Depletion of Membrane Skeleton in Red Blood Cell Vesicles

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ABSTRACT A possible physical interpretation of the partial detachment of the membrane skeleton in the budding region of the cell membrane and consequent depletion of the membrane skeleton in red blood cell vesicles is given. The red blood cell membrane is considered to consist of the bilayer part and the membrane skeleton. The skeleton is, under normal conditions, bound to the bilayer over its whole area. It is shown that, when in such conditions it is in the expanded state, some cell shape changes can induce its partial detachment. The partial detachment of the skeleton from the bilayer is energetically favorable if the consequent decrease of the skeleton expansion energy is larger than the corresponding increase of the bilayer-skeleton binding energy. The effect of shape on the skeleton detachment is analyzed theoretically for a series of the pear class shapes, having decreasing neck diameter and ending with a parent-daughter pair of spheres. The partial detachment of the skeleton is promoted by narrowing of the cell neck, by increasing the lateral tension in the skeleton and its area expansivity modulus, and by diminishing the attraction forces between the skeleton and the bilayer. If the radius of the daughter vesicle is sufficiently small relative to the radius of the parent cell, the daughter vesicle can exist either completely underlaid with the skeleton or completely depleted of the skeleton.

INTRODUCTION

Membranes of some cells and organelles can form, during the budding process, small spherical daughter vesicles that are released from the membrane in the process of vesiculation (Alberts et al., 1989; Bütikofer et al., 1987). This phenomenon has been extensively explored in the case of red blood cells (RBC), for which the mechanism of the budding process is based on the properties of its membrane (Hägerstrand and Isomaa, 1994).

The RBC membrane is essentially composed of two parts, the bilayer and the continuous network of proteins, the membrane skeleton (Steck, 1974; Bennett, 1989). The skeleton is attached to the bilayer integral proteins at the inner side of the bilayer. Under normal conditions the entire bilayer is underlaid with the skeleton (Liu et al., 1989). However, it was found that the major components of the membrane skeleton, spectrin and actin, are in general not present in the RBC vesicles (Allan et al., 1976; Elgsaeter et al., 1976), whereas the lipid composition of the RBC vesicle membrane is very similar to that of the native RBC (Lutz et al., 1977; Hägerstrand and Isomaa, 1994). In addition, RBC vesicles contain the major membrane integral proteins in approximately the same relative amounts as the parent cell membrane (Hägerstrand and Isomaa, 1994). On the basis of these findings it can be concluded that the vesicles released from the RBC membrane are formed by the bilayer part of the membrane, which was confirmed also by direct electron microscope observations (Liu et al., 1989). The aim of the present work is to analyze a possible physical mechanism leading to the formation of the membrane skeleton-depleted vesicles.

A POSSIBLE MECHANISM LEADING TO THE FORMATION OF THE SKELETON-DEPLETED RED BLOOD CELL VESICLES

The problem we address is the formation of a skeleton-free membrane domain that eventually gives rise to the absence of skeleton in the RBC vesicles. The existence of such a domain can be the consequence either of the skeleton disruption (Kozlov et al., 1990) or of the partial detachment of the skeleton from the bilayer. Because membrane integral proteins are corralled by the spectrin network units (Sheetz, 1983), the skeleton disruption would cause the drag of membrane integral proteins out of membrane domains free of skeleton. This is, however, not in accordance with experimental observations (Hägerstrand and Isomaa, 1994). Therefore, in this work we assume that the partial detachment of skeleton is the relevant mechanism leading to the absence of skeleton in RBC vesicles.

To reveal the physical conditions controlling the partial detachment of the skeleton, the elastic properties of the membrane bilayer and the skeleton as well as the interaction energy between these two membrane constitutive elements must be known. On the basis of the experiments of Mohandas and Evans (1994), it can be concluded that the area expansivity modulus of the skeleton is, under normal conditions, a few orders of magnitude smaller than the area expansivity modulus of the bilayer. Therefore, in this work the membrane bilayer is treated as incompressible in the lateral direction; i.e., the area of the bilayer (A) is constant and independent of the state of the skeleton. Furthermore, the bending energy of the skeleton is negligible compared with the bending energy of the bilayer, and the shear energy

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of the skeleton is not taken into consideration. These assumptions give rise to a simple model of RBC behavior in which RBC shape at a given cell volume is determined only by the membrane bilayer, whereas the association state of the skeleton at a given cell shape is determined only by the elastic properties of the skeleton and by its interactions with the bilayer.

In view of adopted simplifications, the elastic energy of the skeleton consists only of the stretching contribution:

$$W_e = \frac{1}{2} K_s (A_s - A_{so})^2 / A_{so}, \qquad (1)$$

where K_s is the area expansivity modulus of the skeleton, A_s is the area of the skeleton, and A_{so} is the area of the relaxed skeleton. The membrane skeleton is taken to be laterally homogeneous, which means that the lateral tension in the skeleton is uniform over the surface of the skeleton. The area of the relaxed skeleton A_{so} is taken to be smaller than the bilayer area A. This notion is based on the observation that the area of the isolated RBC membrane skeleton is, at physiological external conditions, smaller than the area of the RBC (Lange et al., 1982, Svoboda et al., 1992). This implies that, in the intact cell, the skeleton is expanded by means of its interactions with the membrane bilayer (Steck, 1989) to completely underlay the inner surface of the bilayer. In this way, the area of the skeleton is equal to the area of the bilayer A. If the membrane skeleton is partially detached from the bilayer, the area of the skeleton becomes smaller than the area of the bilayer A. Therefore, the lateral tension and the elastic energy of the skeleton are decreased after its partial detachment.

The interaction (binding) energy between the skeleton and the bilayer, W_i, is taken to be proportional to the area of contact between the skeleton and the bilayer. Under normal conditions, when the entire bilayer is in contact with the skeleton, the interaction energy W_i is given by the expression $-\gamma A$, where the positive constant γ is the energy of the skeleton attachment per unit area. After the partial detachment of the skeleton from the bilayer, the interaction energy W_i is increased and is written as

$$W_i = -\gamma (A - A_f), \qquad (2)$$

where A_f is the area of the bilayer that is not underlaid with the membrane skeleton. The area $(A - A_f)$ is the area of contact between the skeleton and the bilayer.

In this work we consider that a possible mechanism for the partial detachment of the skeleton from the bilayer is the membrane tendency to reduce the energy W, which is the sum of the skeleton elastic energy W_e and the interaction energy W_i:

$$W = W_e + W_i. \qquad (3)$$

The partial detachment of the membrane skeleton from the bilayer will take place if the decrease of the elastic energy of the skeleton W_e after skeleton detachment is larger than the corresponding increase of the interaction energy W_i.

ASSOCIATION STATE OF THE MEMBRANE SKELETON FOR A SEQUENCE OF AXISYMMETRICAL PEAR CELL SHAPES

Because vesiculation is in general preceded by cell shape changes, it was of interest for us to investigate whether the partial detachment of the membrane skeleton from the bilayer in the budding region of the cell membrane is induced by cell shape transformations. To reveal the essentials of the partial detachment of the skeleton from the bilayer in the budding region of the cell membrane, we confine our analysis of the budding process to a sequence of axisymmetrical pear cell shapes in which only one daughter vesicle is formed (Svetina and Žekš, 1990).

Pear cell shapes represent a class of stable shapes in the bilayer couple model, where a class of shapes is defined to contain all the stationary shapes of the same symmetry that can be continuously transformed into each other by continuously varying the two model parameters, i.e., the relative cell volume v and the relative difference between the areas of the two membrane lipid layers, Δa (Svetina and Žekš, 1989). Both of these parameters are normalized relative to the corresponding values for the spherical cell that has the same membrane area. At constant relative cell volume, the neck of the pear-shaped cells is narrowing continuously with increasing Δa until it becomes infinitesimally small at some maximal Δa (Δa_ℓ) when the limiting shape composed of a parent-daughter pair of spheres of different radii is reached (as can be seen in Fig. 3).

We assume that, after partial detachment of the skeleton from the bilayer, the skeleton still forms an axisymmetrical closed surface and is flat in the domain that is not in contact with the bilayer (Fig. 1). Thus, at certain skeleton area A_s, which is smaller than the bilayer area A, the area of contact between the skeleton and the bilayer $(A - A_f)$ is the largest

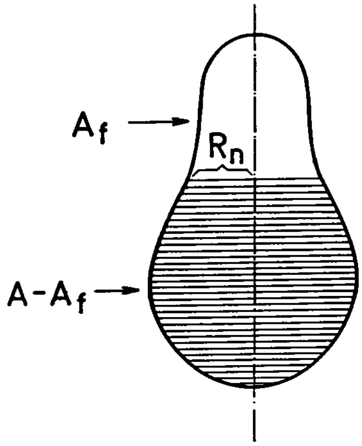

FIGURE 1 Schematic presentation of the axisymmetrical cell and its membrane. The symbol A_f denotes the membrane area where the membrane skeleton (*shaded area*) is detached from the membrane bilayer, whereas R_n is the radius of the horizontal cross section of the cell at the boundary between the membrane region that is underlaid with the skeleton and the region without the skeleton.

possible. We allow the skeleton to form a sharp edge at the border between its attached and detached domains (Fig. 1) as the bending energy of the sketelon is neglected in this work. In the case of axisymmetrical pear cell shape, the area of the membrane skeleton A_s, therefore, can be expressed as (Fig. 1)

$$A_s = A - A_f + \pi R_n^2, \tag{4}$$

where πR_n^2 is the area of the part of the skeleton that is not in contact with the bilayer.

In the following analysis, dimensionless quantities are introduced. All volumes and areas are normalized relative to the corresponding values for the spherical cell with radius $R_o = (A/4\pi)^{1/2}$, whereas the energy W (Eq. 3) is normalized relative to the energy γA. The relative energy $w = W/\gamma A$ can be written in terms of normalized quantities as

$$w = \frac{K_s}{2\gamma a_{so}} (a_s - a_{so})^2 - (1 - a_f), \tag{5}$$

where

$$a_s = A_s/A = 1 - a_f + r_n^2/4. \tag{6}$$

The relative areas a_{so} and a_f are defined as $a_{so} = A_{so}/A$ and $a_f = A_f/A$, and the relative radius is $r_n = R_n/R_o$. In accordance with the previous assumptions, the value of a_{so} is smaller than unity.

To illustrate the effect of the cell shape on the association state of the skeleton, Fig. 2 shows the dependence of the relative energy w of the axisymmetric pear cell shape with the relative volume $v = 0.85$ on the relative area of the bilayer that is not underlaid with the skeleton (a_f) for three different values of Δa and for $\gamma/K_s = 0.45$. The value of a_f is measured from the budding pole of the cell, where the skeleton is detached from the bilayer, and increases as the detachment progresses in the direction toward the opposite pole. When we calculate the energy w as a function of a_f for the given cell shape characterized by relative volume v and relative area difference Δa, the value of the relative radius r_n at each a_f is also determined numerically.

It can be seen in Fig. 2 that the function $w(a_f)$ has for low values of Δa one minimum at $a_f = 0$, which means that, for the pear cell shapes with very wide necks, the bilayer of the cell membrane is completely underlaid with the skeleton. For higher values of Δa (corresponding to the pear cell shapes with a narrower neck) the function $w(a_f)$ has two local minima, one at $a_f = 0$ and one at $a_f \neq 0$. The minimum at $a_f \neq 0$ corresponds to the situation when the membrane skeleton is partially detached from the bilayer. If the minimum of w at $a_f = 0$ is lower than the minimum at $a_f \neq 0$, then the entire bilayer is underlaid with the skeleton. In the opposite case, when the minimum at $a_f \neq 0$ is lower than the minimum $a_f = 0$, the membrane skeleton is partially detached from the bilayer. Above a certain value of Δa, it happens that the minimum of w at $a_f \neq 0$ becomes lower than the minimum at $a_f = 0$, which means that it is more favorable for the membrane skeleton to be partially detached from the bilayer in the budding region of the membrane. The consequent transition of the association state of the skeleton upon the change of Δa from the state when $a_f = 0$ to the state when $a_f \neq 0$ is discontinuous (see also Fig. 3).

At small values of γ/K_s, where the attractive interaction between the skeleton and the bilayer is weak, the function $w(a_f)$ may have an absolute minimum at a_f, which corresponds to $a_f \cong a_{so}$. In this work we do not consider such small values of the ratio γ/K_s as this would mean that the skeleton would be partially detached and relaxed also at the normal RBC shapes, which is not the case.

Fig. 3 shows for the pear-shaped cell the dependence of the association state of the membrane skeleton (shaded area) on the relative area difference (Δa) for different values of

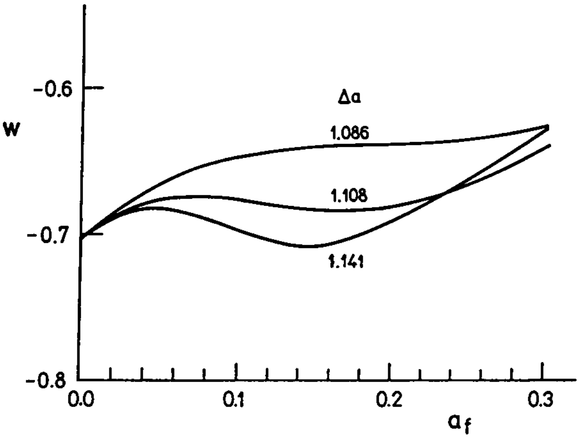

FIGURE 2 Relative energy w (Eq. 5) of the axisymmetric pear cell shape as a function of the relative area a_f for $\gamma/K_s = 0.45$, $v = 0.85$, and $a_{so} = 0.6$ and three different pear class shapes corresponding to three values of relative difference between the areas of the two membrane lipid layers (Δa).

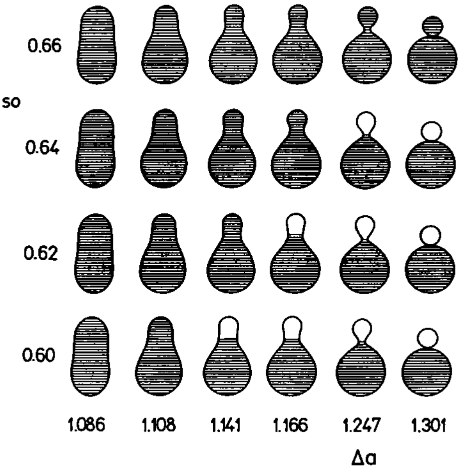

FIGURE 3 The association state of the membrane skeleton (*shaded area*) of the pear-shaped cell determined by minimization of the energy w (Fig. 2) presented for six values of the relative area difference (Δa) and four values of the relative area of the relaxed skeleton (a_{so}) at the relative cell volume $v = 0.85$ ($r_p^2 = 0.86$, $r_d^2 = 0.14$) and $\gamma/K_s = 0.45$.

the area of the relaxed skeleton a_{so} at fixed relative cell volume and fixed ratio γ/K_s. The area and association state of the membrane skeleton at a given axisymmetrical cell shape correspond to the absolute minimum of the function $w(a_f)$ (see Fig. 2). It is seen in Fig. 3 that, at a given value of the ratio γ/K_s, the detachment of the skeleton from the bilayer can be provoked by the narrowing of the cell neck. For cell shapes with narrow cell necks, the partial detachment of the skeleton from the bilayer in the budding region of the cell surface becomes energetically favorable because, for these shapes, the area of the skeleton that is not in contact with the bilayer after skeleton detachment (πR_n^2; Fig. 1) is very small. The effect is dependent on the relative area of the relaxed skeleton, a_{so}, and the ratio γ/K_s. It can be deduced from Fig. 3 that, for the given cell shape characterized by given values of v and Δa and for the fixed value of the ratio γ/K_s, there exists a certain critical value of a_{so}, denoted here as a_{so}^c, so that for $a_{so} \leq a_{so}^c$, the skeleton is partially detached from the bilayer in the convex domain where the daughter vesicle will be formed, whereas for $a_{so} > a_{so}^c$ the skeletion completely underlays the bilayer.

DETACHMENT OF THE MEMBRANE SKELETON IN THE CASE OF THE CELL SHAPES COMPOSED OF A SPHERICAL PARENT CELL AND A SPHERICAL DAUGHTER VESICLE

In this section, the detachment of the membrane skeleton is studied for the case of cell shapes that are composed of a spherical parent cell with radius R_p and a smaller spherical daughter vesicle with radius R_d, connected by an infinitesimally small neck. These shapes are the limiting shapes of the pear class (Fig. 3), corresponding to the limiting Δa (Δa_ℓ), and represent the initial stage of the cell vesiculation process during which, in its final stage, the daughter vesicle would be separated from the parent cell.

In the dimensionless representation, the relative radii $r_p = R_p/R_o$ and $r_d = R_d/R_o$ can be determined from the requirements that the relative cell area is equal to one and that the relative cell volume is equal to v. By solving the corresponding cubic equation, it follows for r_p and r_d that

$$r_p = \frac{1}{2}(\Delta a_\ell + (2 - \Delta a_\ell^2)^{1/2}), \tag{7}$$

$$r_d = \frac{1}{2}(\Delta a_\ell - (2 - \Delta a_\ell^2)^{1/2}), \tag{8}$$

where the relative difference between the areas of the two membrane layers Δa_ℓ is given by

$$\Delta a_\ell = 2\cos\left(\frac{\pi - \arccos(v)}{3}\right). \tag{9}$$

It follows from Eqs. 7–9 that the relative radius of the parent cell r_p increases whereas the relative radius of the daughter vesicle r_d decreases with increasing relative cell volume v. The cell shapes composed of two linked spheres exist only for relative volumes v greater than $2^{-1/2}$ whereas at the latter value of v we have $r_p = r_d = 2^{-1/2}$.

For the cell shapes composed of the spherical parent cell and the spherical daughter vesicle the relative area of the membrane skeleton a_s can be written in the following form:

$$a_{s,\ell} = 1 - a_f^2/r_d^2, \quad a_f \leq r_d^2, \tag{10a}$$

$$a_{s,\ell} = 1 - a_f^2/r_p^2 + 2a_f r_d^2/r_p^2 - r_d^2 - r_d^4/r_p^2, \quad r_d^2 < a_f, \tag{10b}$$

where r_p^2 is the relative area of the spherical parent cell and r_d^2 the relative area of the spherical daughter vesicle. The expression for the relative energy w as a function of a_f in the case of cell shapes composed of two spheres (w_ℓ) can be obtained by inserting the above expressions for the skeleton area $a_{s,\ell}$ in Eq. 5.

The function $w_\ell(a_f)$ has in general three local minima. The first local minimum is always at $a_f = 0$, where the first derivative of w_ℓ with respect to a_f is always positive and corresponds to the situation when the cell membrane is completely underlaid with the skeleton. The second local minimum can exist within the interval $0 < a_f \leq r_d^2$ and corresponds to the situation when the skeleton is completely (at $a_f = r_d^2$) or partially (at $a_f < r_d^2$) detached from the bilayer of the daughter vesicle, whereas it completely underlays the bilayer of the parent cell. The third local minimum of $w_\ell(a_f)$ corresponds to $a_s \cong a_{so}$ and can exist only at very small values of γ/K_s where the skeleton would be partially detached and relaxed, also at the normal RBC shapes, which is, however, not the case. Therefore, in the following we will be interested only in the first and second local minima of $w_\ell(a_f)$.

The first derivative of w_ℓ with respect to a_f is discontinuous at $a_f = r_d^2$,

$$w'_{\ell,-} = -\frac{2K_s}{\gamma a_{so}}(r_p^2 - a_{so}) + 1, \tag{11a}$$

$$w'_{\ell,+} = 1, \tag{11b}$$

where $w'_{\ell,-}$ and $w'_{\ell+}$ are the first derivatives of w_ℓ with respect to a_f at $a_f = r_d^2$, approaching that point from the left (−) and from the right (+), respectively. In the case of negative values of $w'_{\ell,-}$, the second local minimum of w_ℓ is located at $a_f = r_d^2$ whereas in the case of positive values of $w'_{\ell,-}$ it can exist at $a_f < r_d^2$.

Fig.4, showing the dependence of w_ℓ on a_f for three different values of a_{so}, illustrates the case in which $w'_{\ell-}$ is negative; i.e., the second local minimum of $w_\ell(a_f)$ is located at $a_f = r_d^2$. In this case the membrane skeleton is completely detached from the bilayer of the spherical daughter vesicle and completely underlays the bilayer of the spherical parent cell if the absolute minimum of $w_\ell(a_f)$ is at $a_f = r_d^2$. This can happen for the relative area of the relaxed skeleton, a_{so}, which is smaller than a certain critical value of a_{so} ($a_{so,\ell}^c$). To determine $a_{so,\ell}^c$, the

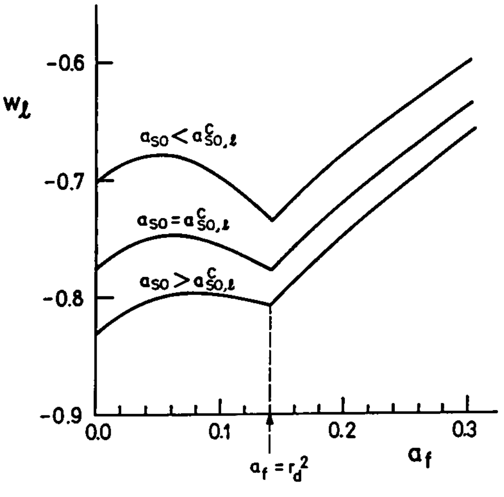

FIGURE 4 Relative energy w_ℓ of the cell shape composed of the w_ℓ spherical parent cell and the spherical daughter vesicle as a function of the relative area a_f for $\gamma/K_s = 0.45$, $v = 0.85$, and three different values of a_{so}: 0.6, 0.641 (where $a_{so} = a^c_{so,\ell}$), and 0.68.

value of a_{so} is calculated at given v and γ/K_s for which $w_\ell(a_f = 0) = w_\ell(a_f = r_d^2)$:

$$a^c_{so,\ell} = \frac{r_p^2 + r_d^2/2}{1 + \gamma/K_s}. \tag{12}$$

However, it follows from Eq. 11a that the value $w'_{\ell,-}$ is negative only for $a_{so} < a^*_{so,\ell}$ where

$$a^*_{so,\ell} = r_p^2/(1 + \gamma/2K_s). \tag{13}$$

Thus, the skeleton is completely detached from the bilayer of the daughter vesicle if a_{so} is, in addition to being smaller than $a^c_{so,\ell}$, also smaller than $a^*_{so,\ell}$. If a_{so} is larger than $a^*_{so,\ell}$, the skeleton can be only partially detached from the bilayer of the spherical daughter vesicle. At a given a_{so}, the condition $a_{so} < a^*_{so,\ell}$ can be fulfilled only at high enough values of $a^*_{so,\ell}$, i.e., at high enough values of r_p and small enough values of γ/K_s. For a constant cell area the relative radius of the daughter vesicle r_d decreases with increasing relative radius of the parent cell, r_p, which means that, at a given a_{so}, the condition $a_{so} < a^*_{so,\ell}$ can be fulfilled only if the radius of the daughter vesicle is much smaller than the radius of the parent cell.

DISCUSSION

The observed absence of membrane skeleton in RBC vesicles can be the consequence of different possible mechanisms such as skeleton disruption, skeleton detachment, or formation of phospholipid blebs by the flow of the membrane material that is not bound to the skeleton. These mechanisms give rise to different compositions of vesicle membranes. We focused here on the skeleton detachment mechanism because RBC vesicles in general contain the integral membrane proteins in approximately the same amount per unit area as the original cell, which would not be the case with the other two mechanisms listed. It would of course be of interest to test the validity of our basic assumption that the formation of a skeleton-free membrane domain is the consequence of partial skeleton detachment and not skeleton disruption (Kozlov et al., 1990) directly, for example, by direct microscope observations of fluorescently labeled RBC skeleton in the course of the budding process.

To elucidate factors affecting the skeleton partial detachment, a simplified model of the skeleton energy was introduced by taking into consideration the skeleton area expansivity and the skeleton-bilayer interaction. The partial detachment of the skeleton from the bilayer was shown to be energetically favorable if the decrease of the elastic energy of the skeleton after skeleton detachment is larger than the corresponding increase of the interaction energy between the skeleton and the bilayer. The partial detachment is promoted by decreasing the energy of the skeleton attachment per unit area (γ), by increasing the area expansivity modulus of the skeleton (K_s), and by decreasing the relative area of the relaxed skeleton (a_{so}), i.e., by an increase of the lateral tension in the skeleton. Here it is important to note that the value of a_{so} is equal to the ratio between the area of the relaxed skeleton, A_{so}, and the area of the bilayer, A, and can therefore be altered by changes of either of these quantities. To each of the parameters of the presented model it is possible to ascribe structural or experimental meaning. For instance, the value of γ can be related to the number of links between the skeleton and the bilayer, which can be different because of different chemical or compositional reasons. For example, it was shown that elevation of intracellular pH above 8.5 enhances the dissociation of ankyrin-band 3 interaction (Low et al., 1991), which plays the predominant role in the linkage of the membrane skeleton to the bilayer. About the relative area of the relaxed skeleton it is possible to gain some insight by studying the behavior of isolated skeletons (Svoboda et al., 1992). Even taking into consideration that the environment of the isolated skeleton differs from its environment in the cell, meaning that a_{so} of the isolated skeleton is not necessarily the same as the cell a_{so}, one can expect that the effects of other factors such as intracellular pH, ionic strength, and temperature are very similar in both cases. The area expansivity modulus of the skeleton K_s of RBC membrane can also be determined independently (Mohandas and Evans, 1994), and it has been shown theoretically (Stokke et al., 1986) that it depends on environmental conditions. Thus, the independent measurements of the parameters of the presented model in correlation with the corresponding studies of the vesiculation process under the same environmental conditions represent a realistic possibility for quantitative experimental tests of the presented ideas.

The main outcome of the presented analysis is that the transition from the state of a cell with completely underlaid skeleton to a state with detached skeleton can be provoked by cell shape changes. By inspecting the skeleton state for a series of pear-shaped cells we have demonstrated that the partial detachment of the skeleton from the bilayer in the budding region of the cell surface arises when the neck between the two parts of the cell becomes sufficiently narrow, so that the increase of the skeleton interaction energy after skeleton detachment is small enough and the gain that results from the decrease of the area expansivity energy prevails.

Already, the introduced simple model for skeleton detachment provides a variety of possibilities about the predicted amount of the skeleton in vesicles, depending on the values of model parameters. Therefore, the precise determination of skeleton amount at the level of individual vesicles and at varying experimental conditions, for example, by varying the external osmotic pressure, which affects the relative size of the released vesicles, could also represent a suitable test of the introduced concepts. In general, if the radius of the daughter vesicle is much smaller than the radius of the parent cell, the daughter vesicle is expected to be completely depleted of cytoskeleton. For certain intermediate sizes of daughter vesicles, and depending on the parameters a_{so} and γ/K_s, it is also possible that there is some skeleton present in vesicles.

It is possible for the model presented to estimate the values of model parameters for which the released daughter vesicle is completely or partially depleted of the skeleton. In view of the presented analysis, skeleton-depleted vesicles can occur if the ratio γ/K_s is smaller than 1 for $a_{so} \approx 0.8$. By taking into account the value 10^{-5} N/m for K_s (Mohandas and Evans, 1994), this would mean that γ must be smaller than 10^{-5} N/m. However, these values can be considered only as rough estimates because the model introduced is a simplification and other contributions to the membrane energy (Waugh and Bauserman, 1995) that we did not take into consideration are involved. An example of a possible neglected contribution is shear deformation. For instance, the partial detachment of the skeleton from the bilayer could be additionally favored as a result of accumulated shear deformations in the region of the cell neck between the daughter vesicle and the parent cell, which are expected to relax at the detached state of the skeleton.

We have for simplicity deliberately separated the phenomenon of partial skeleton detachment from the mechanism of shape changes, which was accomplished by the specific assumptions about the elastic properties of the membrane, in particular, of its bilayer part. For the real RBC membrane, the employed assumption about the elastic properties of the two bilayer monolayers is not valid and consequently any change of the state of the skeleton also affects the cell shape. The detailed analysis of these effects is in preparation.

In the present work the study of the budding process was for the sake of clarity confined to the case in which only one daughter vesicle is created on the whole surface of the axisymmetrical cell. However, as it will also be shown elsewhere, the basic conclusions of this work can be generalized for the case in which many daughter vesicles are created in the budding process.

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