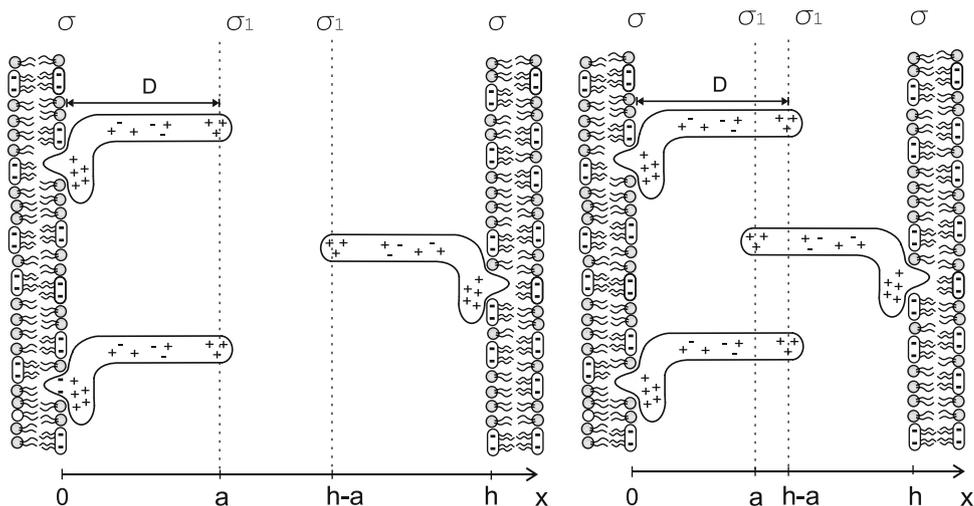
We have shown that a decrease of the free energy due to the orientational ordering of phospholipid molecules and direct interactions between oriented lipid molecules may complement the nonlocal isotropic bending in stabilizing pear vesicle shapes, including shapes with thin neck(s). We suggest that β_2 -GPI induced coalescence of spheroidal vesicles should be also noted that the calculated equilibrium vesicle bud with the parent membrane is of electrostatic nature, i.e. shapes determined by minimization of the membrane free energy given by Eq. 1 can have also a wider neck (see Fig. 7c) corresponding to deep minima of the membrane free energy that would exceed the energies of thermal fluctuations. Minimization of the membrane free energy also given by Eq. 1 predicts the continuous transition between pear-shaped vesicles (Fig. 7c) or discontinuity in the vicinity of limiting shapes composed of spherical parent cell and spherical daughter vesicle (Fig. 7d) which is not possible within the ADE model (Kerker and Sackmann 1991;

Miao et al. 1994) where the transition is always discontinuous (Fig. 7a). To conclude, including the orientational ordering of lipids and their direct interactions in calculating the equilibrium vesicle shapes may render a minimum of the vesicle membrane free energy close to the shape with the narrow neck also for low values of H_0 . Contrary to the predictions of ADE model, both, the continuous shape transitions and discontinuous shape transitions in the vicinity of limiting shapes are possible in accordance with experimental observations.

Coalescence of the bud with the parent vesicle

An important result of this work is that if β_2 -GPI was present in the solution, the membrane bud coalesced with the mother vesicle before it could detach from it (Fig. 7). We suggest that β_2 -GPI induced coalescence of spheroidal vesicles should be also noted that the calculated equilibrium vesicle bud with the parent membrane is of electrostatic nature, i.e. shapes determined by minimization of the membrane free energy given by Eq. 1 can have also a wider neck (see Fig. 7c) corresponding to deep minima of the membrane free energy that would exceed the energies of thermal fluctuations. Minimization of the membrane free energy also given by Eq. 1 predicts the continuous transition between pear-shaped vesicles (Fig. 7c) or discontinuity in the vicinity of limiting shapes composed of spherical parent cell and spherical daughter vesicle (Fig. 7d) which is not possible within the ADE model (Kerker and Sackmann 1991;

Fig. 8 Schematic presentation shows two different regimes regarding the distance between the lipid surfaces $h \geq 2D$ (left) and $h < 2D$ (right)



domain binds strongly to the membrane surface (see Fig. 1) because of electrostatic attraction and also because of the insertion of hydrophobic loop into the membrane (Bouma et al. 1999; Schwarzenbacher et al. 1999).

In our theoretical model we consider two planar charged lipid (containing either cardiolipin, POPS or POPG) surfaces with electrolyte (salt) solution between the surfaces (Fig. 8). Because of positively charged first domains of membrane-bound β_2 -GPI an apparent positively charged region approximately at the distance of β_2 -GPI's length D away from each surface is created (Fig. 8). In the model the positive charge of the tips of the β_2 -GPI molecules bound to both surfaces is represented by two charged surfaces (with the surface charge densities σ) at the distance D from the each of lipid surfaces (Fig. 8).

The electric charge distribution of both cardiolipin containing lipid surfaces is in the first approximation described by surface charge densities of both lipid surfaces (σ), where the contribution due to first domain of bound β_2 -GPI molecules is neglected.

If the distance between the lipid surfaces (h) is larger than $2D$, the space between both lipid surfaces can be divided into three different regions $0 \leq x \leq a$, $a < x \leq (h-a)$ and $(h-a) < x \leq h$ (see Fig. 8). If $h \geq 2D$ the value of $a = D$, while in the case $h < 2D$ the value of a depends on h and is always smaller than D (Fig. 8).

It is well known that linearized Poisson–Boltzmann (PB) theory overestimates electrostatic free energies for lipid membranes. Nevertheless, to keep our model traceable we adopt the result of linearized PB theory, i.e. the electrostatic potential $\psi(x)$ in the system was calculated from linearized PB (LPB) equation:

$$\nabla^2 \phi = \kappa_d^2 \phi, \tag{3}$$

where $\phi = e_0 \psi / kT$ is dimensionless electrostatic potential and the Debye length $\kappa_d^{-1} = \sqrt{\epsilon_w \epsilon_0 kT / (2n_0 N_A e_0^2)}$. Here,

ϵ_w is the dielectric constant of the aqueous solution, ϵ_0 is the permittivity of free space, n_0 is the ionic strength in the bulk solution (i.e. bulk salt concentration; assuming a 1:1 salt such as NaCl), N_A is Avogadro's number and e_0 is the elementary charge.

The bulk solution (outside the space between the surfaces) provides a suitable reference for the electric potential (i.e. $\psi = 0$).

Due to the symmetry of the system we are searching for the solution of Eq. 3 only in the region $0 \leq x \leq h/2$ (see Fig. 8). The solution of Eq. 3 can be then written as

$$\phi = A \exp(-\kappa_d x) + B \exp(\kappa_d x), \quad 0 \leq x \leq a, \tag{4}$$

$$\phi = C \exp(-\kappa_d x) + D \exp(\kappa_d x), \quad a \leq x \leq h/2, \tag{5}$$

where the constants A , B , C and D were determined analytically from the boundary conditions $\frac{d\phi}{dx}(x=0) = -\sigma e_0 / \epsilon_w \epsilon_0 kT$, $\phi(x=a_-) = \phi(x=a_+)$, $\frac{d\phi}{dx}(x=a_-) = \frac{d\phi}{dx}(x=a_+) + \sigma_1 e_0 / \epsilon_w \epsilon_0 kT$ and $\frac{d\phi}{dx}(x=h/2) = 0$.

Including also the configurational entropy of the anions ($i = 1$) and cations ($i = 2$) of the salt dissolved in the water between both planar lipid surfaces, we can write the free energy of the system in the form (Kralj-Iglić and Iglič 1996; Evans and Wennerström 1999):

$$F/A = \int_0^h \left(\frac{1}{2} \epsilon_w \epsilon_0 \left(\frac{d\phi}{dx} \frac{kT}{e_0} \right)^2 + kT \sum_{j=1}^2 \left(n_j \ln \left(\frac{n_j}{n_0} \right) - (n_j - n_0) \right) \right) dx, \tag{6}$$

where n_j are the number densities of anions ($j = 1$) and cations ($j = 2$) in the salt solution, n_0 is the number density of the anions and cations in the bulk solution (i.e. outside the space between the planar lipid surfaces). The bulk solution provides a suitable reference also for electric potential $\phi_{\text{bulk}} = 0$ (see also Evans and Wennerström 1999).

Figure 9 shows the free energy ΔF of the system as a function of the distance h between the two adjacent membrane surfaces with attached β_2 -GPI. As it can be seen in Fig. 9 that for small values of σ_1 the free energy ΔF increases with decreasing inter membrane distance h while for larger values of σ_1 the free energy decreases with decreasing h until the absolute minimum of ΔF close to $h \cong D$ is reached.

The results presented in Fig. 9 reflect the fact that two adjacent membranes without bound β_2 -GPI repel each other while for high enough concentration of membrane bound β_2 -GPI the force between two negatively charged membranes (containing either cardiolipin, POPS or POPG) becomes strongly attractive leading to the equilibrium distance $h \cong D$. The origin of attractive interactions between two like-charged membrane surface is the electrostatic attraction between the positively charged domain on the tip of the membrane-bound β_2 -GPI and negatively charged opposite membrane (Fig. 9). The observed weak β_2 -GPI-induced interaction between zwitterionic POPC–cholesterol vesicles indicates that the proposed attractive Coulomb (charge–charge) interactions between positively charged part of the first domain of membrane bound β_2 -GPI and the negatively charged phospholipids of the adjacent membrane is not the only possible interaction responsible for β_2 -GPI-mediated agglutination between vesicle membrane surfaces. In general, also the charge–dipole and dipole–dipole electrostatic interaction (Israelachvili 1997) between the lipid head-group electric dipole moment and β_2 -GPI may contribute

to the β_2 -GPI-induced agglutination between like-charged membrane surfaces.

It was suggested that adhesion of the buds to the mother membrane due to β_2 -GPI-mediated interactions between the bud and the mother cell membranes may take place also in vivo (Urbanija et al. 2007). According to this suggestion, membranes subjected to stronger mediating effect of proteins in the extracellular solution would shed into the circulation less microvesicles than membranes subjected to weaker mediating effect of extracellular proteins. As increased number of microvesicles was found in peripheral blood of patients with thrombosis (Diamant et al. 2004), compounds that mediate a short ranged attractive interactions between membranes are considered anticoagulant while compounds which impair the effect of adhesion mediators are considered procoagulant. Our results indicate that β_2 -GPI, which agrees with observations that a decrease in plasma β_2 -GPI level (Brighton et al. 1996) and an increase in the level of pro-thrombogenic microvesicles (Dignat-George et al. 2004) was observed in patients with disseminated intravascular coagulation.

Conclusions

Effect of the membrane-attached proteins on the membrane elastic properties can have important physiological consequences. Some proteins that are present in human plasma and are able to attach to the membrane surface were found to mediate interactions between phospholipid-containing structures such as cell membranes, microvesicles and lipoproteins (Bever et al. 2005; Distler 2005). Modifications of membrane bending rigidity due to the attached membrane proteins (Igliet al. 2007) may affect the fluctuations of the membrane and in this way may additionally influence the adhesion process (Helfrich 1995).

In this work, we observed the coalescence of the spherical membrane bud with the parent vesicle before the bud could detach from parent vesicle membrane, i.e. vesiculate. It is proposed that the attachment of positively charged parts of β_2 -GPI to the negatively charged outer surface of POPC–cardiolipin–cholesterol, POPC–POPS–cholesterol or POPC–POPG–cholesterol giant lipid vesicles may cause (at least partially) the observed adhesion of like-charged lipid surfaces. Theoretically β_2 -GPI-mediated interaction between the membrane of the bud and the membrane of the parent vesicle was explained by a specific spatial distribution of charge within β_2 -GPI attached to the negatively charged membrane surfaces containing either cardiolipin, POPS or POPG.

The observed adhesion of phospholipid surfaces, mediated by β_2 -GPI may play an important role in

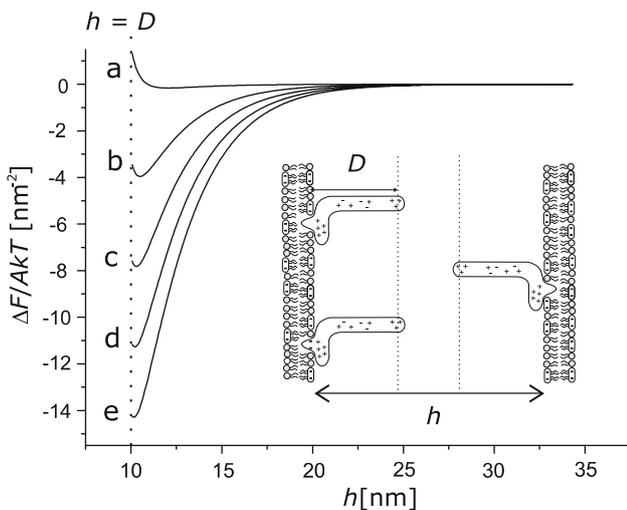


Fig. 9 Free energy $\Delta F = F - F(h \rightarrow \infty)$ as a function of h for five different values of σ_1 : 0.002 As/m² (a), 0.003 As/m² (b), 0.004 As/m² (c), 0.005 As/m² (d), 0.006 As/m² (e). Length of the β_2 -GPI molecule $D = 10$ nm. Values of surface charge density at lipid surface and buffer concentration are constant: $\sigma_2 = -0.05$ As/m². Salt concentration in the bulk solution $m_0/N_A = 15$ mmol/l, where N_A is Avogadro's number

microvesiculation of membranes and therefore in blood clot formation.

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