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Effect of pH on red blood cell deformability

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Abstract The effect of pH on the red blood cell (RBC) deformability, which is a consequence of a change of cell membrane elastic properties is studied experimentally. With the intention to reduce the effects on deformability of cell geometry and cytoplasmic viscosity, we measured the deformability of the cells with the same volume at various pH of cell suspension from 6.2 to 8.0. Constant cell volume was achieved by varying osmolarity. Deformability was quantified by measuring the elongation of RBCs subjected to velocity gradient in a transparent cone-plate rheoscope. Observed significant decrease of deformability at lower pH leads to the conclusion that membrane elastic properties could be affected by pH changes in the range from 6.2 to 8.0.

Key words erythrocyte deformability • pH dependence • membrane elasticity

Introduction

The term cellular deformability is generally used to characterise the RBC's ability to undergo deformation due to applied forces. There are several RBC characteristics that regulate cellular deformability and are influenced by cellular pH. One of them is cell geometry expressed by the ratio of the cell surface area to cell volume. Either membrane loss, which leads to reduction of surface area, or an increase of cell water content, which leads to increase of cell volume, creates less deformable cells. Another cell deformability determinant is cytoplasmic viscosity, due to which the cell deformability is larger at larger cell volumes. Cytoplasmic viscosity mostly depends on intracellular haemoglobin concentration: as the haemoglobin concentration increases from 270 to 370 g/L, the haemoglobin solution viscosity increases from 5 to 15 cp. Haemoglobin solution viscosity increases exponentially with the concentration, reaching 45 cp at 400 g/L and 175 cp at 450 g/L [7]. Membrane elastic properties also affect the cell deformability. Results from micropipette aspiration measurements show that elastic shear modulus is significantly increased at pH lower then 6.2 [1].

Ektocytometric measurements of RBC deformability in isotonic solution show that the deformability obtained by this method remains unaltered when pH is changed from 6.4 to 7.7 [4]. Decreasing of pH from physiological level leads to influx of Cl ions and water due to protonation of negative charges on the haemoglobin [4], and may also influence membrane elastic properties. Thus, ektocytometric results indicate that the cell deformability is affected in a compensating manner by all three mechanisms mentioned above.

The aim of this study was to measure RBC deformability with nearly constant volume in pH range from 6.2 to 8.0 to reduce the effect of geometric and viscosity deformability determinants, with the intention to detect changes of membrane elastic properties that could be a consequence of membrane changes caused by intracellular pH changes.

Materials and methods

Human blood was obtained by standard method for haematological analyses from healthy volunteers. RBCs were washed 3 times by centrifugation (1000 × g for 5 min) with 154 mmol/L NaCl. Washed RBCs were divided into four samples. One volume of each sample was then suspended in 9 volumes of a medium containing 90 mmol/L KCl, 45 mmol/L NaCl, 20 mmol/L Na_2HPO_4/ NaH_2PO_4 at pH values 6.2, 6.8, 7.4 and 8.0, respectively. Medium osmolarity was modified from 380 mOsmol for acid medium to 290 mOsmol for alkaline medium with the addition of sucrose. Osmolarity was measured with Knauer freezing point osmometer. Cell samples were incubated at 37°C for one hour. After incubation the mean cell volume (MCV) and morphological index (MI) were determined. MCV was measured by haematological analyser. MI was estimated by direct microscopic observation of RBCs after fixation in 0.2 % gluteraldehyde. Incubated cells were suspended in dextran solution of the viscosity 23 cp and were then transferred to a transparent cone-plate rheoscope, sheared at different shear rates and captured by CCD camera through an interference contrast microscope and analysed by a computer. Projected length of the cell in the direction of the flow (L) and its width perpendicular to the direction of flow (W) were measured and elongation index D was calculated as D = (L-W)/(L+W). D is a mean value of 50 measurements.

Results

MCV was measured before and after incubation. Changes of MCV after incubation were less than 2 % and show that the water uptake due to pH was reduced due to modified osmolarity calculated by mathematical model of RBC [5] (Fig. 1). Determinations of MI show that morphology remains the same after incubation: MI of all samples was 0.

Elongation index D of incubated cells was measured at three different shear rates: 18 s^{-1}, 37 s^{-1} and 49 s^{-1}. D of RBCs incubated at pH 6.2 is significantly smaller comparing to D of RBCs incubated at higher pH. The influence of pH on deformability decreases at higher pH and is the same at pH 7.4 and 8.0 (Fig. 2).

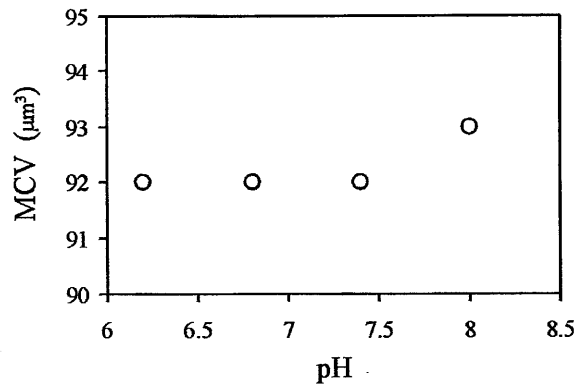

Fig.1 Mean cell volume after incubation at pH 6.2, 6.8, 7.4, 8.0 and osmolarities 386 mOsmol, 354 mOsmol, 322 mOsmol and 291 mOsmol, respectively. MCV before incubation was 92 μm^3.

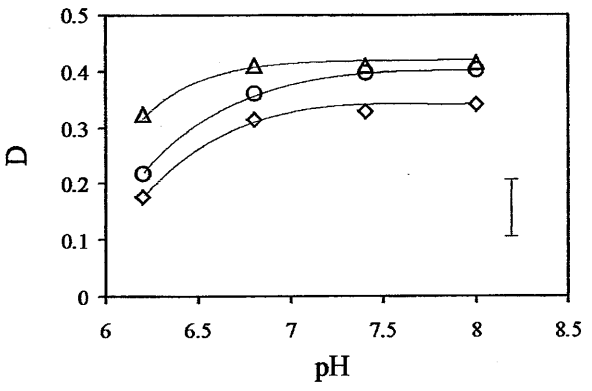

Fig. 2 Elongation index D at pH range from 6.2 to 8.0 for suspension of incubated RBC. Points represent an average for 50 RBCs in shear flow 18 s^{-1} (◇), 37 s^{-1} (○) and 49 s^{-1} (△). Error bar represents the maximum standard deviation of measurements.

Discussion

Presented measurements of RBC deformability in pH region from 6.2 to 8.0 show that deformability is smaller at low pH, if geometric and viscosity deformability determinants are reduced.

Observed pH effect on RBC deformability could be a consequence of pH effect on cell membrane like a change of membrane material properties or a change of RBC shape [2, 7]. Membrane properties could be effected by protonation of negatively charged skeleton at low pH, which is predicted to reduce charge repulsion within skeleton proteins and to consequently increase membrane rigidity [2]. Furthermore, the evidence has been presented [3] that the opposite shape changes of intact RBC and isolated RBC membranes due to pH change can be interpreted on the basis of reversible pH dependent insertion of amphitropic RBC protein glyceraldehyde-3-phosphate dehydrogenase (G3PD) into the inner layer of membrane bilayer. Another mechanism that also predicts a change of the membrane properties might be a pH dependent conformational change of the cytoplasmic domain of band 3 (CDB3) [6, 8]. The effect of CDB3 conformation on the skeleton properties could be intensified by CDB3 – haemoglobin [8] and CDB3 – G3PD interactions [3].

We conclude that the change of skeleton's elastic properties due to contraction of skeleton and the possible interaction of cytoplasmic proteins with a membrane could be the main factors influencing deformability of cells with a constant volume at pH range from 6.2 to 8.0. Which mechanism is principal should be determined in further studies.

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