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Journal of Biomechanics 40 (2007) 2492–2500

JOURNAL OF BIOMECHANICS

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Elastic properties of biological membranes influenced by attached proteins

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Accepted 8 November 2006

Abstract

Positively charged proteins can attach themselves to the negatively charged outer surface of biological cell membranes and liposomes. In this work, the influence of the intrinsic shape of the membrane-attached proteins on the elastic properties of the membrane is considered theoretically. It is shown that attachment of anisotropic proteins to the outer surface of biological membranes may induce tubulation of the membrane. The attachment of semi-flexible rod-like proteins increases the local bending constant, while the attachment of semi-flexible plate-like anisotropic proteins may also reduce the local bending constant of the membrane. The role of the hydrophobic protrusion of the attached protein which is embedded in the outer membrane layer is also discussed.

Keywords: Membrane biomechanics; Membrane-attached proteins; Anisotropic plate-like proteins; Membrane elastic energy

1. Introduction

Intracellular and intercellular traffic requires the generation of highly curved membrane structures in the form of small membrane carrier vesicles and/or long thin nanotubular structures connecting two compartments where the material is transported through nanotubes (Farsad and De Camilli, 2003; Sun et al., 2005 and references therein). Therefore, different mechanisms of membrane deformation which are involved in membrane budding and tubulation have recently gained much attention. Also, important role of membrane budding and tubulation has been found in immune response and pathologic conditions (Farsad and De Camilli, 2003; Greenwalt, 2006). It was shown that membrane-skeleton detached, laterally mobile membrane lipids and integral membrane protein components or their small complexes (raft elements) 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). Clustering of membrane components or raft elements 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).

The deformation of the membrane can be driven also by cytosolic proteins that are attached to the membrane surface. A large number of proteins have been identified that directly bind and deform biological membranes (Farsad and De Camilli, 2003; Bouma et al., 1999). Among them, the banana-like shaped BAR (Bin,Amphiphysin,Rvs) domain containing proteins are the most known (Farsad et al., 2001; Peter et al., 2004; McMahon and Gallop, 2005; Masuda et al., 2006). The binding of proteins to the membrane surface may be driven by electrostatic forces and by penetration of the protein hydrophobic protrusions in the membrane bilayer (Farsad and De Camilli, 2003; Masuda et al., 2006).

Positively charged proteins can bind to the negatively charged outer surface of biological cells, liposomes or lipoproteins, where the electrostatic attractive forces may

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doi:10.1016/j.jbiomech.2006.11.005

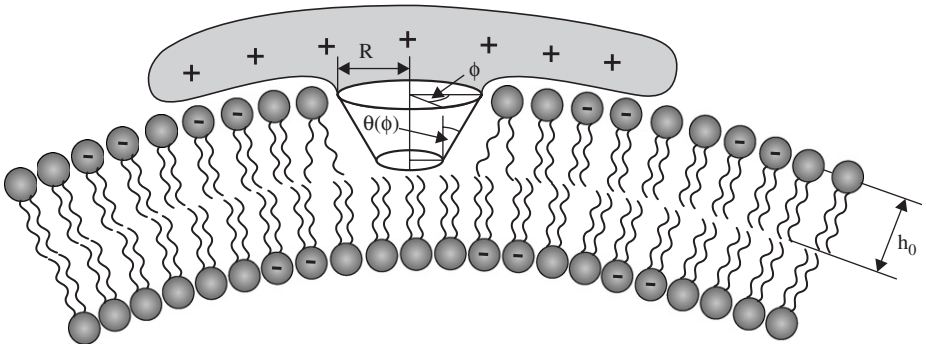

Fig. 1. Schematic figure of a positively charged protein attached to the outer surface of a negatively charged bilayer membrane. The hydrophobic protrusion of the attached protein which is embedded in the outer lipid layer is also shown. The isotropic shape of the hydrophobic protrusion is characterized by the constant "cone-angle" $\theta(\phi)=\bar{\theta}$ and the radius (R) at the lipid head group level. The equilibrium thickness of the monolayer is h_0.

play a decisive role. For example, it was shown recently that the strong attractive interaction of the highly positively charged fifth domain of β_2-glycoprotein I (β_2-GPI) (Bouma et al., 1999) with the membrane surface is possible only if the membrane surface is negatively charged (Willems et al., 1996). 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. Therefore it was suggested that the character of membrane-β_2-GPI interactions (binding) is at least partially of an electrostatic nature, i.e. it is governed by electrostatic attraction between the positively charged domain of β_2-GPI and negatively charged lipid headgroups (Willems et al., 1996). The hydrophobic protrusion(s) of membrane-attached β_2-GPI protein which is embedded in the outer membrane layer (Fig. 1) increases the strength of protein attachment (binding) (Bouma et al., 1999).

The crescent-shaped BAR domain containing proteins present another class of proteins where the binding to the membrane is driven by electrostatics and by insertion(s) of protein hydrophobic protrusions in the outer lipid layer of the membrane (Farsad et al., 2001; Peter et al., 2004; McMahon and Gallop, 2005; Masuda et al., 2006; Gallop et al., 2006).

The proteins attached to the membrane surface may influence the elastic properties of this membrane (Farsad and De Camilli, 2003). Neglecting the anisotropy of lipid molecules, the area density of elastic energy of the bilayer membrane is usually described (see for example Helfrich and Prost, 1988; Kralj-Iglič et al., 2002; Yin et al., 2005 and references therein) as

$$w_b=\frac{k_c}{2}(2H-C_0)^2+k_G C_1 C_2, \tag{1}$$

where $H=(C_1+C_2)/2$ is the mean curvature, C_1 and C_2 are the principal curvatures of the membrane at the given location on its surface, C_0 is the membrane spontaneous curvature, C_1C_2 is the Gaussian curvature, while k_c and k_G are the local bending constant and the Gaussian saddle-splay constant, respectively.

In this work, the influence of the intrinsic shape of membrane-attached proteins on the elastic properties of bilayer membranes is studied theoretically. The intrinsic shape of membrane-attached proteins is described within two different models. In the first, the membrane-attached proteins will be considered as thin rods. In the second model the attached proteins will be considered as small 2-dimensional plates which in general can be anisotropic. At the end the role of the hydrophobic protrusion of the attached protein which is embedded in the outer lipid layer (see Fig. 1) is also discussed.

2. Rod-like proteins attached to the membrane surface

The elastic energy of a rod-like 1-dimensional protein which is attached to the membrane surface of curvature C can be written in the form:

$$E_1=\frac{K_p}{2}(C-C_p^0)^2 L_0, \tag{2}$$

where K_p is the flexural rigidity of the protein and,

$$C=H+D\cos(2\omega), \tag{3}$$

is membrane curvature for a given rotation of the protein (described by the angle ω) with respect to the principal axis of the membrane curvature, $D=(C_1-C_2)/2$ is the curvature deviator at the given location on the membrane surface, C_p^0 is the intrinsic (spontaneous) curvature of the protein and L_0 is the length of the protein.

In the limit of high protein flexural rigidity K_p and strong adhesion, the membrane or part of the membrane should adapt its curvature to the spontaneous curvature of the attached proteins ($C_p^0>0$). Consequently the membrane principal curvatures C_1 and C_2 are constant in these membrane regions. The membrane surfaces with constant C_1 and C_2 can be membrane shapes composed of spheres of equal radii connected by infinitesimal necks (i.e. necklace of spheres) or tubular shapes. The tubular shapes correspond to the minimal possible bending energy of the membrane within the given class of undulated shapes (Iglič et al., 1999). The non-zero principal curvature of the tubular membrane surface ($C_1=1/R_1$) with the attached

semi-flexible or rigid rod-like proteins with spontaneous curvature C_p^0 is in general:

$$C_1 = C_p^0/\cos^2\omega, \tag{4}$$

where ω describes the rotation of the protein in the principal system of the membrane curvature tensor (see Fig. 2). The value $\omega = 0$ corresponds to the minimal possible value of C_1 (Fig. 3). In accordance with experimental observations (Farsad et al., 2001; Farsad and De Camilli, 2003; Peter et al., 2004; Masuda et al., 2006), it can be therefore concluded that semi-flexible or rigid rod-like proteins with spontaneous curvature C_p^0 which are attached to the membrane surface may generate tubular membrane protrusions (Fig. 3). The tubulation is accompanied by the lateral phase separation of proteins with $C_p^0 \neq 0$ (Kralj-Iglič et al., 2005) which are predominantly accumulated in the membrane regions of tubular membrane protrusions.

The membrane-attached proteins can also be less rigid than cellular or lipid bilayer membranes (see for example Nossal, 2001). In this case the contribution of the attached membrane proteins to membrane elastic properties can be estimated in terms of renormalized local membrane bending constant k_c, a renormalized Gaussian saddle-splay constant k_G and a renormalized spontaneous membrane curvature C_0. In order to perform the renormalizations of the membrane constants k_c, k_G and C_0 we first calculate the partition function of a single protein:

$$Q = \frac{1}{\omega_0}\int_0^{2\pi} \exp\left(-\frac{E_1(\omega)}{kT}\right)\mathrm{d}\omega, \tag{5}$$

where different orientational states of the protein on the membrane surface were taken into account (see also Fig. 2), ω_0 is an arbitrary angle quantum, k is the Boltzmann constant and T is the absolute temperature. The free energy of the single attached rod-like protein can be then obtained by the expression (Hill, 1986):

$$f_p = -kT\ln Q. \tag{6}$$

In the limit of small membrane curvatures and constant area density of the proteins (n) we can express the contribution of attached rod-like proteins to the total area density of membrane elastic energy in the terms of the renormalized constants k_c, k_G and C_0 (see Eq. (1)) as

$$\Delta k_c = \tfrac{3}{8}K_p L_0 n, \tag{7}$$

$$\Delta k_G = -\tfrac{1}{4}K_p L_0 n, \tag{8}$$

$$\Delta C_0 = \frac{nK_p L_0 C_p^0}{2k_c}. \tag{9}$$

It can be seen in Eqs. (7)–(9) that within the adopted approximations the local bending constant of the membrane (k_c) always increases with increasing area density of the attached rod-like proteins.

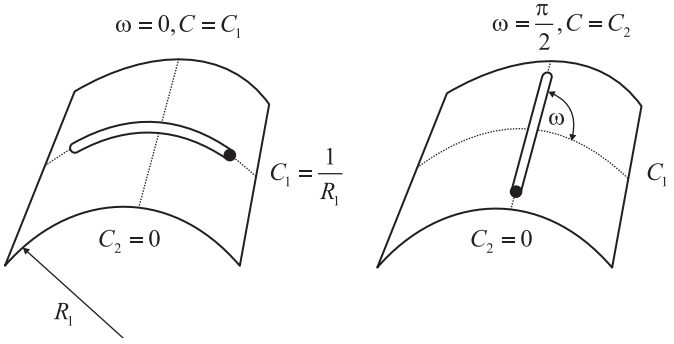

Fig. 2. Schematic figure of two different orientations of a thin rod-like protein strongly attached to a cylindrical membrane surface having $C_1 = 1/R_1$ and $C_2 = 0$. At a given value of the protein rotation angle ω the protein senses the curvature $C = (C_1 + C_2)/2 + ((C_1 - C_2)/2)\cos(2\omega)$ (see Eq. (3)). For positive intrinsic (spontaneous) curvature of the attached protein ($C_p^0 > 0$) (see Eq. (2)) the intrinsic shape of the protein is banana-like.

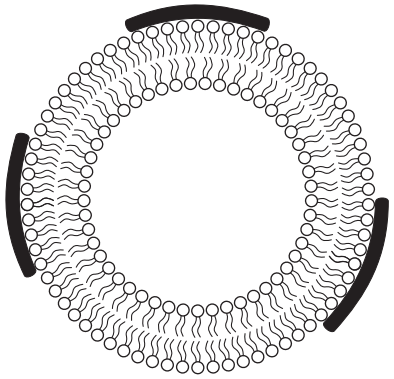

Fig. 3. Schematic figure of a cross-section of a bilayer membrane tubular protrusion with attached rod-like proteins having $C_p^0 > 0$. The rotation angle $\omega = 0$.

3. Plate-like anisotropic membrane protein attached to the membrane surface

Thin shells and strips are in general anisotropic with respect to the curvature of the normal cuts (Helfrich and Prost, 1988; Oda et al., 1999; Iglič et al., 2005) and can attain various equilibrium shapes which are not flat or spherical (Iglič et al., 2005). In this section the membrane-attached protein is treated as a small 2-dimensional thin semi-flexible plate with area a_0 where it is assumed that the protein is in general anisotropic, therefore its intrinsic shape could be described by the two intrinsic principal curvatures C_{1m} and C_{2m} (Fig. 4) and by the orientation of

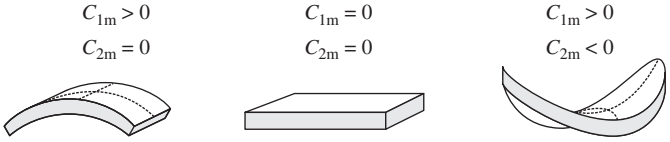

Fig. 4. Schematic figure of the most favourable shapes of membrane-attached proteins having different values of their intrinsic (spontaneous) mean curvatures C_{1m} and C_{2m}.

the protein in the principal systems of the actual local membrane curvature tensor $\underline{C}$.

Accordingly we define the elastic energy of a small plate-like membrane-attached protein with area a_0 as the energy of mismatch between the actual local membrane curvature of the membrane and the intrinsic (spontaneous) curvature of the protein which can be characterized by the tensor $\underline{M} = \underline{R}\,\underline{C}_{\mathrm{m}}\underline{R}^{-1} - \underline{C}$, where the tensor $\underline{C}$ describes the actual curvature, the tensor $\underline{C}_{\mathrm{m}}$ describes the intrinsic curvature of the protein (Fig. 4), while

$$\underline{R} = \begin{bmatrix} \cos\omega & -\sin\omega \\ \sin\omega & \cos\omega \end{bmatrix} \tag{10}$$

is the rotation matrix. In the respective principal systems the matrices that represent curvature tensors include only the diagonal elements

$$\underline{C} = \begin{bmatrix} C_1 & 0 \\ 0 & C_2 \end{bmatrix}, \quad \underline{C}_{\mathrm{m}} = \begin{bmatrix} C_{1\mathrm{m}} & 0 \\ 0 & C_{2\mathrm{m}} \end{bmatrix}. \tag{11}$$

The principal systems of these two tensors are in general rotated in the tangent plane of the membrane surface by an angle ω with respect to each other.

The elastic energy of the protein per unit area (w) should be a scalar quantity. Therefore each term in the expansion of w must also be a scalar (Landau and Lifshitz, 1997), i.e. invariant with respect to all transformations of the local coordinate system. In this work, the elastic energy density w is approximated by an expansion in powers of all independent invariants of the tensor $\underline{M}$ of the second order in the components of $\underline{M}$. The trace and the determinant of the tensor are taken as the set of invariants (Iglič et al., 2005):

$$w = \mu_0 + \frac{K_1}{2}(\mathrm{Tr}\,\underline{M})^2 + K_2\,\mathrm{Det}\,\underline{M}, \tag{12}$$

where μ_0 is the minimal possible value of w, K_1 and K_2 are constants. For the sake of simplicity $\mu_0 \equiv 0$. Taking into account the definition of the tensor $\underline{M}$ it follows from Eqs. (11)–(12) that the elastic energy of the membrane-attached plate-like protein can be written as

$$E = [(2K_1 + K_2)(H - H_{\mathrm{m}})^2 - K_2(D^2 - 2DD_{\mathrm{m}}\cos 2\omega + D_{\mathrm{m}}^2)]a_0, \tag{13}$$

where $H_{\mathrm{m}} = (C_{1\mathrm{m}} + C_{2\mathrm{m}})/2$ is the intrinsic (spontaneous) mean curvature and $D_{\mathrm{m}} = (C_{1\mathrm{m}} - C_{2\mathrm{m}})/2$ is the intrinsic (spontaneous) curvature deviator.

It can be seen from Eq. (13) that the material properties of an anisotropic protein can be expressed in a simple way by only two intrinsic curvatures $C_{1\mathrm{m}}$ and $C_{2\mathrm{m}}$. Fig. 4 shows schematically cylindrical, flat and saddle-like intrinsic (spontaneous) shapes of the thin plate-like protein.

In the limit of high bending rigidity of the plate-like membrane-attached protein and its strong adhesion, the membrane (or a part of it) should adapt its curvatures C_1 and C_2 to the protein intrinsic spontaneous curvatures $C_{1\mathrm{m}}$ and $C_{2\mathrm{m}}$. In this case the values of the membrane mean curvature $H = (C_1 + C_2)/2$, the curvature deviator $D = (C_1 - C_2)/2$ and the protein orientation angle ω corresponding to the minimum of the function E for given values of $H_{\mathrm{m}} = (C_{1\mathrm{m}} + C_{2\mathrm{m}})/2$ and $D_{\mathrm{m}} = (C_{1\mathrm{m}} - C_{2\mathrm{m}})/2$ can be calculated from the necessary conditions for the extremum of the function E:

$$\frac{\partial E}{\partial H} = 2a_0(2K_1 + K_2)(H - H_{\mathrm{m}}) = 0, \tag{14}$$

$$\frac{\partial E}{\partial D} = -K_2 a_0(2D - 2D_{\mathrm{m}}\cos 2\omega) = 0, \tag{15}$$

$$\frac{\partial E}{\partial \omega} = -4a_0K_2DD_{\mathrm{m}}\sin 2\omega = 0, \tag{16}$$

and the sufficient conditions for the minimum of E (Widder, 1947):

$$\frac{\partial^2 E}{\partial H^2} = 2a_0(2K_1 + K_2) > 0, \tag{17}$$

$$\left(\frac{\partial^2 E}{\partial H^2}\right)\left(\frac{\partial^2 E}{\partial D^2}\right) - \left(\frac{\partial^2 E}{\partial H\,\partial D}\right)^2 = -4K_2a_0^2(2K_1 + K_2) > 0, \tag{18}$$

$$\frac{\partial^2 E}{\partial H^2}\left[\left(\frac{\partial^2 E}{\partial D^2}\right)\left(\frac{\partial^2 E}{\partial \omega^2}\right) - \left(\frac{\partial^2 E}{\partial D\,\partial \omega}\right)^2\right] \tag{19}$$

$$= 16K_2^2a_0^3\frac{\partial^2 E}{\partial H^2}(DD_{\mathrm{m}}\cos 2\omega - D_{\mathrm{m}}^2\sin^2 2\omega) > 0, \tag{20}$$

where $\partial^2 E/\partial H\,\partial D = 0$ and $\partial^2 E/\partial H\,\partial \omega = 0$ were taken into account. Considering only positive values of ω, it follows from Eqs. (14)–(16) and (19) that at the minima of E:

$$H = H_{\mathrm{m}}, \quad D = D_{\mathrm{m}}, \quad \omega = k_\omega\frac{\pi}{2} = 0, \quad k_\omega = 0, 2, 4. \tag{21}$$

Considering the case $D = D_{\mathrm{m}}$ (i.e. $k_\omega = 0, 2, 4$) it can be concluded from the above equations that the function E has minimal values for (see also Fig. 5):

$$H = H_{\mathrm{m}}, \quad D = D_{\mathrm{m}}, \quad \omega = 0, \pi, 2\pi, \tag{22}$$

where the $\omega = 0$ and $\omega = 2\pi$ describe the same orientation and where

$$K_1 > -K_2/2, \quad K_2 < 0. \tag{23}$$

If the membrane-attached proteins have $C_{1\mathrm{m}} > 0$ and $C_{2\mathrm{m}} = 0$ (see Fig. 4) the energetically favourable membrane shape would be the tubular membrane shape or a collapsed tubular membrane shape in the form of a twisted strip (helix A, see Fig. 6), where in the last case the proteins would not be distributed at the edges of the strip. For $C_{1\mathrm{m}} > 0$ and $C_{2\mathrm{m}} < 0$ (see Fig. 4) the favourable membrane shape would be the neck connecting the daughter vesicle and the parent cell or the collapsed tubular membrane shape twisted in the form of a helix B strip, where in the last case the proteins would not be distributed at the edges of the strip (see Fig. 6 and Iglič et al., 2005).

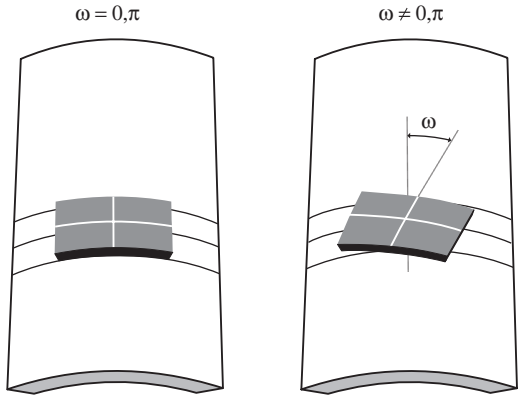

Fig. 5. Schematic figure of a different orientations of membrane-attached plate-like protein with intrinsic principal curvatures $C_{1m}>0$ and $C_{2m}=0$ (see also Fig. 4). The shape of the membrane is cylindrical ($C_1>0$ and $C_2=0$).

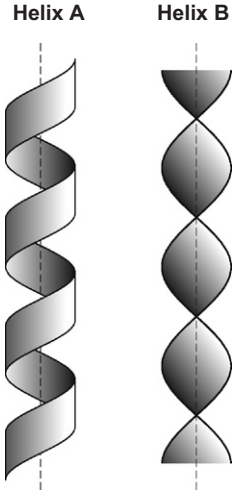

Fig. 6. Schematic presentation of helical (A and B) configuration.

Although there were no experimental data available, it was believed in the past that the bending rigidity of membrane-attached proteins should be larger than the bending rigidity of the membrane. However, as already mentioned above it has been recently estimated (Nossal, 2001) that the bending rigidity of the plate-like proteins attached to the membrane can be similar or smaller than the membrane bending rigidity. In this case also the membrane-attached protein adapts its shape in order to fit its curvature to the actual membrane curvature (which is thus influenced by both, membrane and attached proteins). Since all orientations of an attached protein do not have the same energy (see Eq. (13)), the partition function of a single plate-like protein can be written in the form:

$$Q=\frac{1}{\omega_0}\int_0^{2\pi}\exp\left(-\frac{E(\omega)}{kT}\right)\mathrm{d}\omega, \tag{24}$$

with ω_0 as an arbitrary angle quantum. The free energy of the attached protein is then obtained by the expression $f_p=-kT\ln Q$. Combining Eqs. (13) and (24) allows us to write the contributions of the membrane-attached proteins to the area density of membrane elastic energy $w_p=f_p n$ in the form (up to the constant terms):

$$\begin{aligned} w_p &= (2K_1+K_2)(H-H_m)^2 n a_0 \\ &\quad - K_2(D^2+D_m^2)n a_0 \\ &\quad - kTn\ln\left(\mathrm{I}_0\left(\frac{2K_2 D D_m a_0}{kT}\right)\right), \end{aligned} \tag{25}$$

where we assumed for the sake of simplicity that the lateral distribution of membrane-attached proteins is homogeneous (i.e. n is constant).

For small $x=2K_2DD_m a_0/kT$ the logarithm of the modified Bessel function (see the last term in Eq. (25)) can be approximated as $\ln \mathrm{I}_0(x)\sim x^2/4$. The contribution of attached proteins to the total area density of membrane elastic energy can be then expressed in terms of the renormalized local bending constant k_c, the renormalized Gaussian saddle-splay constant k_G and the renormalized spontaneous curvature C_0 (see Eq. (1)) as follows:

$$\Delta k_c = nK_1 a_0 - nD_m^2 K_2^2 a_0^2/2kT, \tag{26}$$

$$\Delta k_G = n(D_m^2 K_2^2 a_0^2/kT + K_2 a_0), \tag{27}$$

$$\Delta C_0 = H_m n(2K_1+K_2)a_0/k_c, \tag{28}$$

where the relation $D^2=H^2-C_1C_2$ was taken into account.

Comparison of Eqs. (7) and (26) leads us to the conclusion that attachment of 1-dimensional rod-like proteins to biological membranes always increases the local bending constant k_c, while the attachment of 2-dimensional anisotropic (i.e. with $C_{1m}\neq C_{2m}$) proteins may also reduce k_c. Also the effect of the protein attachment on the value of the Gaussian saddle-splay constant k_G could be of opposite sign in both cases (compare Eqs. (8) and (27)).

4. Discussion and conclusions

In the above theoretical analysis we have analysed the influence of the membrane-attached proteins on the membrane elastic properties on the basis of a simple, physically transparent, model. It involves a number of approximations that we discuss in the following.

First, the elastic energy of the bilayer membrane is more complex as assumed in the present work. In addition to local bending energy (Eq. (1)), which accounts for the splay deformation of the lipid molecules, it should include also the term due to the stretching (or compression) of the lipid chains (Helfrich and Jakobsson, 1990; Fournier, 1998), the surface tension energy which describes the contribution due to changes in the lateral density of the lipid polar headgroups (Helfrich and Jakobsson, 1990; Fournier, 1998), the term due to tilt deformation of the lipid chains (Helfrich and Prost, 1988; Fournier, 1998), the non-local bending energy (Evans and Skalak, 1980) and the contribution due

to in-plane orientational ordering of lipids in the membrane regions with large difference between two principal curvatures (Kralj-Iglič et al., 2002, 2006). Among these, the surface tension may play an important role in the case of electrostatic interactions between attached positively charged proteins and the outer surface of a negatively charged bilayer membrane. The negatively charged lipid molecules may migrate towards the protein adsorption site in order to strengthen the adsorption of the protein (Heimburg et al., 1999). In addition to the in-plane redistribution of lipids also the changes in area compression modulus can be expected due to attachment of proteins to the outer membrane surface.

In our analysis the shear energy of the membrane (Evans and Skalak, 1980) was not considered. The effect of shear elasticity may be important in the strongly deformed neck region of spherical membrane invaginations or exvaginations or in thin and long tubular membrane protrusions (Hägerstrand et al., 1999) if these regions are covered by a coat of cytosolic proteins (Kosawada et al., 2005). Considering the shear energy of such protein coat would influence the theoretically predicted shapes of membrane protrusions formed in the budding process (Kosawada et al., 2005), however, it would not influence the basic conclusions of our work regarding the influence of the attached proteins on the bending elasticity of the membrane.

There are also approximations concerning the membrane thickness and local microscopic curvature variations which are considered to be constant in the present paper. In addition to small variations of the membrane thickness (and local membrane curvature) due to the ripple phase formation (Fournier, 1998; Rappolt et al., 2000), formation of the inverse hexagonal phase as intrabilayer inverted micelles (Rappolt et al., 2003 and references therein) or local perturbation by integral membrane proteins due to "hydrophobic mismatch" (Mouritsen and Bloom, 1984; Helfrich and Jakobsson, 1990; Fournier, 1998; Branningan and Brown, 2006), also larger variation of the membrane thickness due to thermal fluctuations which are comparable to membrane thickness (Branningan and Brown, 2006) could occur. In the latter case the membrane curvature becomes a dynamical parameter that changes with time.

Further, we did not take into account the role of the hydrophobic protrusion of the attached protein. Namely, the hydrophobic protrusion of the attached protein which is embedded in the outer membrane layer (Fig. 1) may also influence the elastic properties of the membrane. Typical examples of membrane-attached protein with hydrophobic protrusion(s) are banana-like shaped BAR domain proteins (Masuda et al., 2006) or β_2-GPI proteins (Bouma et al., 1999).

Recently, a simple microscopic interaction model of protein inclusion anchored in the outer lipid layer was suggested (Fosnarič et al., 2005). The energy of the disturbed lipids surrounding the inclusion (Fig. 1) was estimated, where it was assumed that the protein protrusion is anchored within the lipid layer through hydrophobic interactions. In the model the hydrocarbon core of the host lipid layer was allowed to adjust to the shape of the protein insertion (see also (Fig. 1). The corresponding elastic lipid perturbation energy can be expected to depend on the curvature of the membrane. It was shown that the energy of the disturbed lipids around the isotropic protein insertion in the outer lipid layer can be approximately written in the form (Kralj-Iglič et al., 1999):

$$W_{\mathrm{p}} = \mu_{\mathrm{p}} + \frac{\xi}{2}(H - \bar{H}_{\mathrm{m}})^2 + \frac{\xi}{4}D^2 \tag{29}$$

leading to the conclusion that Eq. (13) should be upgraded by additional term (Eq. (29)) in order to take into account also the energy of the disturbed lipids due to the protein insertion in the outer lipid layer.

In the most simple example, the shape of the isotropic hydrophobic protein insertion can be described by the "cone-angle" $\bar{\theta}$ and the radius R of the core of the hydrophobic protrusion at the level of the lipid head group (Fig. 1). The protein insertion is "cone-like" for $\bar{\theta}>0$, cylindrical for $\bar{\theta}=0$ and "inverted cone-like" for $\bar{\theta}<0$. The interaction constant ξ and intrinsic curvature $\bar{H}_{\mathrm{m}}$ can be expressed as (Fosnarič et al., 2005)

$$\bar{H}_{\mathrm{m}} \cong \frac{\bar{\theta}}{R}\left(\frac{R+\lambda_{\mathrm{d}}}{R+2\lambda_{\mathrm{d}}}\right), \tag{30}$$

$$\xi \cong \pi k_{\mathrm{c}} R^2\left(\frac{R}{\lambda_{\mathrm{d}}}+2\right), \tag{31}$$

where $\lambda_{\mathrm{d}}\sim 1$ nm is the monolayer perturbation decay length. The contribution of the hydrophobic insertion of the attached proteins to the total area density of membrane elastic energy can be expressed in terms of renormalized local bending constants k_{c} and k_{G} and renormalized spontaneous curvature C_0:

$$\Delta k_{\mathrm{c}} = 3n\xi/8, \quad \Delta k_{\mathrm{G}} = -n\xi/4, \quad \Delta C_0 = \bar{H}_{\mathrm{m}} n\xi/2k_{\mathrm{c}}. \tag{32}$$

As it can be seen from Eqs. (26)–(28) and (32) the relative contribution of the intrinsic shape of the attached protein to the membrane elastic energy versus the corresponding contribution of its hydrophobic protrusion may substantially vary, depending on the values of the bending constants of the protein (K_1 and K_2), intrinsic (spontaneous) curvatures of the protein ($C_{1\mathrm{m}}$ and $C_{2\mathrm{m}}$), shape of the hydrophobic protrusion (described by angle $\bar{\theta}$ and radius R) and protein area a_0.

In this work we propose that the attachment of rod-like proteins with $C_{\mathrm{p}}^0>0$ (Fig. 2) or the attachment of anisotropic plate-like proteins with $C_{1\mathrm{m}}>0$ and $C_{2\mathrm{m}}=0$ (Figs. 4 and 5) to the membrane surface may induce the formation of membrane tubular protrusions (see also Fig. 7C). The article of Masuda et al. (2006) discusses the similar driving mechanism of the membrane tubulation that is mathematically modelled in this paper. The membrane tubulation is accompanied by accumulation of

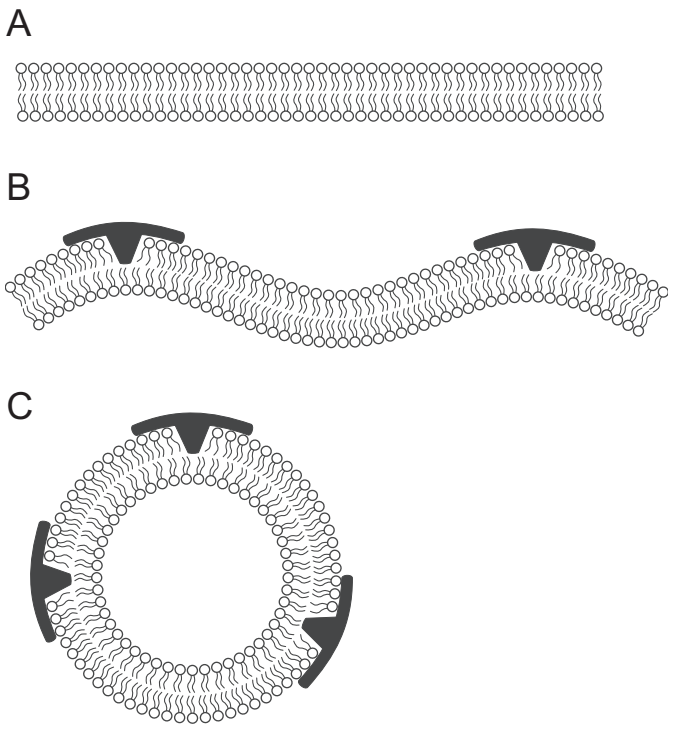

Fig. 7. Schematic figure of a nearly flat bilayer membrane (A), a nearly flat bilayer membrane with attached proteins where the membrane shape is locally changed around each attached proteins (B) and the cross-section of a bilayer membrane tubular protrusion with attached proteins (C).

the attached proteins in the region of the tubular membrane protrusions (see also Kralj-Iglič et al., 2005).

The alternative process to membrane tubulation (as the reaction of the membrane to adhesion of semi-flexible or rigid proteins to its surface) might also be local deformation of the membrane around each of the attached proteins (Fig. 7B). However, this mechanism of membrane shape adaptation around the membrane-attached proteins seems to be less probable (at least if the number of attached proteins is large enough). Namely, the local change of the membrane shape around each of the attached semi-flexible or rigid proteins (Fig. 7B) would greatly increase the non-local bending energy of the bilayer membrane (also called the relative stretching energy since it originates from different stretching of both monolayers during bending of the bilayer at constant average membrane area) (Evans and Skalak, 1980; Stokke et al., 1986; Kralj-Iglič et al., 2005):

$$W_{\mathrm{n}} = k_{\mathrm{n}} A(\langle H\rangle - H_0)^2, \tag{33}$$

where $\langle H\rangle = \int H\,\mathrm{d}A/A$ is the average mean curvature, H_0 is the spontaneous mean curvature, k_{n} is the coefficient of non-local bending rigidity, A is the membrane area and $\mathrm{d}A$ is the membrane area element. For thin and not too strongly curved bilayers the average mean curvature $\langle H\rangle$ is proportional to the area difference between the two membrane monolayers (ΔA): $\langle H\rangle = \Delta A/2A\delta$, where δ is the distance between the two monolayer neutral surfaces. For a closed, nearly flat bilayer membrane ($\langle H\rangle \approx 0, H_0 \approx 0$, Fig. 7A) with N homogeneously distributed attached semi-flexible or rigid proteins the membrane non-local bending energy W_{nb} can be approximately written as

$$W_{\mathrm{n}} \cong k_{\mathrm{n}} A(N\langle H\rangle_{\mathrm{p}} - NH_{0\mathrm{p}})^2 \propto N^2, \tag{34}$$

where $\langle H\rangle_{\mathrm{p}} \neq 0$ belongs to the disturbed membrane patch around the single membrane-attached protein (Fig. 7B). The positive value of $H_{0_{\mathrm{p}}}$ is mainly the consequence of the hydrophobic insertion of the protein in the outer leaflet of the membrane bilayer (see also Masuda et al., 2006; Farsad and De Camilli, 2003), i.e. $H_{0_{\mathrm{p}}} \approx \pi R^2/2A\delta$, where R is the radius of the hydrophobic protrusion (Fig. 1).

Since the energy W_{n} increases quadratically with the total number of membrane-attached proteins (N^2), it can be concluded that the local shape deformations of the membrane around each of the attached semi-flexible or rigid proteins (Fig. 7B) would be energetically less favourable than membrane tubulation and accumulation of the attached proteins on tubular membrane protrusions (Fig. 7C).

As already mentioned above, the hydrophopbic insertion of the membrane-attached proteins (Fig. 1) also increases the spontaneous mean curvature of the membrane (H_0) by $NH_{0_{\mathrm{p}}}$, which is mainly the consequence of the increased area of the outer membrane layer due to hydrophobic insertion of the attached proteins (see Fig. 7B,C). The increase of H_0 due to attached proteins (i.e. the increase of the area difference between the outer and inner membrane layer) is according to the bilayer couple hypothesis (Deuticke, 1968; Sheetz and Singer, 1974) important for membrane tubulation (Kralj-Iglič et al., 2005).

In derivation of Eqs. (26)–(28) we assumed for the sake of simplicity that the membrane-attached proteins are distributed homogeneously over the membrane. We obtained that the presence of membrane-attached proteins cause an increase of the membrane local bending constant while their orientational ordering cause its decrease (first and second term in Eq. (26), respectively). In general, membrane-attached proteins are more or less free to move laterally over the membrane and therefore in equilibrium attain non-homogeneous lateral distribution that is energetically the most favourable. Non-homogeneous lateral distribution of membrane-attached proteins offers an additional internal degree of freedom which would lower the equilibrium free energy of the membrane and contribute to an additional decrease of the membrane local bending constant. This was shown for isotropic membrane inclusions (Kralj-Iglič et al., 1996; Božič et al., 2006) as well as for anisotropic membrane inclusions (Kralj-Iglič et al., 1999).

In conclusion, in this work the mechanical properties of a bilayer membrane with proteins attached to the outer membrane surface were considered theoretically. Regarding the intrinsic shape of membrane-attached proteins, two different kind of proteins were considered: 1-dimensional rod-like proteins and 2-dimensional plate-like proteins which may in general be anisotropic. It is shown that the

attachment of rod-like proteins to the membrane surface may induce the tubulation of the membrane. The most favourable membrane shapes corresponding to a membrane with attached plate-like proteins (Fig. 4) depends on the values of the intrinsic curvatures of the attached proteins C_{1m} and C_{2m}. For $C_{1m}>0$ and $C_{2m}=0$ the attached proteins induce membrane tubulation, while for $C_{1m}>0$ and $C_{2m}<0$, a "saddle-like" membrane is the most favourable.

If the bending rigidity of the membrane-attached proteins is similar or smaller than bending rigidity as the membrane, the attachment of rod-like proteins always increases the local bending constant, while the attachment of plate-like anisotropic proteins may also reduce the local bending constant.

From a biological perspective, a possible application of our model of attachment of semi-flexible plate-like protein on the membrane surface is accumulation and mutual interaction of cytosolic (coat) proteins during the process of spherical membrane budding or membrane tubulation (Farsad and De Camilli, 2003). Here, the predicted local membrane softening induced by membrane-attached plate-like anisotropic cytosolic proteins could facilitate the formation of spherical bud (see also Jülicher and Lipowsky, 1996) or the membrane tubulation.

Effect of the membrane-attached proteins on the membrane elastic properties can have also other important physiological consequences. Some proteins that are present in the blood serum 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 (Bevers et al., 2005; Distler et al., 2005). For example, it was shown that β_2-GPI and antibodies which are present in the sera of patients with antiphospholipid syndrome and can attach to the membrane surface (Asherson et al., 1989; Roubey, 2000), may induce adhesion between negatively charged phospholipid vesicles (Ambrožič et al., 2006). In this work described modifications of membrane bending rigidity due to the attached membrane proteins may affect the fluctuations of the membrane and in this way influence the adhesion process (Helfrich, 1995). The observed adhesion of lipid surfaces, driven by attachment of β_2-GPI and antibodies, may be involved in the mechanisms of blood clot formation.

Acknowledgements

We are grateful to B. Babnik and M. Fošnarič for technical assistance.

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