

Wrinkling-induced fracture of biological membranes under stress

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Abstract. The effect of stress hormones on the structure and the surface morphology erythrocyte membranes is studied. It is shown that structure transformations of membrane proteins cause the development of compressive stress in the membranes leading to their wrinkling. The tensile tangential stress developed at the wrinkle crest is responsible to growth of pores and their coalescence followed by membrane cracking.

Introduction

A mature erythrocyte is a multiscale hierarchically organized system. Its structure lies at the basis of a phospholipid bilayer containing proteins, which fulfill different functions (enzymes, transmembrane carriers, hormone receptors) (Fig. 1,a). Mechanical behavior of such complex hierarchical system is governed by its liquid-crystalline properties. Reversibility of form changes in biological membranes is disturbed in response to environmental conditions (temperature and pH changes, interactions with hormones and mediators), the membranes are subjected to internal stress and suffer irreversible nanostructured transitions leading to their destruction. The aim of this work is to elucidate the effect of stress hormones on the destruction of erythrocyte membranes of rats.

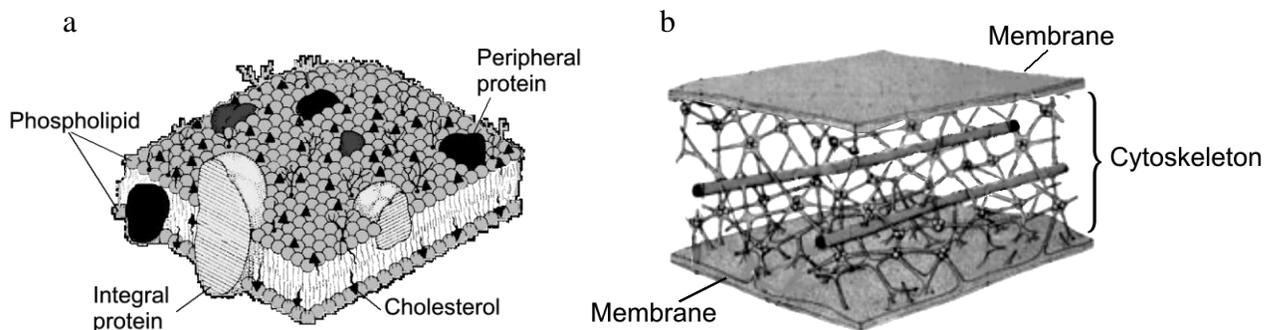


Fig.1. Sketches of a phospholipid bilayer (a) and a cell cytoskeleton (b).

Results and Discussion

Erythrocytes were extracted from fresh blood of Wistar rats after decapitation under light nembutal anesthesia. The blood was diluted to a double volume with isotonic phosphate buffer of pH 7.35 containing 153 mM of NaCl, 5.64 mM of Na₂HPO₄ and 1.03 mM of KH₂PO₄. After sedimentation of cells by centrifugation at 330 g for 10 min, the supernatant was poured off and the washing procedure was repeated two times more. All procedures were performed at 4 °C. Then erythrocytes were incubated with stress hormones at a terminal concentration of 10⁻⁶ M. The obtained erythrocyte suspension of 20 µl volume was transferred to a slide, smeared finely, dried in air and viewed on a Solver Bio NT-MDT atomic force microscope (AFM).

AFM-investigations showed that as-prepared erythrocytes ghosts are biconcave disks in shape (Fig. 2,a). Stress hormones (cortisol, adrenalin, noradrenalin) cause structure changes (denaturation) of proteins containing in the erythrocyte membranes. The latter results in the development of compressive stress in the membranes leading to their wrinkling (Fig. 2,b,c). The origins of compressive stress are different in cases of cortisol and adrenalin incubation.

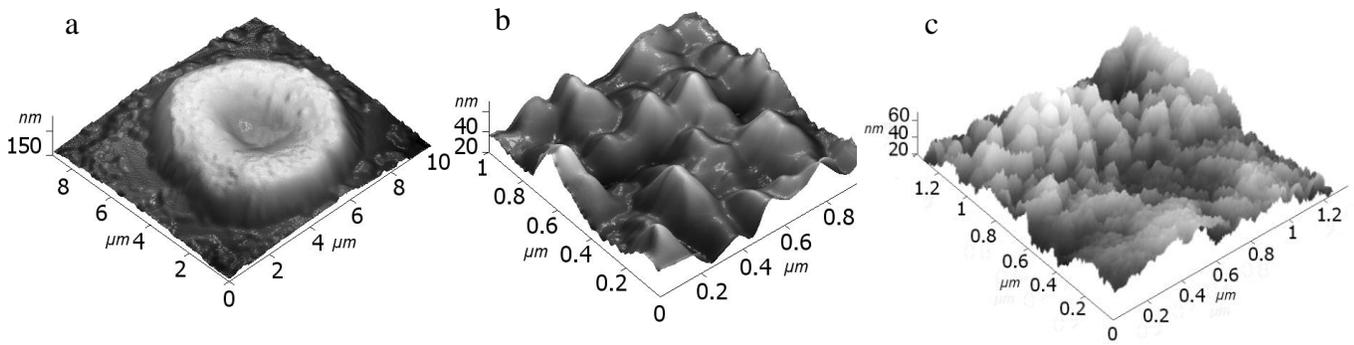


Fig.2. AFM-images of an as-prepared erythrocyte (a) and erythrocytes incubated with cortisol (b) and adrenalin (c).

Cortisol is unable to penetrate deeply into the phospholipid bilayer and interacts with proteins on the surface of cell membranes. Cortisol structure is characterized by three OH-groups and two keto-groups, which form hydrogen bonds with CO- and NH-groups of membrane-bound proteins and phospholipids contained in membranes. Cortisol-induced structural changes of erythrocyte membrane are clearly evident from IR-spectroscopy results [1].

Analysis of IR spectra of rat erythrocyte ghosts inoculated with cortisol revealed an increase in the absorption band intensity of CO- (1655.2 cm⁻¹) and NH-bonds (1548 and 3290 cm⁻¹) by ~ 20 % as well as a shift in stretching vibrations of the peptide bond (NH-bond): 3308 → 3280 cm⁻¹ ($\Delta\nu = 28$ cm⁻¹), and increasing its intensity due to the formation of a hydrogen bond between cortisol and NH-bond of proteins. In addition, shifts of stretching vibrations of CH-bonds: 2848 → 2852 cm⁻¹ ($\Delta\nu = 4$ cm⁻¹) and 2930 → 2925 cm⁻¹ ($\Delta\nu = 5$ cm⁻¹) as well as an increase in absorption band intensity of the C=O-bond of phospholipids and its shift 1748 → 1740 cm⁻¹ were observed. The increase in band intensity points to enhancement of fatty acid ordering and to lowering of phospholipid entropy. The shift of the band owes to the formation of hydrogen bond between the hormone and probably the OH-group at C₂₁ and CO-bond of phospholipids.

The structural transitions in proteins during the interaction of cortisol with erythrocyte membranes can also be judged from fluorescence quenching curves of tryptophan. The maximum decrease in fluorescence was observed at a hormone concentration of 11.6·10⁻⁸ M in the incubation medium

with a protein content of 0.256 mg/ml; ΔF was 34 relative units, i.e. 11 % with respect to control [1].

Because of structural changes of protein molecules contained in the membrane phospholipid bilayer, the proteins tend to enlarge. The more bonds the protein molecule has with other molecules in particular with water ones, the more extended it should be. However, a cell cytoskeleton (Fig. 1,b) is not deformed in such a manner resulting in compression of the enlarged membrane. As a consequence the membrane wrinkles in order to match with cytoskeleton dimensions (Fig. 2,b).

Similar wrinkling is observed on the human skin subjected to long-term water exposure. The outer skin layer (epidermis) consists of necrotic keratin cells, which absorb moisture on immersion in water. Absorption of moisture leads to cell swelling and extension of the outer skin layer. However the outer layer is linked with underlying skin layers, which are unable to extend, therefore, epidermis wrinkles accommodating its lateral dimensions with underlayers [2].

The mechanism of cortisol-induced membrane wrinkling consists in its Euler elastic instability governed by compressive stress [3]. It distinguished from the classic Euler instability only by constraints imposed by the cytoskeleton on bending of the erythrocyte membrane.

While the erythrocyte membrane is subjected to solely elastic deformation on wrinkling, deformation of the cytoskeleton is viscoelastic, i.e. its elastic deformation at the initial stage of wrinkling is followed by viscous flow that is accompanied by redistribution of bound water molecules at the wrinkle crests. Wrinkle parameters are governed first by energetics and then by kinetics of the process. The membrane can wrinkle only on exceeding a critical value of compressive stress [4]:

$$\sigma_w = -E_m \left(\frac{9}{16(1-v_m^2)(1-v_{cs})^2} \right)^{1/3} \left(\frac{\mu_{cs}^R}{E_m} \right)^{2/3}, \quad (1)$$

where E_m is the membrane Young modulus, v_m and v_{cs} are the Poisson's ratios of the membrane and the cytoskeleton respectively, μ_{cs}^R is the cytoskeleton rubbery modulus. If the compressive stress σ is higher than the value determined by Eq. 1, the membrane wrinkles with any wavelength exceeding a critical value:

$$\lambda_c = \pi h \sqrt{-\frac{\bar{E}_m}{3\sigma}}. \quad (2)$$

where h is the membrane thickness and $\bar{E}_m = \frac{E_m}{(1-v_m^2)}$ is the plane-strain modulus of the membrane.

However, amplitude of wrinkles with different wavelengths grows with different rates and the fastest growing mode dominates the initial wrinkling:

$$\lambda_m = \pi h \sqrt{-\frac{2\bar{E}_m}{3\sigma}}. \quad (3)$$

Cortisol-induced membrane wrinkling observed in our experiments is characterized by the wavelength $\lambda=150$ nm. Using the relevant values for erythrocyte membrane ($h=10$ nm and $E_m=128$ MPa) we obtain from Eq. 3 that it is subjected to compressive stress of ~ 5 MPa.

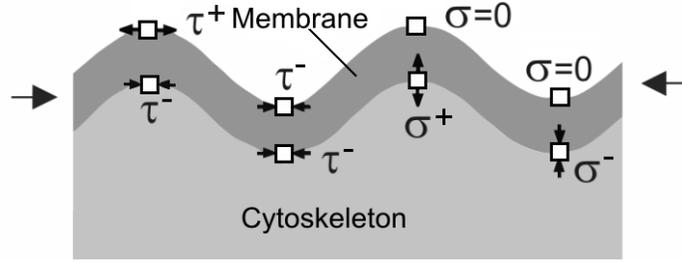


Fig.3. Schematic of the normal (σ) and tangential (τ) stress distribution in the membrane-cytoskeleton system after wrinkling.

As a result of the wrinkling, the wavy membrane-cytoskeleton interface is formed leading to the periodical distribution of normal and tangential stresses (Fig. 3). At the wrinkle crests stress component normal to the membrane-cytoskeleton interface varies from zero on the free surface to a maximum tensile value near the interface. Normal compressive stress acts at the valleys and its value is also maximum near the interface. In-plane stresses in the membrane after wrinkling are also non-uniform. Since the bending momentum tends to increase the wrinkle amplitude at the crests, the stress is compressive near the membrane-cytoskeleton interface, but can turn into tensile ones close to the free surface after a critical value of wrinkle curvature is exceeded.

The tensile tangential stress developed at the wrinkle crests can be estimated similar to bending of a beam [5]:

$$\sigma_{tensile} = \frac{E_m b}{R} \frac{b}{2}, \quad (4)$$

where R is the radius of beam curvature (curvature of wrinkle surface in our case) and b is the distance from the neutral layer to the beam surface (half-thickness of a phospholipid layer). Using the appropriate values of the wrinkle parameters ($E_m=128$ MPa, $R=90$ nm, $b = 5$ nm) we obtain that tensile stress at the wrinkle crests can reach 3.5 MPa.

Because an erythrocyte membrane is a liquid heterocrystal with low shear stability, where structure formation is provided by covalent and hydrogen bonds as well as by hydrophobic and weak electrostatic interactions, tensile tangential stress exceeding the ultimate strength of the membrane (2,55 MPa [6]) can induce its cracking near the wrinkle crests (Figs. 4,a,b). The formation of mode I cracks provides stress relaxation in neighboring areas including a normal tensile component. At the valleys of wrinkles the membrane is subjected to compressive stress, therefore, cracking does not occur there. Similar cracking mechanism was observed under mechanical loading of thin metallic films deposited on compliant polymer substrates [7] (Fig. 4,c).

Similar wrinkling of the erythrocyte membrane followed by its cracking occurs under interaction of the membrane with other stress hormones such as adrenalin and noradrenalin. However, in this case the origin of compressive stress in the membrane is distinguished from the case of cortisol. Unlike cortisol, adrenalin and noradrenalin penetrate deep into the membrane (the presence of adrenoreceptors on the surface of the erythrocyte membranes was first found in [8]) and interact with both proteins incorporated into the membrane and cytoskeleton proteins. EPR spectroscopy investigations showed that the erythrocyte membrane ghosts incubated with adrenalin are characterized by enhancement of the structure ordering of membrane proteins due to the coil \rightarrow β -structure \rightarrow α -helix transition [1]. The latter is accompanied by contraction of a spektrin-actin-

ankyrin network of the cytoskeleton. The effect of contraction was relieved under the action of cytochalasin that stabilizes spectrin [1]. The contraction of the cytoskeleton results in compression of the membrane followed by its wrinkling (Fig. 2,c). The effect is similar to skin wrinkling of an apple observed after its drying and shrinkage. The equations determining the critical value of compressive stress and wrinkle wavelength in this case are the same as for cortisol-induced membrane wrinkling (Eqs. 1 and 2). However the increasing of wrinkle height is due to valley deepening caused by displacement of water dipoles in neighboring areas.

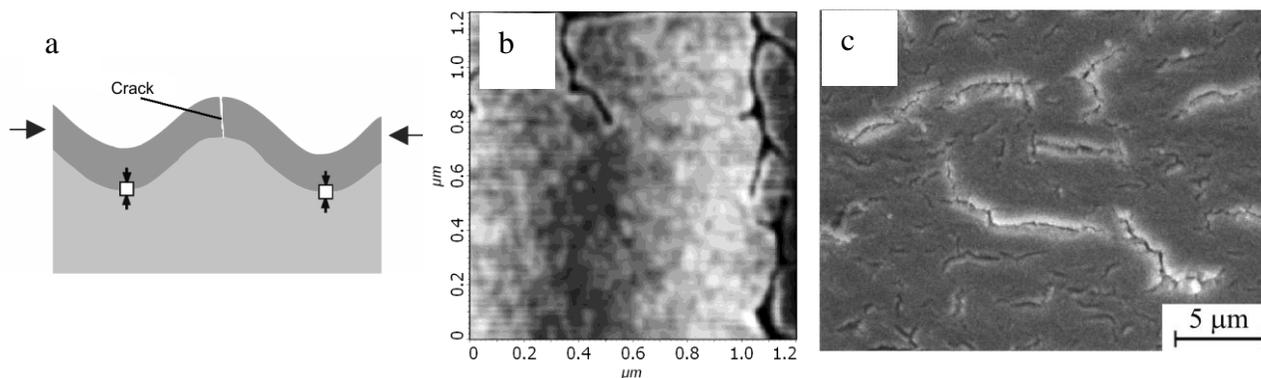


Fig.4. a – mode I cracking of the erythrocyte membrane at the wrinkle crest. b –AFM-image of erythrocyte surface after absorption of cortisol. c – SEM-image of a Cu film on a polypropylene substrate after uniaxial tension.

Cracking of a phospholipid bilayer followed by membrane fracture occurs by means of local structural-phase transitions in the areas of acting compressive stress at the wrinkle crests. In the initial condition the lipid bilayer of biological membranes is a smectic liquid crystal, which molecules are parallel to each other and arranged layer by layer [9]. It is characterized by a low level of intermolecular bonds and low shear stability, i.e. it represents a metastable system. Relaxation of tensile stress occurs by means of formation of an invert pore due to reorientation of lipid molecules on smectic A \rightarrow smectic C transition (Fig. 5,a). As this takes place, the principal axes of the molecules of the liquid crystal are bent in the opposite directions at the same angle.

It is well known that a biological membrane can vary its conformation (configuration of spatial arrangement of molecules) by means of local structural-phase transitions. Thus phospholipid heads are separated on gel-liquid transition under increasing temperature [9].

Fig. 5,b illustrates that in the gel state (the temperature of gel-liquid crystal transition can vary from -20 to -60 °C) all hydrophobic hydrocarbon tails of phospholipid molecules are arranged strictly parallel to each other (trans conformation). On solid-liquid crystal transition the membrane square per molecule considerably increases (from 0.48 to 0.58 nm²) and as a consequence the membrane volume increases too. However, increasing distance between separate phospholipid heads does not result in breaking bonds between hydrophobic tails. The latter results from trans-gosh transitions, which are possible owing to thermal motion of molecules and cause bending of hydrophobic phospholipid tails. Moreover, it leads to breakdown of parallel alignment of the tails especially in the interior of the membrane.

After reaching some critical values of system parameters, displacement of molecules from their equilibrium positions becomes irreversible because of disruption of interaction between phospholipids. It means that there is a critical pore radius such that pore healing becomes impossible. Formation of a lot of pores and their coalescence results in cracking and fracture of the membrane.

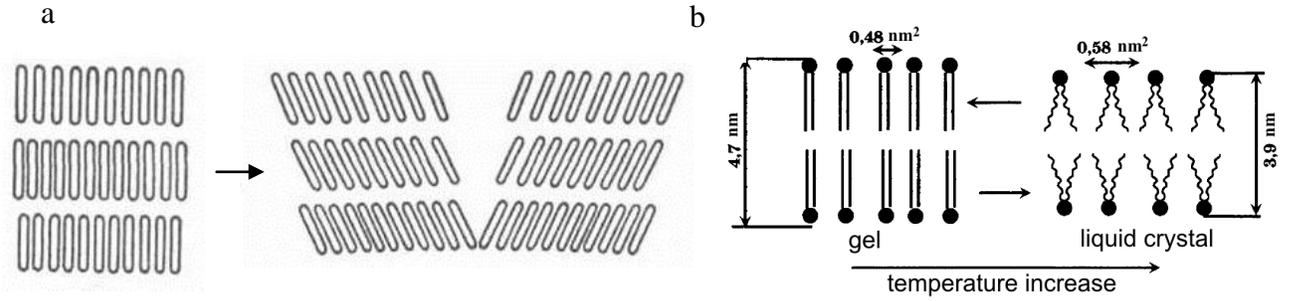


Fig. 5. Rearrangement of lipid molecules on smectic A \rightarrow smectic C transition (a). Structure transformation of a membrane on gel-liquid crystal transition and vice versa under temperature variation (b).

It is possible to calculate from thermodynamic considerations the critical pore radius [9]. Suppose that a transverse pore in the biological membrane is shaped like a cylinder with height h (that equals to the membrane thickness) and radius r . Let the lateral surface of the cylinder is bent with curvature radius $h/2$ (Fig. 7). Bending of lipid bilayer – water interface is known to be accompanied by origination of addition (Laplace) pressure [9]:

$$\Delta P = \frac{2\sigma_n}{r}, \quad (5)$$

where σ_n is the interfacial tension of the inner pore surface. Because the transverse pore is characterized by two curvature radii $h/2$ and r , the considered system is characterized by two Laplace pressures: $P(h/2)$ that favors pore extension and $P(r)$ that promotes its contraction. When $P(h/2) > P(r)$ then the pore will extend but it will be healed if $P(h/2) < P(r)$.

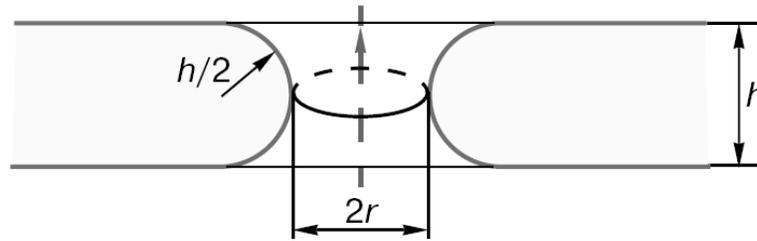


Fig.6. Transverse pore through a biological membrane.

A critical pore radius corresponding to a stable pore can be estimated using the sum energy of a phospholipid bilayer containing a defect (pore). Generally the sum energy E is equal to the work of defect formation minus the energy of the same defectless membrane area [9]:

$$E = 2\pi h\sigma_n r - \pi\sigma r^2, \quad (6)$$

where σ is the membrane surface tension.

Thus two opposite forces act at the pore boundary. The former is the edge linear tension of the pore perimeter that favors pore extension and the latter is the surface tension of the bilayer that promotes

pore contraction. The edge energy of a pore is proportional to its radius and increases the sum energy, while the energy associated with surface tension is proportional to r^2 and decreases the sum energy [9].

Since function $E(r)$ has a maximum, where $\frac{\partial E}{\partial r} = 0$, the Eq. 6 can be rewritten as

$$2\pi h\sigma_n - 2\pi\sigma r = 0. \quad (7)$$

Using Eq. (7) we find that the critical pore radius is

$$r_c = h\sigma_n / \sigma. \quad (8)$$

Because the membrane surface tension and the interfacial tension of the inner pore surface are essentially the same, we obtain $r_c=h$, i.e. the critical pore radius is 10 nm. When pore radius exceeds this value, coalescence of growing pores results in membrane cracking.

Summary

The investigations performed revealed that stress hormones (cortisol, adrenalin and noradrenalin) induce considerable changes in structure of protein molecules contained in erythrocyte membrane. Structure transformation of membrane proteins is accompanied by tension of the phospholipid bilayer or by compression of the cytoskeleton. Both the effects results in wrinkling of erythrocyte membranes accompanied by viscoelastic deformation of the cytoskeleton. The mechanism of membrane wrinkling incubated with stress hormones lies in its elastic instability governed by compressive stress.

The periodic distribution of normal and tangential stresses is formed after perturbation of the initially flat phospholipid bilayer. The stress distribution governs the following growth of membrane wrinkles. After exceeding a critical value of membrane curvature, tangential stress may become tensile in the areas of wrinkle crests that leads to formation of inverted pores. The pore forms by means of reorientation of molecules of the lipid bilayer. When exceeding the critical radius, the pores will extend and their coalescence be followed by membrane cracking.

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