Volume 304, number 1, 32–36 FEBS 11115 June 1992

Correlation between local cell membrane displacements and filterability of human red blood cells

Shmuel Tuvia, Shlomo Levin and Rafi Korenstein

Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, 69978 Tel-Aviv, Israel

Received 23 March 1992; revised version received 16 April 1992

Local mechanical fluctuations of the cell membrane of human erythrocytes were shown to involve MgATP- and Mg^{2+}-driven fast membrane displacements. We propose that these local bending deformations of the cell membrane are important for cell passage through capillaries. In order to verify this hypothesis, we examined cell membrane fluctuations and filterability of erythrocytes over a wide range of medium osmolalities (180–675 mosmol/kg H_2O). The results indicate the existence of a positive correlation between the amplitude of local cell membrane displacements and cell filterability. We suggest that the occurrence of metabolically driven membrane displacements on the side surface of the red blood cell diminishes its bending stiffness and enables it to fold more efficiently upon entrance into blood capillaries. Thus, local cell membrane displacements seem to play an important role in microcirculation.

Erythrocyte deformability; Cell membrane fluctuation; Erythrocyte filterability; Osmosis; Membrane curvature; Microcirculation

1. INTRODUCTION

Mechanical fluctuations of the cell membrane are a newly recognized dynamic activity of the living cell [1–4]. The fluctuations consist of reversible fast local displacements (frequency range of 0.3–30 Hz) of the cell membrane. These cell membrane fluctuations were observed in different types of cells including red blood cells (RBCs) [1,2,4], monocytes, lymphocytes, 3T6 fibroblasts, cardiomyocytes [1] and murine lymphoma cells [3]. These fluctuations reflect local ($\leq 0.25\ \mu m^2$) bending deformation of the cell membrane. They possess the highest amplitude of displacement (≈240 nm) in RBCs. The local bending deformations of the cell membrane in RBCs were shown to depend, to a large extent, on MgATP- and Mg^{2+}-driven dynamic assembly–disassembly of the protein network of the membrane skeleton [2]. Recently, we have shown that oxygenation of the red blood cell leads to an increased amplitude of fluctuation. In addition, oxygenated RBCs displayed slower kinetics of adhesion to a glass substratum than deoxygenated RBCs [4]. In view of these findings we suggested that cell membrane fluctuations correlate with bending deformability of the RBC during its entrance into capillaries. However, this suggestion was based on indirect evidence that the primary mode of RBC deformation in capillaries is a folding or bending about the longitudinal axis of the capillary [5,6]. The correlation between local cell membrane fluctuations of RBCs and their capability to pass through capillaries still needs experimental verification.

The present study examines cell membrane fluctuations and filterability of RBCs through 5 μm pore filters over a wide range of medium osmolarity and establishes a direct correlation between cell membrane fluctuations and filterability of RBCs.

Correspondence address: R. Korenstein, Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, 69978 Tel-Aviv, Israel. Fax: (972) (3) 6409113.

2. MATERIALS AND METHODS

2.1. *Preparation of RBCs for measurement of cell membrane fluctuations*

RBCs were obtained on the day of experiment from healthy donors. Blood (volume of 50–100 μl) was diluted × 50-fold in PBS (130 mM NaCl, 10 mM glucose, 5.5 mM phosphate buffer pH 7.4, 1 mg/ml BSA) and washed twice with PBS followed by two successive centrifugations (1,500 rpm, 2 min) and the buffy coat was gently removed. The RBCs were suspended in a 310 mosmol/kg H_2O Ringer solution (145 mM NaCl, 4 mM KCl, 3 mM $CaCl_2$, 5 mM glucose, Tris buffer pH 7.4, 1 mg/ml BSA). RBC suspensions in Ringer-based solutions of different osmolalities were obtained by a previously used procedure [7]. The RBC suspension (5×10^6 cells/ml) in the original Ringer solution was divided into several 10 ml aliquots. The samples were then centrifuged at 1,500 rpm for 2 min and the supernatant Ringer solution was partially replaced by volumes of distilled water or with 10% NaCl. In this way the osmolality of the medium was varied in the range of 180–675 mosmol/kg H_2O. The change of medium osmolality is accompanied by shifts of ionic strength from 0.1 in hypo-osmotic medium up to 0.34 in hyperosmotic medium. In order to separate the effect of medium osmolality from that of ionic strength we measured the effect of ionic strength changes from 0.1 to 0.16 in iso-osmotic (310 mosmol/kg H_2O) medium and from 0.16 to 0.34 in hyperosmotic (675 mosmol/kg H_2O) solution in which ionic strength was varied. The required medium osmolality was maintained by adding an appropriate amount of NaCl or sucrose. The osmolality was measured in triplicates by a vapor pressure osmometer (Vescor 5500) which was precalibrated with osmometry standard solutions. The RBCs suspension was introduced into the experimental chamber consisting of two cover

Published by Elsevier Science Publishers B.V.

glasses separated by a distance of 0.2 mm. The cells were incubated for 15–20 min in a chamber at 22–24°C allowing them to attach to the bottom cover glass. The shape and size of RBCs in the horizontal and the vertical positions relative to the plane of a microscope stage were measured by phase-contrast microscopy.

2.2. *Measurement of cell membrane displacements of RBCs*

The measurement of local mechanical displacements of the cell membrane was carried out on human RBCs by a novel optical method based on point dark-field microscopy [1]. Using cells attached to a cover glass, we illuminate a very small area (0.25 μm^2) at the cell's edge and record cell membrane displacements by monitoring the time-dependent changes of light reflection and scattering. The fluctuation of the light intensity depends on the changes of the membrane area moving in and out of the focused light spot near the cell's edge. A linear dependence between the relative change in the scattered and reflected light ($\delta F/F$) from the cell surface and the amplitude of the cell's edge displacement was achieved by moving the cover glass, with attached glutaraldehyde fixed cells, by a calibrated vibrator [1]. Linearity of $\delta F/F$ with amplitude of displacement was observed over distances as long as 300 nm ($\delta F/F$ of 1% corresponds to a displacement of 17 nm). The sensitivity of the experimental set-up is about 1%. The amplitude of local membrane displacements was measured on the same RBCs population by successive perfusion of experimental chamber with modified Ringer solutions of different osmolalities. All measurements were carried out at 22–24°C.

2.3. *Preparation of RBCs for filterability measurements*

Blood was obtained on the day of the experiment from healthy donors. 15 ml of vein blood was introduced into a test tube prewashed with 50 mM EDTA. The blood was centrifuged at 3,000 rpm for 10 min. The plasma, buffy coat and upper layer of RBCs were removed. The RBCs in the pellet were washed twice with filtered (through 0.2 μm Millipore filter) PBS and resuspended in a filtered Ringer solution reaching a final hematocrit of 10%. Solutions of different osmolalities were obtained by the procedure described in section 2.1. The possible effect of medium ionic strenght on cell filterability was measured by employing a same procedure to the one described in 2.1, using a hyperosmotic media (675 mosmol/kg H_2O) obtained by adding NaCl or sucrose and mannitol.

2.4. *Measurements of RBCs filterability*

The measurement of RBCs filterability was carried out by recording the time passage of a constant volume (0.15 ml) of 10% RBCs suspension in a Ringer-modified solution through 5 μm pores of a polycarbonate filter (Nucleopore) at 25 mm negative water pressure. The filtration time of 0.15 ml of RBCs suspension as well as the filtration time of the same volume of solution (devoid of RBCs) were measured photoelectrically using a Grass amplifier and recorder. The time resolution of the set-up is ≈ 10 ms. It was possible to perform 3–5 filtration experiments of RBCs suspension with the same filter without plugging it by RBCs. After each filtration of RBCs suspension, the filter was washed out from RBCs by a flow of solution in the opposite direction. Filterability was evaluated as the ratio of the rate filtration of RBCs suspension (V_o) to the rate filtration of the modified Ringer solution (V_s). Since the volume of the different samples being filtered was the same (0.15 ml), filterability was equal to ratio of the filtration time of the solution devoid of RBCs (t_s) to the filtration time of the RBCs suspension (t_o) [8]. The time of filtration of 0.8–0.95 s that we obtained for a 10% RBCs suspension in Ringer solution was similar to values obtained by Hanss [8].

2.5. *Measurment of RBC membrane curvatures*

Two main curvatures of the RBC membrane on the outer side of the cell were measured from phase-contrast microphotographs at magnification 1,200 × (objective 100 ×, NA=1.3). The RBCs were oriented mostly with their larger dimension parallel (x–y plane) to the plane of the microscope's stage (Fig. 1A, B and C, left panel). The curvature of outer surface in the x–y plane is defined by the inverse radius of membrane contour in this plane. The curvature in the z direction (perpendicular to the microscope's stage) was measured on vertically oriented RBCs (with their larger dimension perpendicular to the microscope's stage) as the inverse radius of the external RBC ring.

3. RESULTS AND DISCUSSION

In this study we examined the effects of medium osmolality on local cell membrane displacements in RBCs and their filterability through 5 μm pores of a filter, in an attempt to correlate the amplitude of fluctuation with cell filterability.

Under isosmotic conditions (310 mosmol/kg H_2O) the RBC possesses a displacement amplitude of 225 nm (Fig. 1A). Under these conditions, RBCs maintain a normal biconcave shape with a diameter of 8.2 ± 0.5 μm (mean ± S.D., n=50). The amplitude of local membrane displacement diminished to a value of 140 nm under hypo-osmotic conditions (180 mosmol/kg H_2O) (Fig. 1B). Under these conditions, the diameter of the swollen cells decreased by 3% to a value of 7.9 ± 0.6 μm (n=50; P=0.001 when comparing isosmotic and hypo- osmotic states). Under hyperosmotic conditions (675 mosmol/kg H_2O) the displacement amplitude declines to a value of 140 nm (Fig. 1C). Under these conditions the diame-

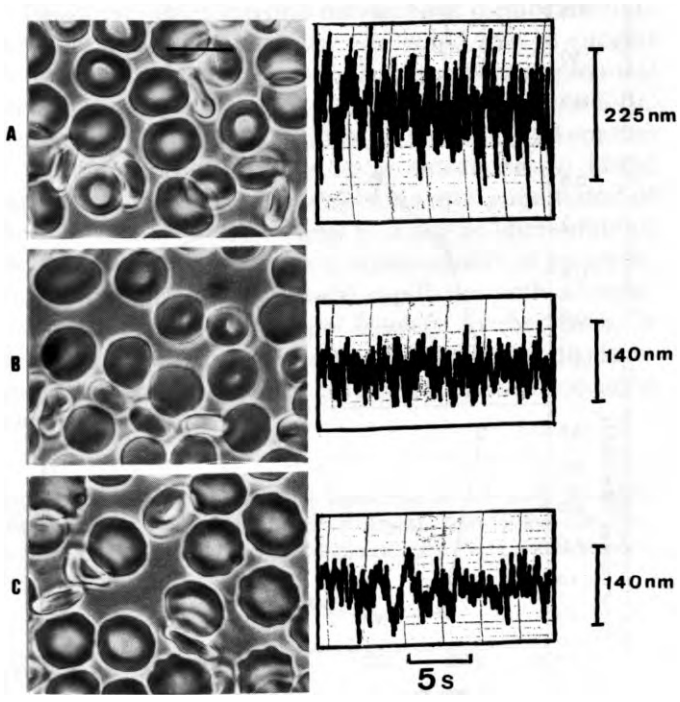

Fig. 1. Influence of medium osmolality on cell membrane displacements and shape of RBCs. (A) RBCs in iso-osmotic solution (310 mosmol/kg H_2O), (B) RBCs in hypo-osmotic solution (180 mosmol/kg H_2O) and (C) RBCs in hyperosmotic solution (675 mosmol/kg H_2O). Left panel (A, B and C), phase-contrast microphotographs of living cells attached to cover glass. The shapes of horizontally and vertically arranged RBCs are seen. Bar = 10 μm. Right panel (A, B and C), typical traces of the displacements (in nm) of a small area (0.25 μm^2) of the RBC outer surface.

ter of the shrinked cells is increased by 6% to a value of 8.7 ± 0.5 μm (n=50; P<0.001 when comparing isosmotic and hyperosmotic states).

The dependence of fluctuation amplitudes and filterability of RBCs on medium osmolality in the range of 180–675 mosmol/kg H_2O is shown in Fig. 2A and B, respectively. The amplitude of cell membrane displacements (225 nm) does not change in the osmolality range of 310–470 mosmol/kg H_2O. However, when medium osmolality varies beyond this range, a strong decrease in amplitude of displacement is observed (≈150 nm at 220–180 mosmol/kg H_2O and 140–170 nm at 675–550 mosmol/kg H_2O). This decline of displacement amplitude is reversible when resuspending the RBCs in an isosmotic medium (data not shown). The large variability of membrane displacements, which amounts to 15% (S.D./mean), reflects variations in the displacement amplitude among the studied cells.

The dependence of the RBCs filterability on medium osmolality is shown in Fig. 2B. We measured RBCs

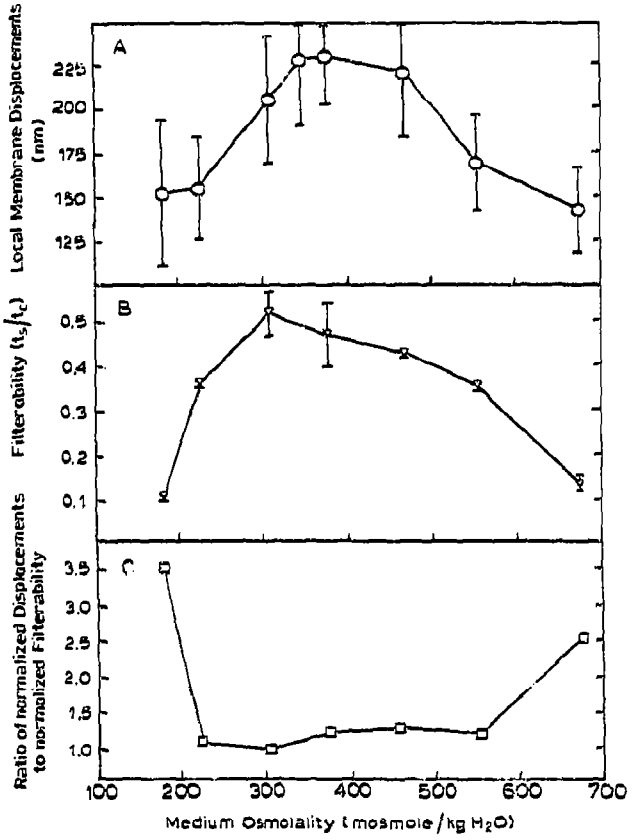

Fig. 2. Relationship between the amplitude of local cell membrane displacement (A) and cell suspension filterability (B) at various medium osmolalities. (A) The dependence of local membrane displacements on medium osmolality. (B) The dependence of filterability on medium osmolality. (C) Correlation between the amplitude of local cell membrane displacements and RBCs filterability (normalized to isosmotic conditions at 310 mosmol/kg H_2O) at various medium osmolalities.

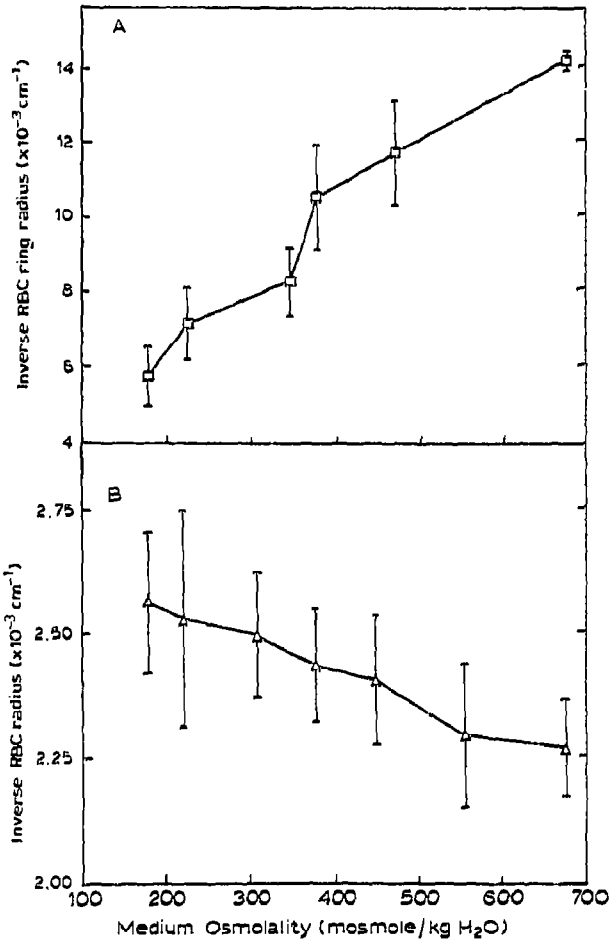

Fig. 3. The dependence of outer membrane curvatures of RBCs on various medium osmolalities. (A) The dependence of the outer RBC curvature in the z direction on medium osmolality. The curvatures in z direction were determined from radius of outer RBC ring. (B) The dependence of the outer RBC curvature in x–y plane on medium osmolality. The curvature in the x–y plane was determined from radius of RBC disc. The measurement of the two curvatures was performed on phase contrast microphotographs of cells oriented perpendicular (z direction) and parallel (x–y plane) to the plane of the microscopic stage.

filterability at a constant pressure by recording the initial flow rates. In the range of 310–470 mosmol/kg H_2O (isosmotic and hyperosmotic conditions), a maximal filterability (0.48–0.50) is maintained. When medium osmolality changes beyond this range limit, a strong decline in filterability is observed (i.e. filterability of 0.36–0.11 at 220–180 mosmol/kg H_2O and filterability of 0.35–0.17 at 550–675 mosmol/kg H_2O). A comparable dependence of RBCs filterability on medium osmolality was previously observed by measuring filterability at a constant flow rate through a 5 μm pore filter by recording the initial pressure rise [7].

The dependence of the RBCs filterability and of local cell membrane displacements on medium osmolality is similar (Fig. 2A and B). The correlation between these two phenomena is shown in Fig. 2C. It can be seen that

the ratio of normalized (to the maximal isosmotic value) cell membrane displacements and RBCs filterabilities is about 1 in the medium osmolality range of 220–580 mosmol/kg H_2O. This indicates the existence of a positive correlation between local cell membrane displacements and cell filterability. It may be suggested that filterability of RBCs through 5 μm pores depends on the amplitude of local displacements of the cell membrane. Only in the two extreme osmolalities of the medium, at 180 mosmol/kg H_2O and 675 mosmol/kg H_2O, the ratio is about 3.5 and 2.2 in hypo-osmotic and hyperosmotic solutions, respectively (Fig. 2C). This reflects the fact that under these two extreme medium osmolalities the relative change in filterability is larger than the corresponding relative change of displacement amplitude. The higher relative decrease in filterability at 180 mosmol/kg H_2O may be due to a large cell volume increase [7] and an extensive stretching of the cell membrane [9]. The large relative drop of filterability at 675 mosmol/kg H_2O could be associated with the increase of cytoplasmic viscosity (due to elevation of hemoglobin concentration) [7] and with the increase of RBC membrane curvature in the z direction (Fig. 3).

The curvature of a membrane determines its passive bending rigidity [10]. Therefore, the curvature of the outer side of the RBC may be expected to affect the amplitude of local displacements and bending deformability of the cell membrane, thus affecting the filterability of RBCs. However, the dependence of either of the two main curvatures of the outer side of RBC does not correlate with either membrane displacement amplitude or with cell filterability. The dependence of displacement amplitude and filterability on medium osmolality shows a bell shaped form (Fig. 2A and B), whereas the two types of curvature change in a monotonic fashion as a function of osmolality (Fig. 3). Thus, it seems that the metabolic-dependent dynamic bending properties of RBC, expressed by the local membrane displacements, are more directly connected to filterability, than static curvature characteristics of the RBC.

The changes of medium osmolality are accompanied with variation of the ionic strength. In order to examine the contribution of the ionic strength changes to the observed variation of membrane displacements and RBC filterability we have carried out measurements under conditions of constant osmolality while varying the ionic strength. Thus, when decreasing the ionic strength of the medium from 0.16 down to 0.1, under iso-osmotic conditions (310 mosmol/kg H_2O) the amplitude of fluctuations (235 ± 42 nm, n=13 and 233 ± 33 nm, n=17, respectively) and RBCs filterability (0.49 ± 0.02, n=4 and 0.49 ± 0.03, n=3, respectively) were not affected. Under hyperosmotic conditions (675 mosmol/kg H_2O), where lower values of cell membrane displacement and RBCs filterability were obtained, variation of medium ionic strength from 0.16 up to 0.34 also did not affect both phenomena. Thus, the amplitudes of cell membrane displacements were 138 ± 27 nm, n=21 and 148 ± 24 nm, n=8 at ionic strength of 0.16 and 0.34, respectively. RBCs filterability also did not change between ionic strength of 0.16 (0.11 ± 0.07, n=4 and 0.11 ± 0.01, n=3, in sucrose and mannitol, respectively) and ionic strength of 0.34 (0.11 ±0.02, n=3). Under both conditions, the change of ionic strength did not affect the RBC's shape. Hence, amplitude changes of cell membrane displacements are caused by medium osmolality changes and are independent of the medium ionic strength in the range of 0.1–0.34.

The observed dependence of the displacement amplitude on medium osmolality may be attributed to the stretching and compression of the membrane skeleton in hypo-osmotic and hyperosmotic conditions correspondingly. Since the membrane skeleton is semi-expanded under isosmotic conditions [11], both stretching and compression of the skeleton network may lead to the attenuation of displacement amplitude [2]. In addition to the induced mechanical stresses of the membrane skeleton due to shape changes, intracellular concentration changes of MgATP and Mg^{2+} may be involved in the attenuation of cell membrane displacements [2,4]. Moreover, we have recently observed a cGMP induced increase of cell membrane displacements, under isosmotic conditions, which was accompanied by an increase of RBCs filterability (submitted).

Based on the correlation between cell membrane fluctuations and cell filterability we would like to suggest that the occurrence of metabolically driven mechanical membrane fluctuations on the side surface of the RBC diminishes the bending stiffness of the RBC and enables it to fold more efficiently upon entrance into blood capillaries. Thus, higher displacements amplitudes of the RBC are expected to lead to a higher filterability of the RBC with a concomitant higher ability of an erythrocyte to penetrate into blood capillaries with a consequent more efficient oxygen delivery to the tissue. A similar interrelation between cell membrane displacements and cell filterability may be expected to occur in other circulating cells.

Acknowledgements: This study is supported by the Basic Research Foundation, The Israel Academy of Sciences and Humanities. The work was carried out in partial fulfillment of a Ph.D. thesis requirement of S. Tuvia.

REFERENCES

[1] Krol, A.Ju., Grinfeldt, M.G., Levin, S.V. and Smilgjavichus, A.D. (1990) Eur. Biophys. J. 19, 93–99.
[2] Levin, S. and Korenstein, R. (1991) Biophys. J. 60, 733–737.
[3] Mittelman, L., Levin, S. and Korenstein, R. (1991) FEBS Lett. 293, 207–210.
[4] Tuvia, S., Levin, S. and Korenstein, R. (1992) Biophys. J., in press.
[5] Bagge, U., Branermark, P.I., Karlsson, R. and Skalak, R. (1980) Blood Cells 6, 231–237.

[6] Gaehtgens, P., Duhrssen, C. and Albrecht, K.H. (1980) Blood Cells 6, 799–811.
[7] Reinhart, W.H. and Chien, S. (1985) Am. J. Physiol. 248, C473–C479.
[8] Hanss, M. (1983) Bioreology 20, 199–211.
[9] Zeman, K., Engelhard, H. and Sackmann, E. (1990) Eur. Biophys. J. 18, 203–219.
[10] Lipovsky, R. (1991) Nature 349, 475–481.
[11] Vertessy, B.G. and Steck, T.L. (1989) Biophys. J. 55, 255–262.