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Amphiphile induced echinocyte-spheroechinocyte transformation of red blood cell shape

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Abstract A possible physical explanation of the echinocyte-spheroechinocyte red blood cell (RBC) shape transformation induced by the intercalation of amphiphilic molecules into the outer layer of the RBC plasma membrane bilayer is given. The stable RBC shape is determined by the minimization of the membrane elastic energy, consisting of the bilayer bending energy, the bilayer relative stretching energy and the skeleton shear elastic energy. It is shown that for a given relative cell volume the calculated number of echinocyte spicula increases while their size decreases as the number of the intercalated amphiphilic molecules in the outer layer of the cell membrane bilayer is increased, which is in agreement with experimental observations. Further, it is shown that the equilibrium difference between the outer and the inner membrane leaflet areas of the stable RBC shapes increases if the amount of the intercalated amphiphiles is increased, thereby verifying theoretically the original bilayer couple hypothesis of Sheetz and Singer (1974) and Evans (1974).

Key words Red blood cell · Spheroechinocyte · Membrane skeleton · Cell shape · Elastic energy

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1 Introduction

The red blood cell (RBC) shape may be altered by varying different chemical and physical conditions which affect the properties of the membrane and the volume of the cell (Deuticke 1968; Gimsa and Ried 1995; Seifert and Lipowsky 1994; Kralj-Iglič et al. 1996). In particular, in terms of the bilayer couple model (Evans 1974; Sheetz and Singer 1974), by keeping the volume of the cell (V) constant, the RBC shape changes due to the change in the conditions which cause the change of the difference between the outer and the inner monolayer areas (ΔA) of the bilayer. Experiments (Sheetz and Singer 1974; Isomaa et al. 1987; Gedde et al. 1997) strongly indicate that lowering the area difference ΔA causes the normal (discocytic) RBC shape to change towards the cup (stomatocytic) shape, while an increase of ΔA causes the transformation of the discocytic shape into the spiculated (echinocytic) shape. The exogeneously induced discocyte-echinocyte transformation is generally reversible by washing (Brecher and Bessis 1972) indicating that this RBC shape transformation is usually not connected to some irreversible change in the conformation of the membrane skeleton or the membrane bilayer.

An increase of ΔA, and the consequent discocyte-echinocyte transformation, can be induced by the intercalation of the amphiphilic molecules in the outer layer of the RBC membrane bilayer (Sheetz and Singer 1974; Isomaa et al. 1987). Spheroechinocytes, having less prominent spicula than echinocytes (Brecher and Bessis 1972; Bessis 1973), may be developed at higher concentration of the echinocytogenic amphiphilic molecules (Hägerstrand et al. 1992). The spheroechinocyte spicula become progressively narrower with increasing amphiphile concentration (Brecher and Bessis 1972; Bessis 1973; Sheetz and Singer 1974; Isomaa et al. 1987). When the concentration of echinocytogenic amphiphilic molecules is further increased, true spheres (spherocytes) appear and finally hemolysis occurs (Brecher and Bessis 1972; Isomaa et al. 1987). It was also observed that after reaching spherocytic shape RBCs release exomicrovesicles from the membrane (Hägerstrand and Isomaa 1989).

It is the goal of the present communication to describe theoretically the process of echinocyte-spheroechinocyte RBC shape transformation driven by the intercalation of the amphiphiles in the outer layer of the RBC membrane bilayer, i.e. by increasing the difference between the areas of the two layers of the RBC membrane bilayer.

2 Theory

Echinocytogenic amphiphiles induced echinocytes immediately, and there are only small alterations of the induced RBC shape during the incubation (Isomaa et al. 1987). Since the experimental results (Mohandas and Evans 1994) show that large deformations of the RBC membrane skeleton can be held over long periods it can be concluded that the membrane skeleton can also be involved in the echinocyte shape transformations. Moreover, it has been shown recently that the skeleton shear elasticity is essential for the stability of true echinocytic shapes (Iglič 1997). Therefore in the present work the stable echinocyte RBC shape is determined by the minimum of its membrane elastic energy (W) consisting of bending (W_b), relative stretching (W_r) and shear (W_s) contributions (Evans 1974; Evans and Skalak 1980; Sackmann 1994; Seifert and Lipowsky 1994). The bending energy of the bilayer is given by (Helfrich 1974)

$$W_b = \frac{1}{2}\, k_c \int (C_1 + C_2)^2 \, dA , \qquad (1)$$

where the integration is performed over the membrane (bilayer) neutral surface area A, while the relative stretching energy of the bilayer is (Evans 1974; Miao et al. 1994; Svetina and Žekš 1996):

$$W_r = \frac{1}{2}\, k_r \, (\Delta A - \Delta A_0)^2/(A\,\delta^2) , \qquad (2)$$

Here, k_c is the local bending modulus of the membrane, k_r is the non-local bending modulus of the bilayer, C_1 and C_2 are the principal membrane curvatures, δ is the distance between the neutral surfaces of both layers of the bilayer, while ΔA_0 is the value of ΔA corresponding to the situation when the stretching energies of both layer neutral surfaces are zero. The parameter ΔA_0 is an increasing function of the number of amphiphilic molecules intercalated in the outer layer of the membrane bilayer (Svetina and Žekš 1996; Seifert 1997).

Recently, a new constitutive model for the membrane skeleton behavior was suggested which takes into account the fact that the membrane skeleton is locally compressible (Mohandas and Evans 1994; Fischer 1992). However, the essential characteristics of the shear elasticity can also be satisfactorily described within the model of immobilized boundaries (Waugh 1996) assuming the lateral incompressibility of the bilayer as well as of the skeleton (Markin and Kozlov 1988). Therefore, owing to simplicity in this work the shear energy of the skeleton is written using an approximate expression (Evans and Skalak 1980):

$$W_s = \mu \int ((\lambda_m^2 + \lambda_m^{-2} - 2)/2) \, dA , \qquad (3)$$

where μ is the membrane skeleton area shear modulus and λ_m is the principal extension ratio along the meridional direction.

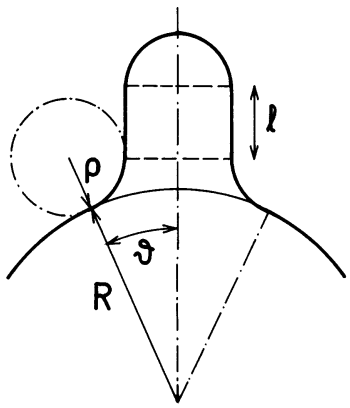

Fig. 1 The model echinocyte shape (Iglič 1997) is described by three parameters characterizing the axisymmetrical spicula (ρ, ϑ, ℓ), the radius of the spherical cell body R and the number of equal spicula distributed on the cell body (n)

The echinocyte shape is described by a geometrical model involving five parameters: the radius of the spherical cell body (R), the number of equal axisymmetrical spicula distributed on the cell body (n), the length of the spiculum cylinder (ℓ), the radius of the spiculum base (ρ) and the angle (ϑ) (Fig. 1). The corresponding mathematical expressions for the cell volume $V(\rho, n, \vartheta, \ell, R)$, membrane area $A(\rho, n, \vartheta, \ell, R)$, area difference $\Delta A(\rho, n, \vartheta, \ell, R)$, bending energy $W_b(\rho, n, \vartheta, \ell, R)$ and shear energy $W_s(\rho, n, \vartheta, \ell, R)$ for the model echinocyte shape are given elsewhere (Iglič 1997).

3 Results

In the following the radius of the sphere R_0 of the membrane area A is chosen as the unit length: $R_0 = (A/4\pi)^{1/2}$. Hence the variables ρ, ℓ and R are redefined as follows, $\rho \to \rho/R_0$, $\ell \to \ell/R_0$ and $R \to R/R_0$. In addition, all volumes and areas are normalized relative to the corresponding values of the spherical cell of the radius R_0. The relative volume is defined as $v = 3V/4\pi R_0^3$, the relative membrane area is $a = A/4\pi R_0^2$, while the relative area differences are $\Delta a = \Delta A/8\pi\delta R_0$ and $\Delta a_0 = \Delta A_0/8\pi\delta R_0$. In accordance with the definition of the radius R_0 of the value of the relative membrane area a is equal to one. The elastic energy of the membrane is normalized relative to the bending energy of the sphere: $w = W/8\pi k_c$.

The stable echinocyte shape is defined as the shape which corresponds to the absolute minimum of the total

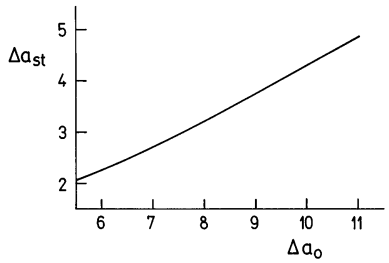

Fig. 2 The calculated relative difference between the areas of the two bilayer layers corresponding to the stable cell shape (Δa_{st}) as a function of Δa_0 for $v=0.6$, $k_r/k_c=4$ (Waugh and Bauserman 1995) and $\mu/k_c=10^{13}$ m^{-2} (Evans 1974; Waugh and Evans 1979)

relative membrane elastic energy $w=w_b+w_s+w_r$ at given v, Δa_0, k_r/k_c and μ/k_c, where $w_b=W_b/8\pi k_c$, $w_s=W_s/8\pi k_c$ and $w_r=W_r/8\pi k_c$. The value of the relative difference between the areas of the two bilayer layers Δa corresponding to the stable cell shape (Δa_{st}) is determined in the minimization procedure and is in general different from Δa_0.

The minimization procedure is performed as follows. First, at given v and Δa, three of the five parameters of the introduced geometrical model of the echinocyte (ϑ, ℓ, R) are determined directly from the constraints for the relative cell volume v and the relative membrane area ($a=1$) and from the requirement for a chosen relative area difference Δa (Iglič 1997). The remaining two parameters (ρ and n) are calculated by the minimization of the enery w_b+w_s (Iglič 1997). In this way the dependencies of the energy w_b+w_s and the parameters ϑ, ℓ, R, ρ and n on the relative area difference Δa can be determined at given v, Δa_0 and μ/k_c. The stable model echinocyte shape and the corresponding relative area difference (Δa_{st}) are then calculated by minimizing the total relative membrane elastic energy $w=w_b(\Delta a)+w_s(\Delta a)+w_r(\Delta a)$ over all possible Δa values.

To illustrate the effect of the intercalation of the amphiphilic molecules into the RBC membrane on the stable echinocyte shape, it is taken in account with the bilayer couple model (Sheetz and Singer 1974), that the change of the shape is caused by the change of Δa_0. It is considered that the RBC membrane parameter Δa_0 is larger if the outer area of the RBC plasma membrane is increased owing to the intercalation of the amphiphilic molecules (Svetina and Žekš 1996; Seifert 1997).

In order to show the effect of increasing Δa_0 (i.e. the increase of the amount of amphiphiles in the outer layer of the bilayer) Fig. 2 shows the dependence of the relative area difference Δa corresponding to the stable cell shape (Δa_{st}) on Δa_0. It can be seen that Δa_{st} is an increasing function of Δa_0.

Figures 3 and 4 show the calculated dependencies of the radius of the cell body R, the angle ϑ, the length of the spiculum cylinder ℓ, the radius determining the spiculum base ρ and the number of the spicula n, corresponding to the minimal total relative membrane elastic energy w, as a function of the parameter Δa_0. It can be seen in Figs. 3 and 4 that the calculated equilibrium number of echinocyte spicula n increases while their average size decreases with increasing Δa_0, i.e. with increasing number of the intercalated amphiphilic molecules in the outer layer of the cell

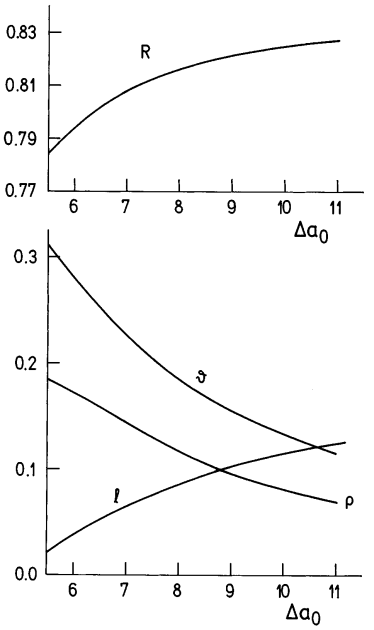

Fig. 3 The calculated equilibrium values of the geometrical parameters of the model echinocyte (spheroechinocyte) shape as a function of the parameter Δa_0 for $v=0.6$, $k_r/k_c=4$ and $\mu/k_c=10^{13}$ m^{-2}

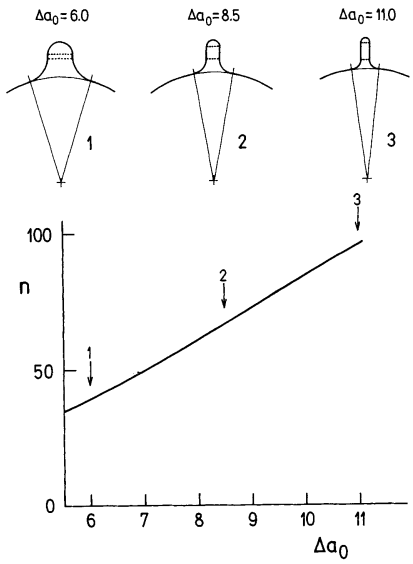

Fig. 4 The calculated equilibrium number of the echinocyte (spheroechinocyte) spicules n as a function of the parameter Δa_0 for $v=0.6$, $k_r/k_c=4$ and $\mu/k_c=10^{13}$ m^{-2}. The stable RBC shapes corresponding to three different choices of the RBC membrane parameter Δa_0 are shown

membrane bilayer. This is in agreement with experimental observations (Sheetz and Singer 1974; Bessis 1973; Isomaa et al. 1987).

The range of ΔA_0 where echinocytic RBC shapes could be expected is estimated. Sheetz and Singer (1974) report values of $\Delta A_0/A$ between 0.005 and 0.019 for different types of amphiphiles. This range of ΔA_0 values corresponds to the interval of Δa_0 values between 3 and 10.5 for $\delta = 3$ nm and $R_0 \cong 3.3$ μm ($A = 138$ μm^2) which is in the range of the values of Δa_0 considered in this work (Figs. 2, 3, 4).

4 Discussion

We have shown that the requirement of the minimal membrane elastic energy that consists of the bilayer bending energy, the bilayer relative stretching energy and the skeleton shear elastic energy can explain the stability of the observed spheroechinocytic shapes. In addition, we have shown, in agreement with experimental observations (Sheetz and Singer 1974), that the number of echinocyte (spheroechinocyte) spicula increases while their size decreases as the echinocytogenic amphiphilic molecule concentration is increased. The final spherical RBC shape at higher amphiphile concentration could arise because of an irreversible loss of membrane in a microvesiculation process where the small daughter vesicles are released from the spicula of spheroechinocytes (Liu et al. 1989; Hägerstrand and Isomaa 1989) or the spheroechinocyte spicula are pinched off from the cell surface as a whole and subsequently disintegrated into smaller spherical vesicles.

In contrast to RBCs, artificial lipid vesicles have never been reported to attain true echinocytic and spheroechinocytic shapes, i.e. spherical shapes with many well defined spicula (Berndl et al. 1990; Seifert 1997). In the case of the lipid vesicles, increasing the area difference can induce the formation of starfish vesicles (Wintz et al. 1993; Seifert 1997). However, the starfish shape is flat and therefore completely different from the echinocytic RBC shape. The starfish vesicles do not exhibit any process resembling spheroechinocytosis. Also, they can exist only at much smaller relative cell volumes ($v \cong 0.3$) than the echinocytic RBCs ($v \cong 0.6$). This indicates that in addition to the bilayer the skeleton of the RBC membrane is also responsible for the formation of the echinocytic shapes, presumably due to shear deformation of the skeleton (Iglič 1997). This is also supported by our recent preliminary observations showing that lamprey erythrocytes, which are deficient in the membrane skeleton (Ohnishi 1985), do not form true spicula upon treatment with echinocytogenic amphiphiles.

The previous studies of echinocyte shapes have been limited to the RBC shapes with short spicula approximated with spherical harmonics (Landman 1984) or to the analysis of the single spicule shape where the constraint for the cell volume was not considered and the number of spicula was not determined in the minimization procedure but was taken as the model parameter (Brailsford et al. 1980; Stokke et al. 1986; Waugh 1996). In both cases the echinocyte and spheroechinocyte shapes with many distinctive spicula can not be studied. In this work however the echinocyte and spheroechinocyte shapes are analyzed by taking into account the constraints for the cell volume and area. In addition, the equilibrium number of spicula under given external conditions is determined in the minimization procedure.

The spontaneous curvature of the membrane bilayer C_0 (Helfrich 1973) was not explicitly considered in the local bending energy term (Eq. (1)). However, the features described by the spontaneous curvature are effectively described by being contained in the RBC membrane parameter ΔA_0 (Miao et al. 1994). The continuum description of the membrane bilayer elastic energy (Eqs. (1) and (2)) can not account for all of the features of the molecular origin and therefore can not clearly reveal the differences between the C_0 and the ΔA_0 RBC membrane parameters.

The relative stretching energy is overestimated using Eq. (2) since the relative stretching deformation of the bilayer can be at least partially relaxed in short time due to redistribution of molecules within each lipid layer in order to equalize the area per molecule and by exchange of lipid molecules between both lipid layers to alleviate curvature induced dilation or compression (Waugh and Hochmuth 1995). It has been suggested that local membrane defects may act as flip sites for phospholipid molecules and related compounds (Classen et al. 1989).

References

Berndl K, Käs J, Lipowsky R, Sackmann E, Seifert U (1990) Shape transformations of giant vesicles: extreme sensitivity to bilayer asymmetry. Europhys Lett 13:659–664

Bessis M (1973) Red cell shapes. An illustrated classification and its rationale. In: Bessis M, Weed RI, Leblond PF (eds) Red cell shapes: physiology, pathology, ultrastructure. Springer, Berlin Heidelberg New York, pp 1–24

Brailsford JD, Korpman RA, Bull BS (1980) Crenation and cupping of the red cell: A new theoretical approach. Part I. Crenation. J Theor Biol 86:513–529

Brecher G, Bessis M (1972) Present status of spiculated red cells and their relationship to the discocyte-echinocyte transformation: critical review. Blood 40:333–344

Classen J, Deuticke B, Haest CWM (1989) Nonmediated flip-flop of phospholipid analogues in the erythrocyte membrane as probed by palmitoylcarnitine: basic properties and influence of membrane modification. J Membr Biol 111:169–178

Deuticke B (1968) Transformation and restoration of biconcave shape of human erythrocyte induced amphiphilic agents and change of ionic environment. Biochim Biophys Acta 163:494–500

Evans E (1974) Bending resistance and chemically induced moments in membrane bilayers. Biophys J 14:923–931

Evans E, Skalak R (1980) Mechanics and thermodynamics of biomembranes. CRC Press, Boca Raton (FL)

Gedde MM, Davis DK, Huestis WH (1997) Cytoplasmic pH and human erythrocyte shape. Biophys J 72:1234–1246

Gimsa J, Ried C (1995) Do band 3 protein conformational changes mediate shape changes of human erythrocytes? Mol Membr Biol 12:247–254

Fischer TM (1992) Is the surface area of the red cell membrane skeleton locally conserved? Biophys J 61:298–305

Hägerstrand H, Isomaa B (1989) Vesiculation induced by amphiphiles in erythrocytes. Biochim Biophys Acta 982:179–186

Hägerstrand H, Isomaa B (1992) Morphological characterization of exovesicles and endovesicles released from human erythrocytes following treatment with amphiphiles. Biochim Biophys Acta 1109:117–126

Helfrich W (1973) Elastic properties of lipid bilayers: theory and possible experiments. Z Naturforsch 28C:693–703

Helfrich W (1974) Blocked lipid exchange in bilayers and its possible influence on the shape of vesicles. Z Naturforsch 29C: 510–515

Iglič A (1997) A possible mechanism determining the stability of spiculated red blood cells. J Biomechanics 30:35–40

Kralj-Iglič V, Svetina S, Žekš B (1996) Shapes of bilayer vesicles with membrane embedded molecules. Eur Biophys J 24:311–321

Isomaa B, Hägerstrand H, Paatero G (1987) Shape transformations induced by amphiphiles in erythrocytes. Biochim Biophys Acta 899:93–103

Landman KA (1984) A continuum model for a red blood cell transformation: sphere to crenated sphere. J Theor Biol 106:329–351

Liu SC, Derick LH, Duquette MA, Palek J (1989) Separation of the lipid bilayer from the membrane skeleton during discocyte – echinocyte transformation of human erythrocyte ghosts. Eur J Cell Biol 49:358–365

Markin VS, Kozlov MM (1988) Mechanical properties of the red cell membrane skeleton: analysis of axisymmetric deformations. J Theor Biol 133:147–167

Miao L, Seifert U, Wortis M, Dobereiner HG (1994) Budding transitions of fluid-bilayer vesicles; the effect of area difference elasticity. Phys Rev E 49:5389–5407

Mohandas N, Evans E (1994) Mechanical properties of the red cell membrane in relation to molecular structure and genetic defects. Annu Rev Biophys Biomol Struct 23:787–818

Ohnishi ST, Asai H (1985) Lamprey erythrocytes lack glycoproteins and anion transport. Comp Biochem Physiol 81B:405–407

Sackmann E (1994) Membrane bending energy concept of vesicle and cell shapes and shape transitions. FEBS Lett 346:3–16

Seifert U, Lipowsky R (1994) Morphology of vesicles. In: Lipowsky R, Sackmann E (eds) Structure and dynamics of membranes. Elsevier, Amsterdam, pp 403–463

Seifert U (1997) Configurations of fluid membranes and vesicles. Advances in Physics 46:13–137

Sheetz MP, Singer SJ (1974) Biological membranes as bilayer couples. A mechanism of drug-erythrocyte interactions. Proc Natl Acad Sci USA 71:4457–4461

Stokke BT, Mikkelsen A, Elgsaeter A (1986) The human erythrocyte membrane skeleton may be an ionic gel II. Numerical analyses of cell shapes and shape transformations. Eur Biophys J 13:219–233

Svetina S, Žekš B (1996) Elastic properties of closed bilayer membranes and the shapes of giant phospholipid vesicles. In: Lasič DD, Barenholz Y (eds) Handbook of nonmedical applications of liposomes. CRC Press, Boca Raton (FL), pp 13–42

Waugh RE (1996) Elastic energy of curvature-driven bump formation on red blood cell membrane. Biophys J 70:1027–1035

Waugh RE, Hochmuth RM (1995) Mechanics and deformability of hematocytes. In: Bronzino JD (ed) The biomedical engineering handbook. CRC Press, Boca Raton (FL), pp 474–486

Waugh RE, Evans EA (1979) Thermoelasticity of red blood cell membrane. Biophys J 26:115–131

Waugh RE, Bauserman RG (1995) Physical measurements of bilayer-skeletal separation forces. Ann Biomed Eng 23:205–211

Wintz W, Döbereiner HG, Seifert U (1996) Starfish vesicles. Europhys Lett 33:403–408