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Membrane skeleton and red blood cell vesiculation at low pH

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Abstract

Shapes of red blood cells at low pH were studied theoretically. It is assumed that the equilibrium shape of the red blood cell corresponds to the minimum of its membrane elastic energy which consists of the bending energy and relative stretching energy of the bilayer, the stretching energy of the skeleton and the interaction energy between the skeleton and the bilayer. It is shown that the aggregation of the skeleton at low pH can cause the red blood cell shape transformation from the stomatocytic shape to the cell shape composed of a spherical parent cell having the bilayer completely underlaid with the skeleton and spherical daughter vesicles without the skeleton. © 1998 Elsevier Science B.V.

Keywords: Erythrocyte; pH; Vesiculation; Membrane skeleton

1. Introduction

The normal equilibrium shape of the red blood cell (RBC) is a biconcave disc. Under certain external conditions the discocytic RBC may be transformed into various other shapes [1]. The RBC shape alterations may be influenced by varying different chemical and physical conditions which affect the properties of the membrane and the volume of the cell [2–5]. In terms of the bilayer couple model [2] the RBC shape can be changed at the constant volume of the cell (V) by the variation of the conditions which cause the change of the difference between the outer and the inner monolayer areas of the bilayer (ΔA). It has been shown theoretically [6–8] and there are strong experimental indications [2,3] for that lowering of the area difference ΔA causes the discocytic RBC shape to change towards the cup (stomatocytic) shape, while an increase of ΔA induces the transformation of the discocytic shape into the spiculated (echinocytic) shape.

These considerations can be adapted to shape alterations occurring when manipulating the intracellular pH of erythrocytes. It has been shown that lowering or elevating intracellular pH gradually takes RBC through stomatocytic or echinocytic shape transformations, respectively [9–11]. Thus, it can be proposed that lowering of intracellular pH decreases ΔA of the membrane bilayer while elevation of pH increases ΔA [11–13]. However, the pH mediated change of the state of the RBC skeleton is in direct contrast to the pH dependence of ΔA and the corresponding RBC shape changes [12], since the RBC membrane skeleton contracts at low pH and expands at high pH [14,15]. These skeleton changes are ex-

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pected to cause spiculation at low pH (an increase of ΔA) and cupping (a decrease of ΔA) at high pH [11]. However, as it was mentioned above, the RBCs are stomatocytes at low pH and echinocytes at high pH, indicating that the membrane skeleton has normally a secondary role in the determination of the area difference ΔA [11,16,17]. This can be at least partially explained by the results of experiments of Mohandas and Evans [5] indicating that the area expansivity modulus and the bending constant of the skeleton are in normal conditions a few orders of magnitude smaller than the corresponding constants of the bilayer.

However, the membrane skeleton may play a significant role in the discontinuous RBC shape transformations at low pH. Namely, it was observed [13,19–21] that incubation of RBCs at pH $\simeq$ 5.4 causes the invaginated stomatocytic RBC shape to change discontinuously into the RBC shape bearing one or several large spherical daughter exovesicles of diameter around 1 μm (Fig. 1). The exovesicles thus formed are free of membrane skeleton [19] which suggests that the skeleton is also involved in the described discontinuous RBC shape transformations at low pH. The discontinuous RBC shape transformation at low pH are, in terms of the bilayer couple model [2] related to a discontinuous increase of area difference ΔA. It was the aim of the present work to describe a role of the membrane skeleton in the determination of the equilibrium RBC shapes at low pH.

It was recently shown for the specific case of the cigar and the pear cell shapes [22] that the aggregation of the skeleton at low pH can cause a discontinuous cell shape transformation from the cigar shape having the bilayer completely underlaid with the skeleton to the cell shape involving a spherical parent cell in which the bilayer is completely underlaid with the skeleton and one spherical exovesicle without the skeleton [18]. The experimental observations (Fig. 1) force to the generalization of the above mentioned theoretical analysis [18] which is given in the present work by taking into account that more than one exovesicle can be created on the surface of the parent cell. Also it is considered that the RBC shape before the discontinuous cell shape transformation at low pH is stomatocytic.

2. Theory

In this work, we study the influence of skeleton aggregation on the stability of the RBC shapes at low pH, which are assumed to be composed of a spherical parent cell with the radius R_p and N equal spherical daughter vesicles with radius R_d, where $N \geq 1$. Such limiting RBC shapes, corresponding to the extreme values of cell ΔA [6,23], resemble well the observed RBC shapes at pH $\simeq$ 5.4 which are presented in Fig. 1.

The equilibrium shapes of RBC are assumed to correspond to the minimum of the total membrane energy [6,18]. The total energy of the RBC membrane (W) is expressed as the sum of three terms [18,23]:

$$W = W_b + W_s + W_i. \quad (1)$$

where W_b is the elastic energy of the bilayer part of

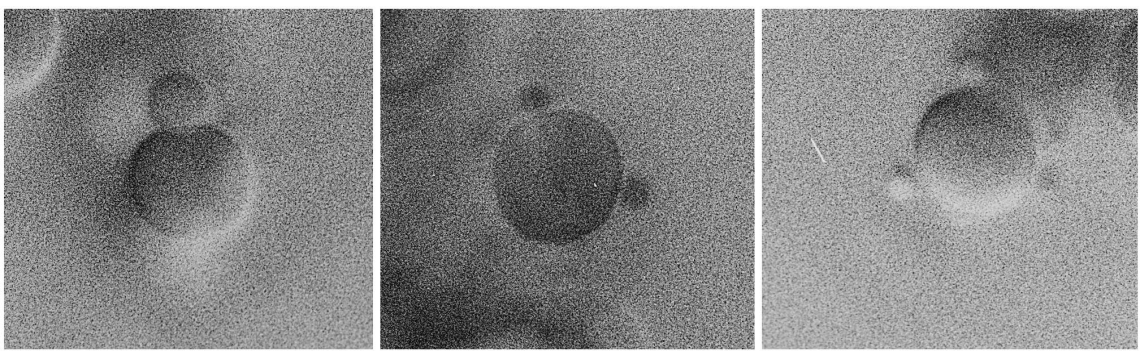

Fig. 1. Micrographs showing red blood cells equilibrated at pH $\simeq$ 5.4 (37°C, 30 min). For photomicrography in an interference contrast microscopy cells were fixed in 1% glutaraldehyde and applied between object glass and cover glass as previously reported [3].

the membrane, W_s is the stretching energy of the skeleton,

$$W_s = K_s(A_s - A_{s0})^2/(2A_{s0}), \quad (2)$$

and W_i is the energy of the interaction between the skeleton and the bilayer [23]

$$W_i = -\gamma(A - A_f). \quad (3)$$

Here K_s is the area expansivity modulus of the skeleton, A_s is the area of the skeleton, A_{s0} is the area of the relaxed skeleton [23], A_f is the area of the bilayer which is not underlaid with the skeleton and γ is the energy of the skeleton attachment per unit area [23,24]. The elastic energy of the bilayer is taken to be the sum of the bending term and the relative stretching term [6,25,26]:

$$W_b = \frac{1}{2}k_c \int (C_1 + C_2)^2 \mathrm{d}A + \frac{1}{2}k_r(\Delta A - \Delta A_0)^2/(A\delta^2), \quad (4)$$

where in the bending term the integration is performed over the membrane (bilayer) area A. Here k_c is the local bending modulus, k_r is the non-local bending modulus, C_1 and C_2 are the principal curvatures, δ is the distance between neutral surfaces of both layers of the bilayer, while ΔA_0 is the reference value of ΔA [26].

At the normal physiological conditions the area of the relaxed skeleton A_{s0} is smaller than the bilayer area A [15,23]. Thus, in the intact cell the skeleton is expanded in order to completely underlay the inner surface of the bilayer [23]. Therefore in the case of the partial skeleton detachment the stretching energy of the skeleton W_s is decreased. The partial detachment of the skeleton from the bilayer is energetically favorable if the decrease of the skeleton stretching energy W_s is larger than the corresponding increase of the skeleton–bilayer interaction energy W_i and bilayer energy W_b.

3. Results and Discussion

At a given cell area (A), cell volume (V) and number of daughter vesicles (N) the radius of the parent cell R_p and the radius of the daughter vesicles R_d of the described limiting RBC shapes can be determined from the constraints for conservation of the cell area and volume:

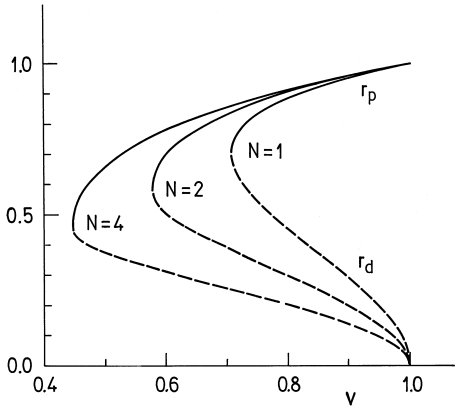

Fig. 2. The calculated relative radius of the parent cell r_p (full lines) and the radius of the daughter vesicles r_d (dashed lines) as a function of the relative cell volume v for three different numbers of daughter vesicles N.

$$4\pi R_p^2 + N4\pi R_d^2 = A, \quad (5)$$

$$4\pi R_p^3/3 + N4\pi R_d^3/3 = V. \quad (6)$$

The system of Eqs. (5) and (6) can be solved analytically only for $N = 1$, describing the RBC shape with a single daughter vesicle [23]. In general, for $N > 1$ the system of Eqs. (5) and (6) is solved numerically. In this work, the tangential method was used. Fig. 2 shows the dependence of the relative radii $r_p = R_p/R_s$ and $r_d = R_d/R_s$ on the relative cell volume $v = V/(4\pi R_s^3/3)$ for different values of number of daughter vesicles N, where as the unit of length the radius $R_s = (A/4\pi)^{1/2}$ is chosen. The limiting RBC shapes composed of a spherical parent cell and N equal spherical daughter vesicles exist only for relative volumes v greater than $(N+1)^{-1/2}$, where at the latter value of volume we have $r_p = r_d = (N+1)^{-1/2}$.

In order to determine the limiting shape of RBC at low pH with minimal membrane energy W and the corresponding association state of the skeleton [23] at given relative cell volume v and given relative area of the relaxed skeleton a_{s0} the minimization procedure is performed as follows. First, at the chosen number of the daughter vesicles N the association state of the skeleton (Fig. 3) is calculated by the

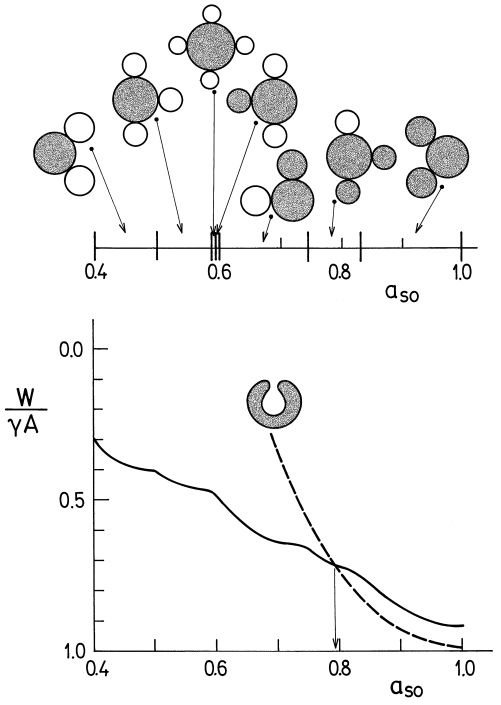

Fig. 3. The total minimal relative energy of the RBC membrane ($W/\gamma A$) for the limiting RBC shapes as a function of the relative area of the relaxed skeleton a_{s0}. The figure also shows schematically the corresponding limiting cell shapes and the association states of the membrane skeleton (shaded area). The dashed line represents the dependence of the energy $W/\gamma A$ on a_{s0} for stomatocytic shape characterized by $\Delta a = 0.51$. The values of the model parameters are: $v = 0.60$, $\Delta a_0 = 0.51$, $k_r/k_c = 4$ [27], $\gamma/K_s = 0.1$ [23], $R_s = 3.3$ μm ($A = 140$ μm^2), $k_c \cong 10^{-19}$ J [27] and $K_s \cong 10^{-5}$ J/m^2 [5].

minimization of the energy W as it is described in detail by Iglič et al. [18,23]. In this way the dependence of the energy W and association state of the skeleton on N can be determined at given v. The equilibrium limiting RBC shape and association state of the skeleton at given a_{s0} (Fig. 3) is then determined by minimizing the membrane energy $W(N)$ over all possible values of N. In the described calculations the relative radii r_p and r_d at given v and given N are determined from Eqs. (5) and (6) as described above.

Fig. 3 shows the calculated dependence of the minimal membrane energy W of the limiting RBC shapes on the relative area of the relaxed skeleton $a_{s0} = A_{s0}/A$ for the relative cell volume $v = 0.6$. Fig. 3 also shows schematically the corresponding equilibrium limiting cell shapes and association state of the skeleton (shaded area) in different regions of a_{s0} values. In accordance with our previous results [23], it was shown that the daughter vesicles can exist either completely underlaid with the skeleton or completely depleted of the skeleton (Fig. 3). In general it is expected that at normal pH the value of a_{s0} is larger than 0.8 [15,23] while it is smaller than 0.8 at pH $\cong$ 5.4 where the skeleton aggregates.

In addition, Fig. 3 shows the dependence of the total membrane energy of the stomatocytic shape on a_{s0} for the same value of the relative cell volume $v = 0.6$. The stomatocytic shape, shown in Fig. 3, was determined by the minimization of the bilayer elastic energy at fixed values of the relative cell volume $v = 0.6$ and the relative area difference $\Delta a_0 = 0.51$ as it is described by Svetina and Žekš [25] where $\Delta a = \Delta A/8\pi\delta R_s$ and $\Delta a_0 = \Delta A_0/8\pi\delta R_s$.

Fig. 3 shows that for values of $a_{s0} > 0.83$ the skeleton completely underlays the inner surface of the cell membrane. On the contrary, for the values of $a_{s0} < 0.59$, corresponding to low pH where skeleton aggregates, the skeleton is completely absent in all daughter vesicles. This agrees well with experimental results which show that the exovesicles formed at pH $\cong$ 5.4 are without the membrane skeleton [19].

Further, it can be seen in Fig. 3 that in the range of higher values of $a_{s0} > 0.8$, the stomatocytic shape has smaller membrane energy W (dashed line) than the limiting RBC shapes while for $a_{s0} < 0.8$ the limiting cell shapes have smaller energy W. This explains why the aggregation of the membrane skeleton at pH $\cong$ 5.4, i.e., the sudden decrease of the area of the relaxed skeleton, can induce the observed discontinuous cell shape transformation from a stomatocytic shape to the limiting cell shapes composed of a spherical parent cell and spherical daughter vesicles (Fig. 1).

It can also be seen in Fig. 3 that the calculated equilibrium limiting RBC shapes have at smaller values of a_{s0} only a few large daughter vesicles similar to the observed RBC shapes at pH $\cong$ 5.4 (Fig. 1).

In this work the energy of the skeleton due to shear deformation was not considered. However, the inclusion of the shear energy would not alter the basic conclusions of this work since the partial detachment of the skeleton in the budding region of the

cell membrane is additionally favored due to the accumulated shear deformations in the region of the cell neck between the daughter vesicle and the parent cell [28]. Also the membrane energy due to the lateral redistribution of the membrane components [26,29,30] was not taken into account. The detailed analysis of the role of these phenomena in the vesiculation process is in preparation.

The observed (Fig. 1) discontinuous cell shape transition of the stomatocytic RBC into the RBC composed of a nearly spherical parent cell and nearly spherical daughter vesicles would be at normal conditions energetically unfavorable since the decrease of the skeleton elastic energy W_s at partial skeleton detachment in daughter vesicles would be smaller than the corresponding increase of the skeleton-bilayer interaction energy W_i and the increase of the bilayer elastic energy W_b. However, it is established in this work that after sufficient increase of the lateral tension in the skeleton, i.e., after sufficient decrease of the unstretched area of the skeleton A_{s0}, the decrease of W_s after partial skeleton detachment may prevail over the corresponding increase of W_i and W_b. Consequently, the discontinuous transition of the stomatocytic RBC shape into the limiting RBC shape composed of spherical parent cell and N equal spherical daughter vesicles becomes energetically favorable.

The molecular mechanism of the pH associated change of ΔA is still unknown [12]. Changes in the electrostatic interaction between the negatively charged phospholipid headgroups in the inner monolayer do not appear relevant for the pH dependent ΔA changes [12] and the active phospholipid translocation seems too slow to account for the observed fast pH induced RBC shape transformations [31]. Recently, it was proposed that pH changes may induce conformational changes of the transmembrane protein band 3 which may rapidly change ΔA value [31].

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