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Shapes of bilayer vesicles with membrane embedded molecules

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Abstract The interdependence of the lateral distribution of molecules which are embedded in a membrane (such as integral membrane proteins) and the shape of a cell with no internal structure (such as phospholipid vesicles or mammalian erythrocytes) has been studied. The coupling of the lateral distribution of the molecules and the cell shape is introduced by considering that the energy of the membrane embedded molecule at a given site of the membrane depends on the curvature of the membrane at that site. Direct interactions between embedded molecules are not considered. A simple expression for the interaction of the membrane embedded molecule with the local membrane curvature is proposed. Starting from this interaction, the consistently related expressions for the free energy and for the distribution function of the embedded molecules are derived. The equilibrium cell shape and the corresponding lateral distribution of the membrane embedded molecules are determined by minimization of the membrane free energy which includes the free energy of the membrane embedded molecules and the membrane elastic energy. The resulting inhomogeneous distribution of the membrane embedded molecules affects the cell shape in a nontrivial manner. In particular, it is shown that the shape corresponding to the absolute energy minimum at given cell volume and membrane area may be elliptically non-axisymmetric, in contrast to the case of a laterally homogeneous membrane where it is axisymmetric.

Key words Membrane embedded molecules · Vesicle shape · Lateral distribution

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Introduction

Equilibrium shapes of cells with no internal structure (such as phospholipid bilayer vesicles and mammalian erythrocytes) are determined by the minimum of the energy of their membranes. For laterally homogeneous closed membranes, composed of layers which are unconnected but in contact, the relevant energy is the elastic energy of such membranes (Svetina and Žekš 1992). The corresponding equilibrium shapes are well represented by the generalized bilayer couple model (see Heinrich et al. 1993, Miao et al. 1994 and references therein). The generalized bilayer couple model includes as the two opposite limits the spontaneous curvature model (Deuling and Helfrich 1976) and the strict bilayer couple model (Svetina and Žekš 1989).

Membranes can in general be viewed as a matrix composed of lipid molecules in which large molecules (such as integral membrane proteins) are embedded. The embedded molecules can, with greater or smaller mobility, move around in the plane of the membrane (Gennis 1989). Different experimental data suggest that the most energetically favorable distribution of the embedded molecules is laterally inhomogeneous. The spontaneously released vesicles of in vitro aged sheep erythrocytes and sheep erythrocyte ghosts are enriched in integral glycoproteins with respect to intact cells (Lutz et al. 1977), suggesting an inhomogeneous distribution of membrane proteins which could arise from significantly higher curvature of vesicle membranes. Weitz et al. (1982) studied human erythrocyte membrane vesiculation induced by addition of dimyristoylphosphatidylcholine liposomes. Also here, inhomogeneous distribution of membrane proteins was observed, as the vesicles released from the membrane were enriched in some integral membrane proteins. The observations of microvilli formed on lymphocytes (de Petris 1978) show that some molecules which are relatively free to move laterally over the cell membrane tend to leave the cell body and accumulate on microvilli where the membrane curvature is larger. It was likewise observed (Weiss and Subjeck

1973) that the glycoprotein area density was higher on the microvilli than on the intermicrovillous membrane surface of Ehrlich ascites tumor cells. These observations indicate a possibility that a property which varies over the surface, and could be the cause for the inhomogeneous lateral distribution of the molecules over the membrane, is the membrane curvature.

It is of interest to investigate how membrane curvature affects the distribution of membrane constituents over the membrane area. Besides that, it is also of interest to investigate how this distribution affects the membrane curvature and the cell shape. In this work, a bilayer membrane with embedded molecules which span both layers of the membrane is considered. These molecules are treated as equal and the direct interactions between them are neglected. The only relevant contribution to the energy of the embedded molecule is therefore the energy of the interaction between the molecule and the local membrane curvature which depends on the shape of the molecule. Our preliminary results (Svetina et al. 1990) indicate that inhomogeneous distribution of the membrane embedded molecules produces equilibrium shapes that are, in general, different from the shapes of the laterally homogeneous membrane which were obtained by minimization of the membrane elastic energy within the bilayer couple model. Therefore it seemed to be of interest to examine in detail how the presence of the embedded molecules affects the shape within the framework of the bilayer couple model.

Related systems and problems have been studied before. The inhomogeneous distribution of membrane constituents was related to the vesicle shape. Most of these studies consider the membrane as a single layer. The basic idea for the description of the interdependence of the inhomogeneous distribution of the membrane constituents and the cell shape was put forward by Markin (1981). The expression for the free energy of the membrane composed of two species of molecules was derived by employing the spontaneous curvature model where in addition to the membrane bending energy were included the contributions due to the membrane constituents. For the dependence of the membrane bending constant and of the spontaneous curvature on the area density of the membrane constituents, the phenomenological expressions were proposed. The equilibrium shapes of a two-dimensional analog of the cell (an elastic ring) were calculated. More recently, a phenomenological coupling between the membrane curvature and the area densities of membrane constituents was applied (Leibler 1986; Leibler and Andelman 1987; Kawakatsu et al. 1993; Seifert 1993; Taniguchi 1994). The free energy of the membrane was expressed by means of a Ginzburg-Landau expansion in powers of the order parameter related to the concentration of the membrane constituents. Static and dynamic phenomena of a membrane containing a certain amount of laterally diffusing embedded particles and of a two component membrane were considered. Assuming that the energy of interaction of the embedded particles with the phospholipids, as well as among themselves, is given by a harmonic function of the particle concentration, and that the membrane curvature changes only very little with the position on the membrane, Leibler (1986) established by using the above formalism that the membrane free energy could be written in the form of the membrane bending energy of the spontaneous curvature model with renormalized expressions for the membrane bending constant and the spontaneous curvature of the membrane. Further, by considering two component vesicles, intramembrane phase separation of the molecules was obtained and the stability of shapes was studied in the strong segregation limit (Kawakatsu et al. 1993) and in the weak segregation limit (Taniguchi et al. 1994).

Considering that the membrane is composed of two layers Mitov (1981) derived an expression for the free energy of the two component membrane starting from some basic parameters of molecules such as the surfaces per head and per hydrophobic part of the molecule at a given deformation of the membrane and the constants characterizing the interaction between heads and chains, respectively, of the lipid molecules. Seifert (1993) considered shapes of two component vesicles where the spontaneous curvature was assumed to depend linearly on the local compositions of the two membrane layers. The membrane free energy could then be expressed in the form of the elastic energy of the generalized bilayer couple model with renormalized expressions for the parameters of the elastic bending energy: the spontaneous curvature and the local and non-local bending constants of the membrane as well as the equilibrium area difference of the two membrane layers.

In our model the membrane is treated as a laterally homogeneous bilayer in which the embedded molecules span both layers. The formalism proposed here differs from the previous models as it starts from the energy of the interaction of an individual embedded molecule with the membrane curvature at the site of the molecule. An expression for this interaction is proposed where it is taken that an embedded molecule is symmetric with respect to the axis perpendicular to the membrane. The macroscopic quantities such as the equilibrium lateral distribution of the embedded molecules, the membrane free energy and the equilibrium cell shape are then derived using the methods of statistical physics. Following the procedure, in the next section, the consistently related expressions for the molecular distribution function and for the free energy of the membrane independence of the local membrane curvature are derived. The variational problem is defined, in which the equilibrium shape and distribution corresponding to the minimum of the membrane free energy (including the membrane elastic energy in addition to the contribution of the embedded molecules) at given constraints are sought. It is then shown how the curvature dependent interaction of the embedded molecules with the membrane influences the cell shape, the distribution of embedded molecules and the membrane free energy. Possible relations of these features to similar systems studied and also to biological phenomena such as the existence of elliptic erythrocytes are indicated.

Theory

The membrane is treated as a two-dimensional continuum in which laterally mobile molecules such as proteins are embedded. The embedded molecules are taken to be equal and also independent in the sense that the direct interactions between them are neglected. It is assumed that the addition of the embedded molecules does not affect the membrane area. In this model a molecule interacts with the membrane by simply "sensing" its local curvature. The energy of interaction between the membrane and the molecule (E) reflects the mismatch in the shape of the part of molecule immersed in the membrane and the local membrane curvature which causes an increase in the energy. It is assumed that the energy E is a function of the two principal curvatures C_1 and C_2 of the membrane at the site of the molecule. In general, the principal curvatures vary over the vesicle surface. Because the embedded molecules are expected to be more abundant at energetically more favorable sites, they are distributed nonuniformly over the vesicle surface.

Here, the expressions for the distribution of the membrane embedded molecules and for the corresponding equilibrium free energy are derived from the same basic assumptions and from the same statistical mechanical origin. In the first step, the distribution function for membrane embedded molecules which is energetically the most favorable at a given cell shape is sought. In this procedure (which is described in Appendix I) the expression for the free energy of membrane embedded molecules is minimized with respect to the distribution function of the molecules. The Boltzmann distribution function is obtained as the most favorable distribution function (AI.14),

$$n/n_u = \frac{1}{\Sigma} e^{-E(C_1, C_2)/kT}, \tag{1}$$

where

$$\Sigma = \frac{1}{A}\int e^{-E(C_1, C_2)/kT} dA, \tag{2}$$

and

$$n_u = N/A, \tag{3}$$

n is the number surface density of the embedded molecules at a given position, n_u is the number surface density corresponding to the uniform distribution, k is the Boltzmann constant, T is the temperature, dA is the area element, and N is the total number of the membrane embedded molecules. Integration is performed over the entire membrane area A.

The free energy of the membrane embedded molecules F_d which corresponds to the above distribution function for a given vesicle shape is (AI.16)

$$F_d = -kTN \ln\Sigma . \tag{4}$$

The membrane elastic energy W in the generalized bilayer couple model (in which the membrane is considered to be composed of two layers which are unconnected but in close contact) is (see Heinrich et al. 1993; Miao et al. 1994, and references therein)

$$W = \frac{1}{2}k_c \int (C_1 + C_2 - C_0)^2 \, dA + \frac{1}{2}\frac{k_r}{A}\left[\frac{\Delta A - \Delta A_0}{h}\right]^2, \tag{5}$$

where k_c and k_r are the local and the non-local bending moduli, respectively, C_0 is the spontaneous curvature of the membrane, ΔA is the area difference between the outer and the inner membrane layer areas

$$\Delta A = h \int (C_1 + C_2)\, dA , \tag{6}$$

with h the distance between the neutral surfaces of the two membrane layers, and ΔA_0 the area difference of the two unstretched membrane layers.

The equilibrium principal curvatures as functions of the position on the membrane (determining the equilibrium vesicle shape) are obtained by minimization of the membrane free energy F which includes the membrane elastic energy W and the free energy of the membrane embedded molecules F_d

$$F = W + F_d . \tag{7}$$

Dimensionless quantities are introduced. If R_s is the radius of the sphere with the membrane area A,

$$R_s = \left(\frac{A}{4\pi}\right)^{1/2}, \tag{8}$$

the dimensionless curvatures are

$$c_1 = R_s C_1, \quad c_2 = R_s C_2, \quad c_0 = R_s C_0 \tag{9}$$

and the relative area element is

$$da = dA/4\pi R_s^2 . \tag{10}$$

The area difference of the two membrane layers ΔA and the equilibrium area difference ΔA_0 are normalized with respect to the area difference of a spherical cell $8\pi R_s h$,

$$\Delta a = \frac{1}{2}\int (c_1 + c_2)\, da , \tag{11}$$

and $\Delta a_0 = \Delta A_0/8\pi R_s h$, respectively. The free energy (7) is normalized with respect to the bending energy of the spherical cell $8\pi k_c$, $f = F/8\pi k_c$,

$$f = \frac{1}{4}\int (c_1 + c_2 - c_0)^2 da + \frac{k_r}{k_c}(\Delta a - \Delta a_0)^2 - p \ln \int e^{-E(c_1, c_2)/kT} da \tag{12}$$

where

$$p = NkT/8\pi k_c \tag{13}$$

and we express the interaction of a membrane embedded molecule with the membrane curvature in terms of dimensionless curvatures $E(c_1, c_2)$.

The equilibrium shape and the corresponding distribution of membrane embedded molecules which yields the minimal value of the membrane free energy is sought. While performing the minimization it is taken into account that the relative membrane area $a = A/4\pi R_s^2$

is constant

$$\int da = 1, \tag{14}$$

that the relative volume $v = V/(4\pi R_s^3/3)$ is constant (where V is the cell volume),

$$\int dv = v, \tag{15}$$

and that the number of all the membrane embedded molecules is constant,

$$\int \frac{n}{n_u}\, da = 1. \tag{16}$$

In the examples calculated and presented in this work the value of the non-local bending constant k_r is taken to be infinite. This is identical with the assumption that the relative area difference is constant

$$\Delta a = \Delta a_0, \tag{17}$$

which means that we are applying the strict bilayer couple model in which the shapes are characterized by the values of v and Δa_0 (Svetina and Žekš 1989).

In order to calculate the shapes the interaction between the membrane embedded molecule and the membrane E has to be specified. A simple expression is proposed in which the interaction is expanded up to quadratic terms of the principal curvatures (Appendix II, Eq. AII.8),

$$E = K\left((C_1 + C_2 + C_s)^2 - 4/3\, C_1 C_2\right), \tag{18}$$

where K and C_s are constants. The constant C_s determines both principal curvatures that are the most favorable for the molecules. In deriving Eq. (18) we considered for the sake of simplicity that the part of the molecule which is immersed in the membrane is axially symmetric with respect to the local axis perpendicular to the membrane area so that the energy E is minimal when $C_1 = C_2 = 3C_s/4$ (AII.13).

The distribution function of the membrane embedded molecules expressed by means of dimensionless quantities is obtained by inserting the interaction E (18) into (1)

$$n/n_u = \frac{1}{\Sigma} \exp\left(-\kappa\left((c_1 + c_2 - c_s)^2 - \frac{4}{3} c_1 c_2\right)\right) \tag{19}$$

where

$$\kappa = K/kTR_s^2, \tag{20}$$

$$c_s = C_s R_s. \tag{21}$$

Results

In the following it is shown how the parameters κ, c_s and p (Eqs. (20), (21) and (13)) influence the equilibrium distribution of the membrane embedded molecules and the cell shape. At given relative volume v we study the interval of the relative membrane area difference Δa_0 where the cells with laterally homogeneous membrane have relatively low energies (Svetina and Žekš 1989). Within this interval of Δa_0 the shapes are oblate axisymmetric, non-axisymmetric and prolate axisymmetric, while they are symmetric with respect to the equatorial ($z=0$) plane as well as with respect to $x=0$ and $y=0$ planes (Heinrich et al. 1993; Kralj-Iglič et al. 1993).

The variational problem of shape determination is solved by using a simple parametric model (Kralj-Iglič et al. 1993) as in the treated interval of Δa_0 the approximative solutions of the variational problem for the laterally homogeneous membrane obtained by this model are sufficiently close to the shapes and energies obtained by the exact solution of the variational problem (Kralj-Iglič et al. 1993). In the model, the contour of the shape cross section in the $y=0$ plane, $z=\zeta(x)$, is described by the Cassini function modified by Canham (1970)

$$\zeta(x) = \pm\beta\left[(\gamma^4 + 4\alpha^2 x^2)^{1/2} - \alpha^2 - x^2\right]^{1/2}, \tag{22}$$

where α, β and γ are the characteristic parameters and $\pm$ accounts for the symmetry of the contour with respect to the x axis. The curve (22) is rotated either around its symmetry axis z or around its symmetry axis x (to yield oblate discoid shapes and prolate shapes, respectively) in such a way that a view along the symmetry axis of the obtained shape is an ellipse with a given semiaxis ratio ϑ, this being the fourth characteristic model parameter (Kralj-Iglič et al. 1993). The values of the parameters α, β, γ and ϑ are found, for which the membrane free energy f (12) is minimal within the strict bilayer couple model (conditions (14)–(17)). The values of $\vartheta \geq 1$ account for all different shapes which can be generated by this model. For the value of $\vartheta = 1$ the equilibrium shape is axisymmetric while the values $\vartheta > 1$ represent non-axisymmetric elliptic shapes.

We first examined how the interaction of membrane embedded molecules with the membrane curvature affects the elliptic deformation of the cell (Figs. 1, 2). As an example, a vesicle with $v=0.6$ and $\Delta a_0 = 1.069$ was considered. These values of v and Δa_0 were chosen since the vesicle with a laterally homogeneous membrane (in which the embedded molecules are uniformly distributed) with these values of v and Δa_0 is moderately elliptically deformed ($\vartheta = 1.43$, Kralj-Iglič et al. 1993). Therefore we can study whether the non-uniform distribution of membrane embedded molecules would cause an increase or a decrease of the already existing elliptic deformation. Molecules are characterized by different values of the parameter c_s which may attain any positive or negative value and which determines the most favorable membrane curvatures for the molecules (Appendix II). A high value of c_s means that molecules favor high values of both principal curvatures while a low value of c_s means that molecules favor low values of both principal curvatures. Figure 1 shows the ellipse semiaxis ratio ϑ as a function of the parameter κ [reflecting the strength of the interaction, Eq. (20)] at constant value of p for the three chosen values of c_s: $c_s=4$, $c_s=2$ and $c_s=0$. Figure 2 shows the ellipse semiaxis ratio ϑ as a function of the parameter p (which is proportional to the total number of membrane embedded molecules N (Eq. 13)) at con-

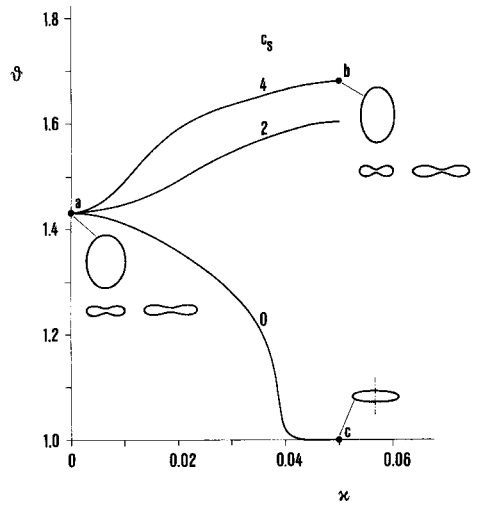

Fig. 1 The ellipse semiaxis ratio ϑ of equilibrium shapes as a function of the strength of the curvature dependent interaction of the membrane embedded molecules and the membrane (κ), for three values of the parameter c_s: 0, 2 and 4. Three characteristic shapes are also depicted: *a* the shape in which the lateral distribution of the embedded molecules is uniform ($\alpha=0.74$, $\beta=0.8$, $\gamma=0.76$, $\vartheta=1.43$); *b* the shape with membrane embedded molecules which favor high curvatures ($c_s=4$; $\alpha=0.68$, $\beta=0.97$, $\gamma=0.68$, $\vartheta=1.68$); *c* the shape with membrane embedded molecules which favor low curvatures ($c_s=0$; $\alpha=0.37$, $\beta=0.28$, $\gamma=1.27$, $\vartheta=1$). The non-axisymmetric shapes *a* and *b* are represented by their front, side and top views, while the axisymmetric shape *c* is represented by its side view and the rotation axis. Values of other parameters are $v=0.6$, $\Delta a_0=1.069$ and $p=25$

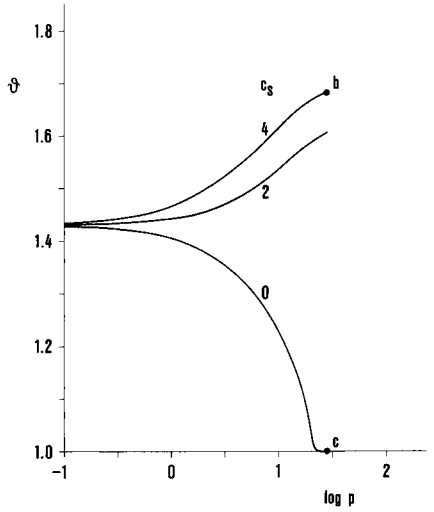

Fig. 2 The ellipse semiaxis ratio ϑ as a function of the parameter p reflecting the total number of membrane embedded molecules, for the values of parameter c_s: 0, 2 and 4. The positions of the characteristic shapes *b* and *c* shown in Fig. 1 are also marked by the corresponding letters. Values of other parameters are $v=0.6$, $\Delta a_0=1.069$ and $\kappa=0.05$. The shape *a* corresponds to the limit $\log(p) \to -\infty$

stant value of κ for the same values of c_s. For the local membrane bending modulus $k_c=10^{-19}$ J, temperature $T=310$ K and vesicle area 140 10^{-12} m^2, and chosen values of $\kappa=0.05$, $\log p=1.4$ and $c_s=4$, the energies of the membrane embedded molecule are in the range of kT values, the number of the embedded molecules N is about 1.5 10^4 while the parameter C_s is about 1.2 10^6 m^{-1}.

Considering the non-uniform distribution of membrane embedded molecules which favor high curvature ($c_s=4$) it can be seen in Figs. 1 and 2 that the elliptic deformation is larger (ϑ is larger) than in the case of uniform distribution of membrane embedded molecules and that it increases with increasing κ and p, respectively. The elliptic deformation is also larger when $c_s=2$. For the embedded molecules favoring low curvature ($c_s=0$) the elliptic deformation is smaller than in the case of uniformly distributed molecules and it decreases with increasing κ and p. For strong enough interaction the cell with $c_s=0$ is axisymmetric.

The effect of the membrane embedded molecules on the cell shape and on the distribution of the molecules can also be looked at by analyzing the distribution of the membrane area and the number of the embedded molecules over the membrane curvatures. As an example Fig. 3 shows the distributions of the relative membrane area (full lines) and of the relative number of membrane embedded molecules (broken line) with respect to c_1+c_2 for the shape (b) marked in Fig. 1 (molecules in favor of high curvatures) and for the shape (a) where the embedded molecules are uniformly distributed over the membrane (in this case the two distributions are identical). Whereas the distribution of the relative membrane area over c_1+c_2 of a sphere is a delta function at $c_1+c_2=2$, deviation of the shape from the sphere causes the distribution to broaden and become more complex. The shape (b) where the molecules favor larger curvatures is distorted relatively to the shape (a) in a way that exhibits a larger portion of membrane area pertaining to higher values of c_1 and c_2. Also, the area density of membrane embedded molecules is higher in these regions as $d(n/n_u)/d(c_1+c_2)$ (broken curve) exceeds the $da/d(c_1+c_2)$ (full curve).

For illustration, the distribution of the membrane embedded molecules over the cell surface for the shape (b) viewed from above is shown in Fig. 4. The molecules accumulate closer to the rim where the curvature is larger while they are expelled from the central region where the curvature is smaller.

In order to compare the equilibrium shapes which were calculated by taking into account inhomogeneous lateral distribution of membrane embedded molecules with the equilibrium shapes of cells with uniformly distributed molecules the calculated dependence of the membrane free energy of the equilibrium shapes f on the relative area difference Δa_0 for a chosen value of the relative volume $v=0.6$ is given (Fig. 5). It was established for the cells with laterally homogeneous membrane (Kralj-Iglič et al. 1993) and is also shown in Fig. 5 (curve (A)), that for this value of the relative volume ($v=0.6$) the $f(\Delta a_0)$ curve exhibits two local minima in the treated Δa_0 interval. The local energy minimum at lower Δa_0, corresponding to the oblate discoid shape is at chosen value $v=0.6$ lower than the local minimum at higher Δa_0 which corresponds to the prolate shape. The shapes pertaining to the minima are both axisymmetric. There is a region of non-axisymmetric el-

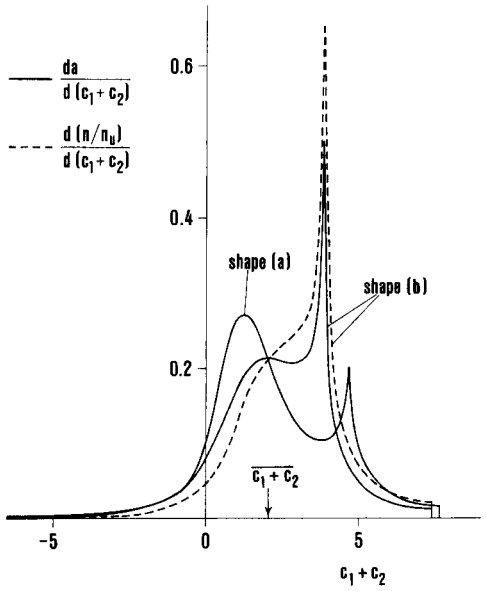

Fig. 3 Distributions of the relative membrane area (*full lines*) and the relative number of membrane embedded molecules (*broken line*) with respect to $c_1 + c_2$ for the shapes *a* and *b* marked in Fig. 1. For the shape *a* the distribution of the embedded molecules is laterally uniform so that the distributions of the relative membrane area and of the number of membrane embedded molecules are identical. The average value of the sum of the principal curvatures $\overline{c_1 + c_2} = 2\,\Delta a_0$ is marked

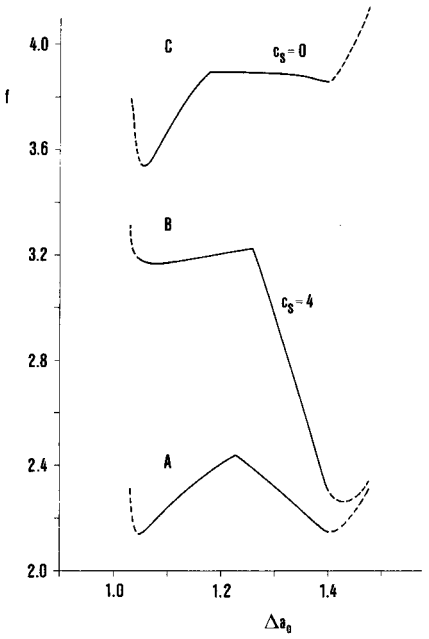

Fig. 5 The relative free energy of the equilibrium shapes f as a function of the relative area difference Δa_0 for three cases which correspond to three different sets of parameters regarding the membrane embedded molecules; *A*: $\kappa = p = 0$, *B*: $\kappa = 0.05, p = 5, c_s = 4$; *C*: $\kappa = 0.05$, $p = 5$, $c_s = 0$. In all cases $c_0 = 0$ and $v = 0.6$. The *broken lines* represent free energies of axisymmetric shapes while the *full lines* represent free energies of nonaxisymmetric elliptic shapes

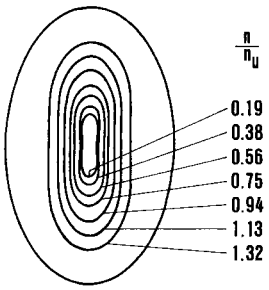

Fig. 4 The top view of the shape *b* marked in Figs. 1 and 2. A family of curves of equal number density of the embedded molecules is shown. The number density is calculated relative to the density of the corresponding uniform distribution

liptic shapes between the minima. At both transitions from axisymmetric to non-axisymmetric shape (at the two minima) the shapes and energies change in a continuous manner. Figure 5 (curves B and C) shows the free energy of the equilibrium shapes f of the cell with $v = 0.6$ and $c_0 = 0$ as a function of the relative area difference of the two membrane layers for the membrane with embedded molecules favoring (B) high curvature ($c_s = 4$) and for the embedded molecules favoring (C) low curvature ($c_s = 0$).

It can be seen in Fig. 5 that the values of f are larger owing to the presence of the membrane embedded molecules and that the dependence of f on Δa_0 is considerably affected. In case B ($c_s = 4$) the absolute energy minimum lies in the range of the prolate shapes and is significantly lower than the minimum in the range of the oblate shapes. The equilibrium shape corresponding to the absolute minimum is prolate and axisymmetric. In the range of oblate shapes the lower bound of the Δa_0 interval of non-axisymmetric shapes is shifted towards smaller Δa_0 with respect to the case of uniform distribution of membrane embedded molecules (Fig. 5). In case *C* ($c_s = 0$) the minimum in the range of oblate shapes is considerably lower than the minimum in the range of prolate shapes so that the equilibrium shape corresponding to the absolute minimum is oblate and it is non-axisymmetric.

Discussion

The main subject of this work is the interdependence of the lateral distribution of the membrane embedded molecules and the cell shape. The macroscopic quantities: distribution of the membrane embedded molecules over the membrane area and the corresponding free energy are derived starting from the energy of an individual embedded molecule which is taken to be a second order polynomial in the two principal curvatures. The state of an embedded molecule in the membrane is in this case characterized only by the two degrees of freedom determining its lateral position on the membrane. Namely, in order to show the consequences of the ideas presented in the simplest manner we did not take into consideration the other four degrees of freedom of a molecule, i.e. the three degrees of freedom for the molecular rotation and the molecular position in the direction normal to the plane of the membrane. The ex-

pression derived for the molecule energy given in Appendix II (AII.5) actually involves also the rotation of the molecule around the axis directed along the membrane surface normal and could be applied in cases of non-axisymmetric molecules where the mismatch in the shape of the immersed part and the membrane changes along the contact rim. Our simplified treatment corresponds to the situation where the immersed part of the molecule is axisymmetric. In this case the interpretation of the results obtained is more transparent in particular because for the embedded molecule the two most favorable principal membrane curvatures have equal values (AII.13).

The curvature dependent interaction between the embedded molecules and the membrane can have a strong effect on the lateral distribution of the molecules and also on the cell shape. It is obvious that for simple thermodynamic reasons the embedded molecules are more abundant in the membrane regions with favorable curvatures and less abundant in the regions with unfavorable curvatures. It can also be understood that a cell attains the equilibrium shape which involves an increased amount of regions of the favorable values of principal curvatures.

The cell shapes are obtained by the criterion of the minimum membrane free energy which contains, in addition to the usually considered elastic energy of the membrane, the contribution due to the laterally inhomogeneous distribution of the embedded molecules. The set of equilibrium shapes thus obtained consists of shapes that are in general different from the shapes obtained by the minimization of membrane elastic energy of the homogeneous membrane.

Regarding the membrane elastic energy, all the results presented were calculated in the limit of the strict bilayer couple model, i. e. by assuming the non-local bending constant k_r to be infinite. Analogously to the case of laterally homogeneous membrane (Heinrich et al. 1993) the results obtained within the strict bilayer couple model can be generalized in a straightforward manner which allows for the finite k_r.

The inclusion of curvature dependent energy of membrane embedded molecules brings in additional parameters: c_s (21) determining the membrane curvatures which are the most favorable for the membrane embedded molecules, κ (20) expressing the strength of the molecule – membrane interaction, and p (13) reflecting the number of the membrane embedded molecules. The parameter c_s determines whether the shape will be distorted in a way to exhibit larger regions with larger curvatures or with smaller curvatures. The values of parameters κ and p determine the extent of the distortion (Figs. 1, 2). It is expected that the shapes obtained would differ the least from the corresponding shapes obtained in the case of the laterally homogeneous membrane if we assume that the average curvature is equal to the most favored one. By considering that the relative average curvature is equal to the relative area difference Δa_0 (11) and that for the embedded molecules both the most favorable relative curvatures were shown to be $3c_s/4$ (AII.13), it can be expected that the values of c_s close to $4\Delta a_0/3$ would cause the least deformation while for the c_s values significantly different from $4\Delta a_0/3$ the effects would be considerable. For the example presented in Figs. 1 and 2 for which $\Delta a_0 = 1.069$ so that $4\Delta a_0/3 = 1.42$ it was shown (Figs. 1, 2) that if $c_s = 2$ or 4, larger elliptic deformations are promoted while for $c_s = 0$ the shapes are less elliptically deformed than the corresponding shapes of the laterally homogeneous membrane.

Our results show that the intrinsic curvature of the membrane embedded molecules characterized by the parameter c_s determines whether the cell shape corresponding to the absolute energy minimum at given cell volume and membrane area is in the region of oblate or prolate shapes. If the parameter c_s is large in comparison to the average of the mean curvature over the membrane area, the prolate shape corresponding to the minimum at higher Δa_0 in the $f(\Delta a_0)$ curve (Fig. 5) is energetically more favorable while for low c_s the oblate shape corresponding to the minimum at lower Δa_0 is preferred. This effect is in qualitative terms the same as the effect of the non-zero spontaneous curvature c_0 on the energies of the shapes of the cell with a laterally homogeneous membrane. In the case of homogeneous membranes, owing to the non-zero spontaneous curvature, a linear function of Δa_0 has to be added to the $f(\Delta a_0)$ curve, by which the positions and the heights of the two minima are shifted whereas the corresponding shapes remain the same (Svetina and Žekš 1989). Consequently, the absolute minimum of the $f(\Delta a_0)$ curve corresponds to an axisymmetric shape. However, besides this effect of relative shifting of the energy of the prolate and oblate shapes, it was shown here that the inhomogeneous distribution of membrane embedded molecules may cause the absolute energy minimum to correspond to a non-axisymmetric shape (Fig. 5).

It is clear from the above that the nonaxisymmetric shape cannot be predicted for the absolute energy minimum state of the system on the basis of those curvature dependent interactions for which the effect of nonhomogeneous distribution can be described by renormalization of the values of the parameters of the original elastic energy model. In order to reveal the features of the model presented which are responsible for its more complex behavior, it is of interest to see under what conditions its predictions can be described by the renormalization procedure. As presented in Appendix III, the membrane free energy can be expressed in the form of the generalized bilayer couple model with renormalized model parameters for weak interactions of the embedded molecules with the membrane ($K/kT \to 0$) and $|C_s|$ much larger than $|(C_1 + C_2)|$. The membrane free energy has in this limit essentially the same form as the free energy introduced by Seifert (1993). There is a slight difference in the renormalized model parameters due to the difference between the two models. In the model presented the membrane is composed of two layers and the embedded molecules span both of them whereas in the model of Seifert (1993) the membrane is asymmetric with regard to the composition of its layers. In our case for $c_s \neq 0$ the inhomogeneous distribution of the embedded molecules gives rise to the nonzero spontaneous membrane curvature whereas in the case of

Seifert, if the membrane is symmetrical and the spontaneous curvature is zero, in the unstressed state of a two component mixture of lipids the membrane would tend to be flat. The stable nonaxisymmetric shapes can also be obtained within the model of Seifert by shifting within the phase diagram of possible stable shapes of the generalized bilayer couple model. However, a nonaxisymmetric stable shape corresponding to the absolute energy minimum cannot be obtained by this model.

The result that the redistribution of membrane constituents may shift the absolute minimum of the system free energy into the region of shapes with different symmetry properties can bear a significant relevance with regard to the behavior of real biological systems. It is plausible to assume that cells in general attain shapes which correspond to the absolute minimum of their membrane energy, provided that there exists an appropriate coupling between the cell biochemical processes and the parameters determining cellular mechanics. As has been suggested previously (Svetina and Žekš 1990), the polar asymmetry in the distribution of membrane embedded molecules could thus be the basis for the stabilization of the polarized state of a cell in the process of establishing cellular polarity. Another pertinent example is the occurrence of elliptic erythrocyte shapes in some human blood disorders (Palek 1987) and in some healthy animals, e.g. in llama (Khodadad and Weinstein 1983). The elliptic erythrocyte shapes were characterized, with respect to the discoid shape of normal human erythrocytes, either by altered structure of some membrane proteins and therefore altered interaction with the membrane, or by the altered abundance of membrane embedded proteins (Liu et al. 1991; Khodadad and Weinstein 1983). Our analysis shows that changes in the structure or in the abundance of the membrane proteins may give rise to changed values of one or more of the system parameters which influence the shape (κ, c_s and p) in such a way that the absolute energy minimum is shifted towards the region of non-axisymmetric shapes. The effect of the distributional free energy presented here can be considered only as one of the possible mechanisms which would make the erythrocyte shape elliptic. In explaining the existence of elliptic erythrocytes other contributions to the membrane free energy arising from the bilayer – cytoskeleton interactions and by the interactions between the molecules within the cytoskeleton such as the membrane shear energy (Stokke et al. 1986a, b; Elgsaeter and Mikkelsen 1991; Strey et al. 1995) should however be taken into account. Further studies providing quantitative data on the values of the relevant parameters will be needed to evaluate the relative effects of different contributions to the membrane energy.

The analysis presented was performed by using an approximative solution of the variational problem defined by the minimization of the membrane free energy at given geometrical constraints. Because in the case of a homogeneous membrane the shapes calculated with the parametric model used here were shown to be in the treated Δa_0 region sufficiently close to the shapes obtained by the exact methods (Kralj-Iglič et al. 1993) we believe that the general conclusions about the effect of the embedded molecules on the behavior of the system are correct. However, it is expected that for Δa_0 values outside the treated range, for strong interaction between the embedded molecules and the membrane, and for large numbers of embedded molecules, the possible stable cell shapes would significantly differ from the corresponding stable shapes of the elastic energy models. Therefore, work to solve the variational shape problem including the membrane embedded molecules exactly is also in progress.

Appendix I

Shape dependent free energy of membrane embedded molecules

In order to derive the free energy of the membrane embedded molecules the membrane surface is divided into m small regions of area A_i, $i=1, 2, \ldots, m$. It is assumed that the dimensions of A_i are small, so that the principal curvatures C_{1i} and C_{2i}, and the interaction energy $E_i = E_i(C_{1i}, C_{2i})$ are taken to be constant over A_i. In a particular region chosen there are N_i membrane embedded molecules. The local equilibrium state of N_i equal, independent and indistinguishable membrane embedded molecules in a region having an area A_i at temperature T is described by the canonical partition function Q_i (Hill 1962),

$$Q_i = e^{-N_i E_i / kT} \frac{1}{N_i!}, \tag{AI.1}$$

where k is the Boltzmann constant and T is temperature. The corresponding free energy of the membrane embedded molecules of the i-th region of the membrane is then $F_{d,i} = -kT \ln Q_i$. Using the Stirling approximation yields

$$F_{d,i} = N_i E_i + kT N_i (\ln N_i - 1). \tag{AI.2}$$

To obtain the free energy of the membrane embedded molecules of the entire membrane, contributions to the free energy of all the regions are summed ($\sum_i F_{d,i}$) over the membrane area $A = \sum_i A_i$.

If the interaction energy is constant everywhere in the membrane, the distribution of the membrane embedded molecules is uniform. The uniform distribution is considered as the reference distribution and is described by a constant number surface density n_u,

$$n_u = \sum_i N_i / A. \tag{AI.3}$$

In the reference state there are $N_{u,i}$ molecules in the i-th region of the membrane,

$$N_{u,i} = n_u A_i, \tag{AI.4}$$

so that the contribution to the free energy of this state of the i-th region $F_{d,i}^{\text{ref}}$ is

$$F_{d,i}^{\text{ref}} = kT N_{u,i} (\ln N_{u,i} - 1). \tag{AI.5}$$

To obtain the free energy of the reference state ($\sum_i F_{d,i}^{ref}$), the contributions of all the regions are summed. The free energy F_d is defined as the difference between $\sum_i F_{d,i}$ and $\sum_i F_{d,i}^{ref}$. In the summation of the contributions (AI.2) the number surface density of the membrane embedded molecules n_i is used,

$$n_i = N_i/A_i. \tag{AI.6}$$

It is also taken into account that in the entire membrane there are altogether N molecules,

$$N = \int_A n(A)\, dA = n_u A, \tag{AI.7}$$

where the summation is replaced by the integration, so that n_i transforms into $n(A)$ and dA is the area element. Using (AI.2) to (AI.7), the free energy of the membrane embedded molecules is

$$F_d = \int_A [E(C_1, C_2)\, n + kTn \ln(n/n_u)]\, dA\,. \tag{AI.8}$$

The requirement of local equilibrium was already considered by using the canonical partition function in any of the m small regions. However, to obtain the equilibrium state of the entire system, the distribution function of the membrane embedded molecules $n(A)$ is sought for which the free energy F_d is minimal. While performing the minimization of F_d with respect to $n(A)$ the total number of membrane embedded molecules N is kept constant (AI.7). This is obtained by variation of the functional

$$G = F_d - \lambda\left(\int_A n\, dA - N\right), \tag{AI.9}$$

where λ is the Lagrange multiplier.

The functional can be expressed as

$$G = \int \bar{G}(C_1, C_2, n)\, dA, \tag{AI.10}$$

where

$$\bar{G} = E(C_1, C_2)\, n + kTn \ln(n/n_u) - \lambda n + \text{const.} \tag{AI.11}$$

The corresponding Euler equation is

$$\frac{\partial \bar{G}}{\partial n} = 0. \tag{AI.12}$$

Using (AI.11) and (AI.12) yields the Boltzmann distribution function

$$n = \exp\left(-E(C_1, C_2)/kT + \lambda/kT - 1\right). \tag{AI.13}$$

The parameter λ is determined by using the condition (AI.7) and the expression (AI.13), and we get

$$n/n_u = \frac{1}{\Sigma} e^{-E(C_1, C_2)/kT}, \tag{AI.14}$$

where

$$\Sigma = \frac{1}{A}\int e^{-E(C_1, C_2)/kT} dA\,. \tag{AI.15}$$

The free energy of the membrane embedded molecules F_d minimized with respect to the distribution function of these molecules for a given vesicle shape (which is determined by knowing the principal curvatures C_1 and C_2 over the entire surface) is then obtained by inserting (AI.14) and (AI.15) into the expression (AI.8) and some rearranging,

$$F_d = -kTN \ln\Sigma. \tag{AI.16}$$

Appendix II

The dependence of the energy of membrane embedded molecules on the local curvature of the membrane

The membrane free energy is expressed in terms of the interaction of the membrane embedded molecule with the membrane which depends on the local membrane curvature $E(C_1,C_2)$. In order to specify this interaction, a simple expression is proposed based on the notion that it is energetically favorable that the shape of the part of the molecule which is immersed in the membrane is fit to the local membrane curvature. Any mismatch in the shape, which may change along the rim of the immersed part, causes an increase of the energy.

The normal membrane curvature C in a chosen direction is expressed in terms of the two principal membrane curvatures C_1 and C_2,

$$C = C_1 \cos^2\phi + C_2 \sin^2\phi, \tag{AII.1}$$

where ϕ describes the orientation of the chosen direction in the principal axes system. The shape of the immersed part of the molecule (which may change along the rim) is approximated at a chosen direction by

$$C_m = C_{1m}\cos^2\psi + C_{2m}\sin^2\psi, \tag{AII.2}$$

where C_{1m} and C_{2m} are the principal curvatures characterizing the shape of the molecule and ψ is the angle of rotation with respect to the molecule principal axes system. We also introduce the angle ω which describes the rotation of the molecule principal axes system with respect to the membrane principal axes system so that

$$\phi = \psi + \omega. \tag{AII.3}$$

To obtain the energy of the interaction of the immersed part of the molecule at a given ω the contributions over all angles ψ are summed,

$$E(\omega) = \frac{\xi}{4\pi}\int_0^{2\pi} (C - C_m)^2\, d\psi, \tag{AII.4}$$

where ξ is the constant of the local interaction. By inserting the expressions (AII.1) and (AII.2) into (AII.4) and performing the necessary integrations we get

$$E(\omega)=\frac{3\xi}{16}\left[(C_1+C_2)^2-\frac{4}{3}C_1C_2\right]+\frac{3\xi}{16}\left[(C_{1m}+C_{2m})^2-\frac{4}{3}C_{1m}C_{2m}\right]-\frac{\xi}{4}(C_{1m}+C_{2m})(C_1+C_2)-\frac{\xi}{8}(C_{1m}-C_{2m})(C_1-C_2)\cos(2\omega). \quad \text{(AII.5)}$$

The energy E in general depends on the orientation of the immersed part of the molecule with regard to the rotation of the molecule around its axis along the membrane surface normal. However, for the sake of simplicity, in this work only the molecules which are axisymmetric with respect to this axis are considered, so that

$$C_{1m}=C_{2m}=C_{0m}, \quad \text{(AII.6)}$$

and the energy does not depend on the angle ω

$$E=\frac{3\xi}{16}\left[(C_1+C_2)^2-\frac{4}{3}C_1C_2\right]-\frac{\xi}{2}C_{0m}(C_1+C_2)+\frac{\xi}{2}C_{0m}^2. \quad \text{(AII.7)}$$

Omitting the constant term the energy E can be written in the form

$$E=K\left((C_1+C_2-C_s)^2-\frac{4}{3}C_1C_2\right), \quad \text{(AII.8)}$$

where

$$K=3\xi/16, \quad \text{(AII.9)}$$

and

$$C_s=4C_{0m}/3. \quad \text{(AII.10)}$$

The principal curvatures which are the most favorable for the membrane embedded molecules $C_{1,\min}$ and $C_{2,\min}$, respectively, are determined by the conditions

$$\frac{\partial E}{\partial C_1}=2(C_{1,\min}+C_{2,\min}-C_s)-\frac{4}{3}C_{2,\min}=0, \quad \text{(AII.11)}$$

$$\frac{\partial E}{\partial C_2}=2(C_{1,\min}+C_{2,\min}-C_s)-\frac{4}{3}C_{1,\min}=0, \quad \text{(AII.12)}$$

which give

$$C_{1,\min}=C_{2,\min}=3C_s/4. \quad \text{(AII.13)}$$

Appendix III

The generalized bilayer couple model as a limit of a weak interaction between the membrane embedded molecules and the membrane

It is shown that by taking into account some additional assumptions about the interaction between the membrane embedded molecules and the membrane curvature the equations having the form of the membrane elastic energy of the generalized bilayer couple model with renormalized values of elastic constants, C_0 and ΔA_0 can be obtained from the expression for the membrane free energy (12).

It is assumed that the interaction between the membrane embedded molecules and the membrane curvatures is very small in comparison to kT over the entire membrane area

$$K/kT \to 0 \quad \text{(AIII.1)}$$

so that we can expand the exponential function in Σ (Eq. (2)) as well as the logarithmic function in the free energy term due to the membrane embedded molecules (F_d) (Eq. (4)) in terms of E/kT. The expansion up to quadratic terms in E/kT yields

$$F(E\ll kT)=\frac{1}{2}k_c\int(C_1+C_2)^2dA+\frac{NkT}{4\pi R_s^2}\int[E/kT-E^2/2(kT)^2]dA+\frac{NkT}{2}\left(\int[E/kT-E^2/2(kT)^2]\frac{dA}{4\pi R_s^2}\right)^2. \quad \text{(AIII.2)}$$

The expression for the interaction of the membrane embedded molecule (18) is inserted into (AIII.2). Further, it is assumed that $|C_s|$ is much larger than any $|(C_1+C_2)|$. In this respect, retaining only the terms proportional to KC_s, and omitting the constant terms, the free energy becomes

$$E_b=\frac{1}{2}k_{c,\mathrm{eff}}\int(C_1+C_2-\bar{C}_0)^2dA+\frac{1}{2}k_{r,\mathrm{eff}}(\Delta A-\overline{\Delta A}_0)^2/Ah^2, \quad \text{(AIII.3)}$$

with renormalized local bending constant $k_{c,\mathrm{eff}}$

$$k_{c,\mathrm{eff}}=k_c-4n_u K^2 C_s^2/kT, \quad \text{(AIII.4)}$$

renormalized non-local bending constant $k_{r,\mathrm{eff}}$

$$k_{r,\mathrm{eff}}=k_r+4n_u K^2 C_s^2/kT, \quad \text{(AIII.5)}$$

renormalized spontaneous curvature $\bar{C}_0$

$$\bar{C}_0=C_0 k_c/k_{c,\mathrm{eff}}+2n_u KC_s/k_{c,\mathrm{eff}} \quad \text{(AIII.6)}$$

and renormalized equilibrium area difference of the two membrane layers

$$\overline{\Delta A}_0=\Delta A_0 k_r/k_{r,\mathrm{eff}}. \quad \text{(AIII.7)}$$

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