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Time-dependent elastic extensional RBC deformation by micropipette aspiration: redistribution of the spectrin network?

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Abstract. The time dependence of small elastic extensional RBC deformation by micropipette aspiration has been analyzed. This process shows two-phases which are characterized by time constants of the order of some tenths of seconds and about ten seconds, respectively. The equilibrium tongue length is reached after about 30 s. For the first, fast step we assume that the membrane model of immobilized boundaries holds, i.e., the skeleton is tightly associated with the lipid bilayer and no redistribution of the skeleton with respect to the lipid bilayer is allowed. This lipid-spectrin interaction or anchorage is characterized by some association force density. It has been shown that at a given tongue length the force generated owing to the membrane deformation and acting to redistribute the spectrin, overcomes (in some membrane area) the association force density and results in an additional increase of the sucked membrane length. Equations have been derived to describe this process. From the experimental conditions of an RBC aspiration and the determined tongue length corresponding to the second slow aspiration step, the association force density between the lipid bilayer and the spectrin network may be determined. From literature data and our own results a force density of between 40 and 50 Pa has been estimated.

Key words: Erythrocyte – Spectrin – Membrane model – Membrane viscosity

Introduction

Extensive biochemical investigations have resulted in a detailed knowledge of RBC membrane composition (Cohen 1983; Lutz et al. 1986; Low 1987; Yeagle 1987). Nowadays, attention is centered much more on the molecular organization and its functional manifestations. With regard to mechanical membrane properties, biophysical experiments testing the viscoelastic behaviour of the erythrocyte membrane for different modes of deformation (Evans and Skalak 1980; Dormandy 1985; Hochmuth 1987; Lerche et al. 1988) are an adequate tool for gaining functional information. In addition to basic interest, these mechanical membrane properties are of special interest owing to the important role they play in influencing blood flow in healthy and diseased people (Cokelet et al. 1980; Mohandas et al. 1983; Chien et al. 1986; Ernst 1986).

In this paper we focus our attention on the small elastic extensional RBC deformation by micropipette aspiration (originally proposed by Rand (1964)) and the corresponding time behaviour. In brief, by applying a negative pressure difference individual RBC are partly sucked into a cylindrical glass micropipette with an inner diameter of about 1 μm. The length of the cell tongue in the micropipette is measured under TV control (for details see: Chien et al. 1978; Evans and Skalak 1980; Hochmuth 1987; Meier 1988). From the final length of the tongue, the diameter of the pipette and the pressure difference an apparent elastic shear modulus for a normal RBC of 4 μN/m to 7 μN/m (cf. Hochmuth 1987; Meier 1988) can be calculated according to an algorithm previously described (Chien et al. 1978; Evans and Skalak 1980).

The important problem in this topic is the relationship between the equilibrium conformation of lipid and protein molecules, the structure of the membrane and its mechanical properties. In a previous paper (Lerche et al. 1988) on a mechanical three-layer model we discussed evidence that the glycocalyx, the membrane bilayer and the membrane skeleton are quite differently involved in membrane compressibility, elastic membrane shear and bending rigidity as well as membrane spontaneous curvature. With respect to a small elastic extensional RBC deformation, there is no doubt that the main structural basis for the elastic shear modulus is attributed to the cytoskeleton (Mohandas et al. 1983; Stokke et al. 1986a; Kozlov and Markin 1987; Meier 1988). It is formed by a slow association between spectrin heterodimers (spectrin dimer consists of two polypeptides with molecular weights of about 220 000 and 240 000 Daltons) forming

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heterotetramers (spectrin dimers associated head-to-head to form 200 nm long flexible units). The entire membrane skeleton is build by "elementary unit meshes" each containing an actin protofilament with several heterodimers (Scheven and Stibenz 1983; Sheetz 1983; Stokke et al. 1986a; Kozlov and Markin 1987). The loose ends of such a unit mesh can interact as described above with dimers belonging to adjacent meshes and in such a way an integrated meshwork (network) is formed via heterotetramers.

Despite detailed investigations (Sheetz 1983; Lutz et al. 1986; Meier et al. 1989; Nakao et al. 1989), the nature of the interaction between the spectrin network and the lipid bilayer is not fully understood. It has been shown that there is a specific binding site on each spectrin dimer by which it interacts with integral proteins (band III) via ankyrin. The nature of the direct interaction of spectrin with the internal lipid monolayer is not yet clear, but this interaction does define the freedom of motion of lipid molecules in the monolayer with respect to a unit spectrin mesh. Two mechanical models have been proposed (Markin and Kozlov 1986): the model of free boundaries (MFB) and the model of immobilized boundaries (MIB). Within the framework of the MIB, the membrane skeleton unit mesh cannot redistribute with respect to the cytoplasmic surface of the lipid bilayer. This model assumes an interaction of each spectrin dimer over the entire length with the lipids of the bilayer and this means that the lipid molecules are inside a "corral" formed by spectrin molecules. Owing to the "liquid" like properties of this lipid membrane bilayer, the high compressibility modulus prevents any area change due to forced deformation both of the lipid area as well as the spectrin mesh. In the MIB the spectrin network resists any shear deformation and this can be quantified by the apparent elastic shear modulus μ (Stokke et al. 1986a; Kozlov and Markin 1987). In fact, the MIB is based on the shell theory of mechanical membrane properties introduced by Evans and Skalak (1980).

In the MFB the skeleton is assumed to be attached to the lipid bilayer only at a finite number of points (e.g. spectrin-ankyrin-band III). Under these conditions the attachment points can move in the plane of the lipid bilayer and this results in a redistribution of the membrane skeleton. Therefore, a spectrin unit mesh and the corresponding area fragment of the lipid membrane are in some way independent of each other. In the MFB we have to analyze separately the lateral equilibrium for the spectrin network and the lipid bilayer to describe the mechanical equilibrium due to the deformation force (Markin and Kozlov 1986). In contrast, in the case of the MIB, the equation of the mechanical lateral equilibrium is solved once for the whole membrane fragment.

Both models, applied to the RBC aspiration technique, should result in different tongue length with the same suction pressure (Markin and Kozlov 1986; Stokke et al. 1986b). Whereas in the framework of the MIB length of the membrane tongue L_p is nearly proportional to the suction pressure even at small values, in the MFB we find that a critical tongue length is already obtained at very low aspiration pressure drops (necessary to overcome the elastic energy due to membrane bending). As for the MIB, further RBC aspiration demands increasingly higher suction pressure. Quantitatively this means that with the same suction pressure applied we get a longer tongue for the MFB in comparison to the MIB.

There have only been a few investigations designed to obtain quantitative data on the time course of RBC deformation during its aspirational entry into a micropipette (Chien et al. 1978; Chabanel et al. 1985; Meier 1988). It was shown that the deformational entry of RBC into a micropipette in response to a step aspiration pressure exhibits a two-phase behaviour (Fig. 1). After an initial rapid phase of deformation of less than 1 s, one can obtain a continued, slower phase of deformation. The steady state tongue length was attained within a 30 s period with an applied pressure step for normal RBC (Meier 1988). This steady state length is the experimental basis for the determination of the elastic shear modulus μ, which we propose to call the apparent elastic shear modulus to direct attention to the fact that the corresponding algorithm for calculation is used, but the underlying constitutive material equation for this deformation process has to be worked out. On the basis of the two relaxation times (τ_1, τ_2) and assuming the membrane to be a visco-elastic body, two "membrane viscosities" can be estimated ($\eta_{m1} = \mu \cdot \tau_1$; $\eta_{m2} = \mu \cdot \tau_2$). Obtained viscosities are of the order of 1 μNs/m and 20 μNs/m for η_{m1} and η_{m2}, respectively.

Apart from some qualitative hypotheses assuming molecular reorganization of the membrane material due to loading (Meier 1988), there is no biophysical treatment of this two-phase aspirational entry into a micropipette. The aim of this paper is, therefore, to discuss the physical nature of the two different time-dependent steps of the suction processes and to give a theoretical model based on the redistribution of the spectrin network and a force (loading) dependent interaction of spectrin unit meshes with the lipids of the bilayer.

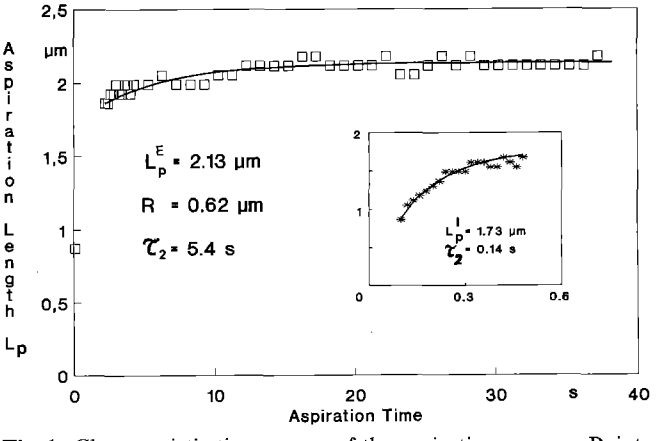

Fig. 1. Characteristic time course of the aspiration process. Points represent the digitized values obtained by means of a line-TV-analyzer (Meier 1988). The curve corresponds to the fitted regression based on two exponential terms. The insert shows the rapid phase with an enlarged time scale. Pipette radius was 0.62 μm and the pressure drop amounts 53.9 Pa. τ_1 and τ_2 are the two relaxation times. L_p^I and L_p^E are the tongue lengths related to the first phase and to the final equilibrium state, respectively

Statement of problem

Let us consider a red cell membrane consisting of a membrane bilayer and an associated membrane skeleton. The membrane skeleton can be treated as a network consisting of "elementary unit meshes" (Cohen 1983). Let us further assume that the unit mesh is rectangular in shape with sides (lengths l_x and l_y) parallel to the principal axes. The elastic energy of a mesh which exhibits elastomeric properties (Kozlov and Markin 1987) is equal to

$$W_s = \frac{\mu}{2}(l_x^2 + l_y^2) \tag{1}$$

where μ is a structural parameter of the mesh and can be interpreted as the apparent elastic shear modulus of an RBC. According to Kozlov and Markin (1987) this modulus is given by $\mu = k \cdot T\, m/3\, n \cdot \delta^2$, where $n \cdot \delta^2$ is the mean square distance between the ends of the spectrin filaments, m the average number of associated heterodimers per unit mesh and $k \cdot T$ is the product of Boltzmann's constant and the absolute temperature.

The tensions acting on an elementary unit mesh of the skeleton from the neighbouring meshes (due to membrane deformation into the micropipette) were analyzed in detail elsewhere (Markin and Kozlov 1986) and are given by:

$$T_{sx} = \mu \cdot \frac{l_x}{l_y}, \qquad T_{sy} = \mu \cdot \frac{l_y}{l_x}. \tag{2}$$

Let us assume that the MIB applies at the beginning of the deformation process. This means that association (interaction) of the whole spectrin dimer molecule with lipids and penetrating proteins (ankyrin-band III complex) prevents any redistribution of an elementary unit mesh with respect to the corresponding lipid area. We shall characterize this spectrin-lipid interaction by an association force F_a belonging to one unit mesh. The membrane deformation leads to the appearance of a force F_s, acting on a spectrin unit mesh to move it with respect to the lipid bilayer. If this moving force F_s is greater than F_a, the spectrin-lipid-anchorage ruptures and such a unit mesh will be uncoupled from "its" lipid bilayer part and possibly translocated along the lipid bilayer. In this case, the mechanics have to be analyzed within the framework of the MFB.

Furthermore, let us assume that the membrane of the RBC may be characterized by two different viscosities. The first one η_b described the energy dissipation due to the redistribution (flow) of lipid molecules in the two-dimensional lipid bilayer itself. The second viscosity η_s characterizes those dissipation processes which are associated with a displacement (slip) or the spectrin network relative to the lipid bilayer. We point out here that experiments have shown that $\eta_b \ll \eta_s$ (Chien et al. 1978; Meier 1988). Within the framework of our membrane model this means that any flow in the plane of the lipid bilayer is quicker than the redistribution of the spectrin network relative to the lipid bilayer.

The aspiration process of a small part of an RBC into a micropipette may be subdivided into the following events: Before pressure application all elementary unit meshes have a symmetrical shape with area a and characteristic length $l_0 = \sqrt{a}$. The first, fast process of aspiration is governed by the MIB. Because of the characteristic times, $\tau_s \sim \eta_s/\mu \gg \tau_b \sim \eta_b/\mu$, there is no redistribution of the spectrin network even if there are free boundary conditions ($F_a \simeq 0$) and the tongue length in the micropipette is determined as the MIB predicts (Markin and Kozlov 1986). Examination of tensions acting on an elementary unit mesh of the skeleton from the neighbouring unit meshes and arising because of their deformation according to (2) reveals that the resulting moving force F_s acting on such a unit mesh may be expressed by the tension of the membrane skeleton as follows:

$$F_s = \oint T_s \, dl. \tag{3}$$

Integration has to be done over the whole contour of such a unit mesh. T_s equals the tension vector of the skeleton.

Within the framework of the MFB the equilibrium state of the membrane skeleton corresponds to $F_s = 0$. For the problem under analysis, i.e. the deformation during the first phase of aspiration, the MIB has to be applied. In this case the moving force F_s acting on the unit mesh is not equal to zero. In an equilibrium this force is compensated for by the force of interaction between spectrin molecules and the lipid bilayer F_a. If it is true, despite the membrane deformation, that $F_s < F_a$ at all points on the membrane, then no redistribution of the skeleton occurs and the aspiration process stops after the first fast step. If, however, there exists (owing to the RBC suction) an area of the membrane where $F_a < F_s$, then this part of the skeleton redistributes with respect to the lipid bilayer. In this case one observes the second, much slower step of the aspiration process (Fig. 1).

The aim of this paper is, therefore, to analyze the conditions of a two-phase process for micropipette aspiration and to deduce the equilibrium RBC tongue length. In order to do this we have to calculate the distribution of the force of skeleton reorganization F_s over the membrane at the end of the first aspiration phase. If there is a membrane region where a skeleton redistribution is possible, then, in a second step we have to analyze the reorganization of the skeleton and to determine the additional membrane tongue length, which is reached during the second phase of such an aspiration process.

Conditions of redistribution of the membrane skeleton

In the following we consider the mechanics of the RBC membrane at the end of the first, fast aspiration process. Under this condition, there is no redistribution of the membrane skeleton and the mechanical description of this situation has to follow that usually employed (Evans and Skalak 1980); in our terminology this is the approach of the MIB. Therefore, the tongue length L_p^{I} of an RBC in the micropipette (Fig. 2) depends on the aspiration pressure drop Δp and the apparent membrane shear modulus μ. It may be expressed (Chien et al. 1978; Evans and Skalak 1980) as

$$\frac{\Delta p \cdot R_p}{\mu} = (2 L_p^{\mathrm{I}}/R_p) - 1 + \ln(2 L_p^{\mathrm{I}}/R_p). \tag{4}$$

Assuming that $\Delta p \cdot R_p/\mu \gg 1$ one gets an approximate but simple equation:

$$L_p^I = \frac{1}{2}\frac{\Delta p \cdot R_p^2}{\mu}. \tag{5}$$

In the above situation each aspirated elementary unit mesh deforms with respect to its initial shape in such a way that each mesh is elongated in its meridional direction. It can be shown that the meridional length of such a deformed mesh at the position z inside the micropipette equals

$$l_z = \sqrt{a}\sqrt{\frac{2(L_p^I - z)}{R_p} + 1}. \tag{6}$$

For the perpendicular direction the corresponding length is

$$l_p = \frac{\sqrt{a}}{\sqrt{\frac{2(L_p^I - z)}{R_p} + 1}}. \tag{7}$$

Equations (6) and (7) describe the situation for unit meshes sucked into the cylindrical part of the pipette, i.e. for z-values of $0 < z < L_p^I - R_p$. Furthermore, (6) and (7) were derived without taking into consideration the deformation of unit meshes at the hemisphere of the aspirated membrane projection.

For the plane part of the RBC membrane (i.e., the part not sucked in) we use the polar coordinate r, with the centre of the micropipette as the origin (Fig. 2). It is easy to show that the length l_r, directed parallel to r, of such a mesh is equal to

$$l_r = \sqrt{a\left(\frac{2L_p^I R_p}{r^2} + 1\right)} \tag{8}$$

and the perpendicular width l_φ is

$$l_\varphi = \sqrt{\frac{a}{\left(\frac{2L_p^I \cdot R_p}{r^2} + 1\right)}}. \tag{9}$$

From (2), the tension (due to cell deformation) acting on the unit mesh boundary from the neighbouring meshes is (in the micropipette):

$$T_{sz} = \mu\left[\frac{2(L_p^I - z)}{R_p} - 1\right], \quad T_s = \mu^2/T_{sz}. \tag{10}$$

For the plane part of the membrane one gets:

$$T_{sr} = \mu\left[\frac{2L_p^I R_p}{r^2} + 1\right], \quad T_{s\varphi} = \mu^2/T_r. \tag{11}$$

The total force in the meridional direction, acting on an elementary unit mesh from neighbouring ones and attempting to redistribute the unit mesh with respect to the lipid bilayer, is (inside the micropipette):

$$F_s = a\frac{\partial T_{sz}}{\partial z} = -\frac{2\mu a}{R_p} \tag{12}$$

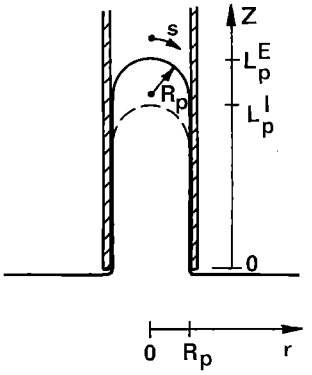

Fig. 2. Schematic illustration of an aspirated RBC membrane projection in its equilibrium state (L_p^E). The projection length L_p^I after the first, fast suction process is marked by the dashed line. Beside the coordinate z directed parallel to the pipette, the polar coordinate r for the plane membrane part and the meridian distance s are shown

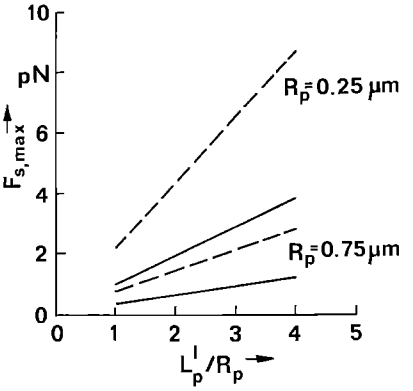

Fig. 3. Maximum force $F_{s,max}$ generated by RBC pipette aspiration and acting to redistribute the skeleton with respect to the lipid bilayer as a function of deformation ratio L_p^I/R_p for different pipette radii and membrane shear elasticity moduli in accord with (13). ($a = 0.03$ µm^2, *dashed lines*: $\mu = 4$ µN/m (Meier 1988; Chien et al. 1978), *full lines*: $\mu = 9$ µN/m (Hochmuth and Waugh 1987)

and correspondingly, in the plane part of the membrane:

$$F_s = a\left[\frac{\partial T_{sr}}{\partial r} + \frac{T_{sr}}{r} - \frac{T_{s\varphi}}{r}\right] = -4a\mu\left[\frac{L_p^{I2} R_p^2}{r^3(2L_p^I R_p + r^2)}\right]. \tag{13}$$

Equation (12) shows that the force F_s is directed from the hemispherical end of the membrane projection to the tip of the pipette and that it does neither depend on the z-position or on the length of aspirated cell projection. In contrast, (13) shows that in the plane part of the membrane (i.e., outside the micropipette) there is a strong dependence of the generated force F_s on the radial position of the elementary unit mesh. The force of redistribution itself is also directed to the tip of the pipette (opposite to the r-coordinate). This force reaches its maximum value directly at the micropipette tip ($r = R_p$) and its value may be estimated from (13) as $F_{s,max} = -2a\mu L_p^I/R_p^2$, assuming $L_p^I \gg R_p$. It should be mentioned that, as Fig. 3 shows, even if the experimental extension ratios are chosen to be equal, the generated forces to redistribute elementary unit meshes are different and they depend on the radius (even though the membrane properties are the same).

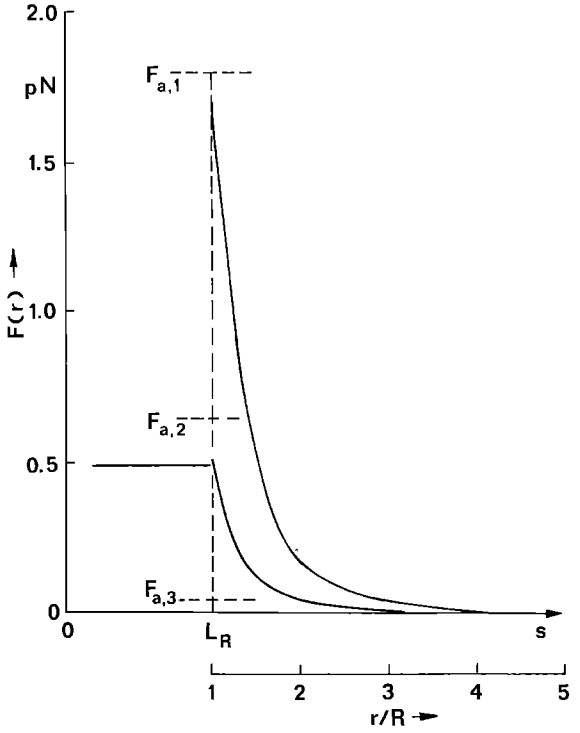

Fig. 4. Force distribution with respect to the meridian distance s from the middle of the RBC projection hemisphere in accord with (12) and (13). F_{a1}, F_{a2} and F_{a3} are assumed spectrin-lipid association forces, which oppose the generated force F_s acting to redistribute the spectrin with respect to the lipid bilayer. ($a=0.03$ μm^2, $\mu=4$ μN/m, $L_p^I/R_p=1.4$ (lower curve), $L_p^I/R_p=3$ (upper curve)). Note, that in contrast to the r-abscissa the s-abscissa is not in scale (drawn only schematically)

The above results can be comprehensively illustrated (Fig. 4), if the absolute value of the force acting to redistribute the skeleton with respect to the lipid bilayer is plotted as a function of the meridional distance s, measuring from the top of the membrane projection inside the micropipette. In Fig. 4 the segment $s<L_R$ represents the independence of the acting force F_s on the position of the unit mesh inside the cylindrical pipette, but the segment $s>L_R$ illustrates the decline of the force with increasing distance from the capillary tip in the plane membrane outside the pipette.

Analysis of the tongue length of the second aspiration phase

In the following the increase of the RBC membrane projection during the second phase of membrane aspiration is investigated. Let us analyze the events for the case where the generated force F_s acting on an elementary unit mesh is of some intermediate value ($F_{a,3}<F_s<F_{s,\max}$). Then the redistribution process of the skeleton occurs in the aspirated part of the membrane and a limited region of the plane membrane ($F_a<2\,\mu a/R_p$) or only within a plane part of the membrane outside the pipette (case $F_{a,2}$ in Fig. 4). As a consequence, with the same suction pressure (Δp) the cell projection in the micropipette lengthens further and part of the plane membrane region where the MFB works is sucked into the pipette. On the other hand, during this second phase of aspiration, the area where redistribution occurs increases and the projection lengthens further and finally reaches an equilibrium projection length $L_p^E=L_p^I+L_p^{II}$. L_p^I characterizes the length of cell projection due to the first step, but L_p^{II} is the additional length reached during the second phase. In the Appendix we derive the corresponding equations for the final equilibrium tongue length L_p^E and the value of L_p^{II}. For the additional projection length of the RBC membrane in the micropipette as a result of the second phase of aspiration this analysis yields

$$L_p^{II}=\frac{\Delta p\cdot R_p^2}{4\,\mu}\ln\left(\frac{\Delta p\cdot a}{F_a}\right)=\frac{L_p^I}{2}\ln\left(\frac{\Delta p\cdot a}{F_a}\right) \tag{14}$$

and therefore the total equilibrium tongue length is

$$L_p^E=\frac{\Delta p\cdot R_p^2}{2\,\mu}\left[1+\frac{1}{2}\ln\left(\frac{\Delta p\cdot a}{F_a}\right)\right]. \tag{15}$$

It is interesting to discuss the above equation with respect to the value of F_a, i.e. the interaction or association force between the spectrin heterodimers of one elementary unit mesh and the corresponding lipid bilayer as well as integral proteins penetrating the bilayer. If this force is applied to the area of one unit mesh F_a/a, we get the force density f_a. The results obtained for different values of this force density may be summarized as follows:

1. If the force per unit mesh (13), generated by the applied suction pressure Δp, acting to redistribute the cytoskeleton, is smaller than the force density f_a no second aspiration process occurs. This means that, for

$$f_a>\Delta p=2\,\mu\cdot L_p^I/R_p^2=F_{s,\max}/a$$

it is true that

$$L_p^E\equiv L_p^I\,.$$

2. If there is only a very small force density ($f_a\approx 0$), the model of free boundaries has to be applied and any force per unit mesh F_s/a generated by the suction pressure Δp redistributes the membrane skeleton. Therefore, even for very small Δp it is true that

$$0\approx f_a<\Delta p=2\,\mu\,L_p^I/R_p^2=F_s/a.$$

This means that the MFB has to be applied and according to Markin and Kozlov (1986) the equilibrium projection length can be obtained from the following equation:

$$L_p^E=R_p(K+1)\left\{1+\ln\left[1.3\cdot\frac{(K+2)}{(K+1)}\right]\right\}-\frac{\pi-2}{2} \tag{16}$$

where

$$K=\Delta p\cdot R_p/2\,\mu\,.$$

3. If for a given Δp it holds that

$$2\,\mu/R_p<f_a<\Delta p=\frac{2\,\mu\,L_p^I}{R_p^2}=F_{s,\max}/a$$

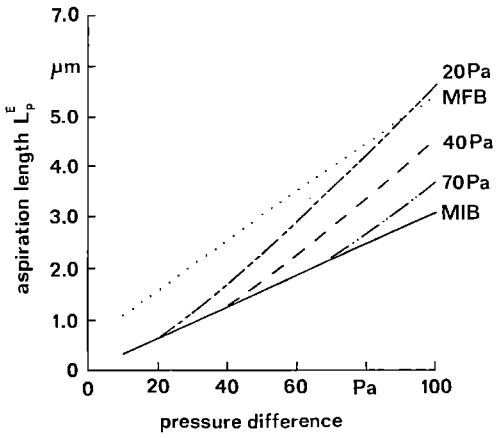

Fig. 5. Dependence of the equilibrium projection length L_p^E of part of an aspirated RBC membrane on the suction pressure difference Δp. Calculations are done for the MFB (16), for the MIB (4) and for a membrane, where a partial redistribution of the skeleton results in a second phase of aspiration (17). Parameters used: $R_p = 0.50$ μm, $\mu = 4$ μN/m, $f_{a1} = 20$ Pa, $f_{a2} = 40$ Pa, $f_{a3} = 70$ Pa

then the membrane skeleton redistributes in some part of the membrane and the equilibrium projection length is:

$$L_p^E = L_p^I + \frac{\Delta p \cdot R_p^2}{4\,\mu} \ln\left(\frac{\Delta p}{f_a}\right). \tag{17}$$

Figure 5 summarizes the equilibrium projection lengths as a function of the applied aspiration pressure calculated for the cases discussed above.

Discussion

From the biophysical viewpoint, currently available information on RBC membrane structure (Cohen 1983; Mohandas et al. 1983; Stokke et al. 1986a; Low 1987; Lutz et al. 1986) favours a three-layer membrane model (Schmid-Schönbein et al. 1983; Lerche et al. 1987; Lerche et al. 1988). These layers are: the glycocalyx, the conventional lipid bilayer membrane including penetrating proteins and the spectrin-actin skeleton. Different kinds of membrane deformation involve different layers of this three-layer membrane model (Lerche et al. 1988).

In an analysis of the extensional deformation of RBC membranes by means of micropipette aspiration, the membrane properties are usually treated as a summation of the mechanical contributions of the laminar lipid bilayer and the skeleton network (Evans and Hochmuth 1977; Evans and Skalak 1980). The underlying theory results from the deformation of strongly interacting shells. This approach was criticized as not being applicable to the real biological membrane (Schmid-Schönbein et al. 1983).

Recently, Markin and Kozlov (1986) investigated the mechanics of membrane deformations for strongly intercalated shells (MIB) and for freely sliding layers (MFB). This approach was not, however, able to describe the experimentally demonstrated two-phase deformation depicted in Fig. 1 and also obtained by other authors (Chien et al. 1978; Sung et al. 1985; Hochmuth and Waugh 1987). Usually, qualitative explanations such as "the material properties of the erythrocyte membrane are altered during the 20 s period of deformation in the micropipette" have been offered (Chien et al. 1978).

For the first time the approach described in this paper allows one to analyze the two-phase process of membrane aspiration and to move towards some molecular understanding. The most significant proposal is that we suggest a force density for the association (interaction, anchorage) of the spectrin molecules forming an elementary unit mesh with the lipid bilayer and that at a given membrane deformation (projection length in the pipette) the generated force per unit mesh of the skeleton becomes critical and a redistribution of such a mesh with respect to the lipid bilayer may occur.

The force generated near the tip (the maximal one) depends proportionally on the apparent membrane shear modulus μ but also on the pipette radius. Therefore, even if the extension ratios L_p^I/R_p are chosen experimentally to be equal, a different force is generated for a different pipette radius. If this force is in excess of the force of interaction (anchorage) between the spectrin molecules and the lipid bilayer F_a, then the aspiration process follows the two step kinetics described by (17). If we know the equilibrium length L_p^E and the first plateau-value L_p^I an interaction force density f_a may be estimated (from (17)). From (17) we get:

$$f_a = \Delta p \cdot \exp\left(-\frac{4\,\mu \cdot (L_p^E - L_p^I)}{\Delta p \cdot R_p^2}\right). \tag{18}$$

Referring to work of Chien and co-workers (Chien et al. 1978, Fig. 7–9) we find the following data (not necessarily for the same experiment) $\mu = 4.2$ μN/m, $R_p = 0.68$ μm, $L_p^I = 1.40$ μm, $L_p^E = 1.60$ μm and $\Delta p = 50$ Pa. These data results in an interaction force density of about 43.2 Pa. From our data (Fig. 1), which are representative for normal RBC, we get (with $\mu = 4.3$ μN/m, $R_p = 0.62$ μm, $L_p^I = 1.73$ μm, $L_p^E = 2.13$ μm and $\Delta p = 53.8$ Pa) a value of some 44.2 Pa. This is a remarkable coincidence for two experiments in different laboratories.

Unfortunately, no other detailed investigations have been reported. Nevertheless, from this analysis it may be predicted that the smaller the pipette radius the higher the generated force and the larger that region where the anchorage between spectrin and the lipid bilayer breaks, and consequently the second phase of the aspiration process takes place. On the other hand, for small extensional ratios L_p^E/R_p, the generated force per unit mesh $F_{s,\max}/a$ may be less than f_a and therefore no second phase will be obtained (cf. Fig. 5).

The above analysis also raises the question of what the apparent membrane shear modulus means when it is computed on the basis of L_p^E (Chien et al. 1978; Evans and Skalak 1980; Hochmuth and Waugh 1987; Meier 1988). If we want to probe the elastic properties of the resting (unloaded) membrane, we should compute the value of μ on the basis of L_p^I. Otherwise, we get the material characteristic of a membrane, where the spectrin-lipid bilayer intercalation is, at least in some region, broken and the two layers may more or less slide freely with respect to each other. Meier (1988) showed that a cyclic aspiration and release of the RBC membrane into a micropipette

does indeed result in continuously increasing tongue length for small suction pressures (about 45 Pa). The first loading process already results in some disruption of the anchorage between the spectrin layer and lipid bilayer and, therefore, the second loading cycle already works over a membrane to which the model of free boundary has to be applied. Correspondingly we get a larger deformation (tongue length) with the same pressure drop. This result also has to be taken into account if the apparent shear modulus is determined by increasing the pressure drop consecutively, as is usually done (Chien et al. 1978; Evans and Skalak 1980).

The origin of the proposed interaction or anchorage force f_a is not specified as yet, but very recent data show that beside the spectrin-ankyrin-band III interaction, fatty acid acylation of peripheral (cytoskeletal) proteins could condition and/or regulate the association of these proteins with the membrane (Marinetti and Cattieu 1982; Lutz et al. 1986; Maretzki et al. 1990). Within this framework, the "shear thinning" behaviour of the RBC membrane (Chien et al. 1978; Hochmuth and Waugh 1987), i.e. the decrease of the relaxation time with increasing strain rate, can be discussed. In the case of micropipette aspiration high strain rates also imply high extensional rates L_p/R_p. But this also means that $F_{s,\max}$ increases in the same manner and therefore, as Fig. 4 shows, in a large region of the plane part of the sucked in membrane the disruption of the anchorage also takes place immediately. This gives the membrane additional freedom to dissipate the strain and may result in an apparent decrease of the relaxation time.

In conclusion, it can be stated that for the experimentally obtained two-phase aspiration process a mechanical membrane model is proposed which derives from the conventional model of the strongly intercalated spectrin "shell" and lipid bilayer "shell". It has to be applied to small deformations. For large membrane deformations due to micropipette aspiration the anchorage of these two "shells" is loose or completely disrupted and the deformation process has to be described by a membrane model with freely sliding "shells", the so-called model of free boundaries.

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Appendix

Equilibrium length of RBC projection in the case of membrane skeleton redistribution

Consider the situation where a projection of the RBC membrane is sucked into the pipette of radius R_p by the pressure difference Δp. The system reaches its equilibrium (Fig. 2). Let us further assume that the spectrin-lipid association force per elementary unit mesh f_a is larger than $2\,\mu/R_p$ (cf. Fig. 4). Then the system may be divided mechanically into four regions. In the first region, where $L_p^E > z \geq L_p^I$, we have to apply the MIB. For the second region, where $L_p^I > z \geq 0$, the MFB is applied. The same is true for the third region, i.e. the plane part of the membrane outside the pipette characterized by $R_p < r < r_{cr}$. The RBC membrane outside this circle ($r > r_{cr}$) will not undergo any restructuring with respect to the skeleton and the bilayer and has therefore to be described within the framework of the MIB.

Let us analyze the tension T and the deformation of an elementary unit mesh of the membrane skeleton for all four membrane regions. The total membrane tension is characterized by T_z, T_r, T_φ. In contrast, as in the main paper, T_{sr} and $T_{s\varphi}$ describes the tension acting on an elementary unit mesh from the neighbouring unit meshes. The value of the tension T_r and T_φ has to be summed from the skeleton tension T_{sr} and $T_{s\varphi}$ as well as the tension of the lipid bilayer T_b. Referring to Kozlov and Markin (1987) for detailed information, we obtain the following results:

Region I:

$$T_z = \Delta p\, R_p \tag{A 1}$$

$$l_z = \sqrt{a\,\frac{2}{R_p}(L_p - z) + 1} \tag{A 2}$$

$$l_\varphi = \sqrt{\frac{a}{\frac{2}{R_p}(L_p - z) + 1}} \tag{A 3}$$

where a is the area of a unit mesh ($a = l_\varphi \cdot l_r$).

Region II:

$$T_z = \Delta p\, R_p \tag{A 1}$$

$$l_z = A_2 \exp\left[\frac{\mu z}{R_p(\Delta p\, R_p - T_b)}\right] \tag{A 4}$$

$$l_\varphi = \frac{\mu A_2}{(\Delta p\, R_p - T_b)} \exp\frac{\mu z}{R_p(\Delta p\, R_p - T_b)} \tag{A 5}$$

where μ is equal to the apparent shear elastic modulus and A_2 is an integration constant. It should be stressed that the tension of the bilayer T_b does not vary in the membrane regions II and III because the lipid bilayer may be treated as a two-dimensional fluid.

Region III:

$$T_r = \mu\sqrt{1 + \frac{A_3}{r^2}} + T_b\,, \qquad T_{sr} = \mu\sqrt{1 + \frac{A_3}{r^2}}\,, \tag{A 6}$$

$$T_\varphi = \frac{\mu}{\sqrt{1 + \frac{A_3}{r^2}}} + T_b\,, \qquad T_{s\varphi} = \frac{\mu}{\sqrt{1 + \frac{A_3}{r^2}}}\,, \tag{A 7}$$

$$l_r = A_3' \frac{A_3 + r^2}{r + \sqrt{A_3 + r^2}} \tag{A 8}$$

$$l_\varphi = A_3' \frac{r}{r + \sqrt{A_3 + r^2}}\,. \tag{A 9}$$

As above, A_3 and A_3' are some constants of integration.

Region IV:

$$T_r = \frac{\mu A_4}{r_2} - \mu \ln \frac{\mu}{\sqrt{A_4 + r^2}}, \tag{A 10}$$

$$T_\varphi = T_r - \mu\left(1 + \frac{2A_4}{r^2} - \frac{r^2}{A_4 + r^2}\right), \tag{A 11}$$

$$l_r = \sqrt{a\left(1 + \frac{A_4}{r^2}\right)}, \tag{A 12}$$

$$l_\varphi = \sqrt{\frac{a}{1 + \frac{A_4}{r^2}}}, \tag{A 13}$$

$$T_{sr} = \mu \sqrt{1 + \frac{A_4}{r^2}}, \tag{A 14}$$

$$T_s = \frac{\mu}{\sqrt{\left(1 + \frac{A_4}{r^2}\right)}}, \tag{A 15}$$

where A_4 is another constant of integration.

The force generated due to the aspiration of the membrane projection into the micropipette outside the pipette is given by (11) of the main paper and is for regions III and IV

$$F = -\frac{\mu \cdot a}{r^3} \frac{A_4^2}{(A_4 + r^2)}. \tag{A 16}$$

To solve (A 1–A 16) we have to apply the corresponding boundary conditions. For regions I and II for $z = L_p^{\mathrm{I}}$, the l-values should be the same. One gets therefore:

$$\sqrt{\frac{a}{\frac{2}{R_p}(L_p - L_p^{\mathrm{II}}) + 1}} = \frac{\mu A_2}{(\Delta p\, R_p - T_b)} \times \exp\left[\frac{\mu L_p^{\mathrm{II}}}{R_p(\Delta p\, R_p - T_b)}\right]. \tag{A 17}$$

At the border of region II and III, i.e. at the coordinate $z = 0$, there should be a continuous value of l_r and l_φ. The corresponding equation is:

$$A_3' \frac{\sqrt{A_3 + R_p^2}}{R_p + \sqrt{A_3 + R_p^2}} = A_2, \tag{A 18}$$

$$A_3' \frac{R_p}{R_p + \sqrt{A_3 + R_p^2}} = \frac{\mu A_2}{\Delta p\, R_p - T_b}. \tag{A 19}$$

Between region III and IV ($r = \varrho$) this also holds for the boundary for T_r and l_φ. We obtain

$$\mu \frac{\sqrt{A_3 + \varrho^2}}{\varrho} + T_b = \mu \frac{A_4}{\varrho^2} - \ln \frac{\varrho}{A_4 + \varrho^2}, \tag{A 20}$$

$$A_3' \frac{\varrho}{\varrho + \sqrt{A_3 + \varrho^2}} = \frac{\varrho \sqrt{a}}{A_4 + \varrho^2} \tag{A 21}$$

At this boundary ($r = \varrho$) the generated force acting to redistribute the skeleton according to (A 16) is

$$F_s = -\frac{\mu \cdot a}{\varphi} \frac{A_4^2}{(A_4 + \varphi^2)} \tag{A 22}$$

Finally, we have to take into account that for the continuity of the skeleton network the overall quantity of elementary unit meshes in the zone (area) between region II and III is to be preserved. After corresponding integration we find:

$$\frac{R_p^2(\Delta p\, R_p - T_b)^2}{\mu^2 A_2^2}\left\{1 - \exp\left[-\frac{2\mu L_p^{\mathrm{II}}}{R_p(\Delta p\, R_p - T_b)}\right]\right\} + \frac{1}{A_3'^2} \cdot (\varrho + \sqrt{A_3 + \varrho^2})^2 - (R_p + \sqrt{A_3 + R_p^2})^2 = (2R_p L_p^{\mathrm{II}} + (\varrho^2 - R_p^2))/\sqrt{a}. \tag{A 23}$$

By means of (A 17–A 23) we are able to evaluate the constants of integration, the radius ϱ of the boundary between region III and IV, the tension of the lipid bilayer T_b in zone II and III and the equilibrium length of the cell projection L_p^E, as well as the additional projection length L_p^{II} of the second aspiration phase. Analytical solution of these equations may be obtained if one assumes that $\Delta p \cdot R_p/\mu \gg 1$. Doing so we obtain:

$$\varrho = R_p \sqrt{2\frac{\Delta p \cdot a}{F_a}} \tag{A 24}$$

$$L_p^{\mathrm{II}} = \frac{\Delta p \cdot R_p^2}{4\mu} \ln\left(\frac{\Delta p \cdot a}{F_a}\right) \tag{A 25}$$

$$L_p^E = \frac{\Delta p \cdot R_p^2}{2\mu}\left[1 + \frac{1}{2} \ln\left(\frac{\Delta p \cdot a}{F_a}\right)\right]. \tag{A 26}$$

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