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Effects of incubation pH on the membrane deformation of a single living human red blood cell

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Abstract

The effects of incubation pH on the morphology, the surface charge and the deformability of a single living human red blood cell (RBC) were studied. A novel multi-dimensional microscope was employed to perform real time, non-invasive *in situ* measurements on the cell shape and size, as well as the membrane bending and shearing elastic moduli of the cell. A phase-analysis micro-electrophoresis laser scattering technique was used to measure the surface charge density. It was shown that the incubation pH markedly influences the surface charge density and the membrane elastic properties of the RBCs and thus leads to a change in their morphology and deformability.

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Keywords: Red blood cell (RBC); Morphology; Deformability; Bending and shear elastic modulus; Surface charge density

1. Introduction

The flow of a red blood cell (RBC) through capillary vessels in the human body depends mainly on the mechanical deformation ability of its membrane. Changes of environmental conditions, such as pH, will strongly influence the deformability of the cell. Incubation pH is significant in the regulation of a biological cell's living states, such as proliferation, division, differentiation and metabolism, etc. [1]. In recent years, many investigations on the effects of pH on the stability and deformation of the membrane [2,3], the cytoskeleton and lipid bilayer [4,5], and the shape and size of RBCs [6,7] have been reported. However, most of these studies were executed for a RBC population. Only a few experiments have determined the membrane mechanical properties of a single RBC, though in these experiments the RBCs were strongly disturbed by external forces acting by means such as micropipette aspiration, atomic force microscopy and optical tweezers, etc. [8]. Based on our knowledge, heretofore, no one has reported how pH affects the spontaneous deformation of a single living RBC, and nothing is known about its mechanism. So, the purpose of this study is to perform non-invasive, real-time, *in situ* measurements on the effects of environmental pH on the shape and size, the surface charge density and the mechanical deformation of a single living RBC by using the techniques of multi-dimensional microscopy [9] and phase-analysis micro-electrophoresis laser scattering.

2. Material and methods

2.1. Preparation of PBS

An isosmotic (290 mosM) phosphate buffered saline (PBS), consisting of 138 mmol/L NaCl, 5 mmol/L KCl, 7.5 mmol/L Na phosphate, 1 mmol/L $MgSO_4$, and 5 mmol/L glucose (pH 7.4), was employed in the experiment. In order to study the effect of pH, the incubation

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medium was slowly titrated with 0.1 mol/L HCl or 0.1 mol/L NaOH to a pH range from 5.6 to 9.0.

2.2. Preparation of RBCs

Heparinized vein blood from healthy adult men was centrifuged at 2000*g* for 10 min at 4 °C three times with PBS (pH 7.4), during which the plasma and buffy coat were removed by aspiration. Then, the washed RBCs were separated by using density gradient centrifugation. The RBCs were put into the density gradient solution, which consists of different content (v/v) of dextran, at 23%, 22%, 21%, 20%, and 19% from the bottom to the top, and centrifuged at 3000*g* for 40 min at 4 °C. After centrifugation, the top 5–10% of the cells were harvested to provide a young-RBCs fraction. Young RBCs were washed in PBS (pH 7.4) three times by centrifugation (2000*g* for 10 min, 4 °C). Then, the washed young RBCs were incubated in PBS with various pH at 37 °C for 1 h, and kept cold until they were tested on the same day. All preparations of cell suspension were carried out at room temperature, while the measurements were carried out at 37 °C in a specially designed sample chamber.

2.3. Observation and analysis of RBCs

The morphology of single living young RBCs was observed and measured by a multi-dimensional microscope [9]. The microscope is capable of performing observations on the stereo and phase-contrast images of living cells and making measurements on their sizes and dynamic properties. In order to describe the RBC's shape, we used the classification of morphological index (MI) designated by Bessis et al. [10]. In brief, ten shapes were considered in the classification: discocyte (MI score 0), three types of stomatocytes (MI scores −1, −2, and −3) to spherostomatocyte (MI score −4), and four types of echinocytes (MI score +1, +2, +3, and +4) to spheroechinocyte (MI score +5). At least 100 RBCs at each incubation pH were scored for each sample. The diameter of the RBC was also determined by image-analyzing software provided by the multi-dimensional microscope. Eighty RBCs were analyzed for each incubation pH.

Two dynamic properties, the membrane bending elastic modulus, k_c, and the membrane shearing elastic modulus, μ_c, were measured based on the flicker phenomenon of the RBC [11,12]. Fifteen erythrocytes were analyzed for each incubation pH. The surface charge density of the RBC was determined by a Zeta PLUS analyzer (Brookhaven Corp., USA).

3. Results

3.1. Effect of pH on the shape and size of RBCs

At physiological pH 7.4, a human RBC takes a biconcave discoid shape (discocyte) as shown in Fig. 1b. However, they will transform into stomatocytes (shown in Fig. 1a) or echinocytes (shown in Fig. 1c) on decreasing or increasing the pH value, respectively. This is consistent with the changes in the MI with pH value. It is shown in Fig. 2 that MI is negative and increases from -0.60 ± 0.02 to 0.00 ± 0.07 in the pH range from 5.6 to 7.0. The negative MI denotes that there are more stomatocytes than discocytes or echinocytes in the RBCs suspension at low pH. In the pH range 7.0–7.8, the MIs are slightly greater than 0; this means that there are more discocytes than stomatocytes or echinocytes in the suspension. However, MI increases abruptly from 0.07 ± 0.07 to 0.88 ± 0.07 in the pH range 7.8–9.0. This means that the number of echinocytes increases markedly. At the same time, the diameter of a single young RBC decreases from 8.08 ± 0.59 μm to 5.75 ± 0.23 μm as the pH increases from 5.6 to 9.0 (as shown in Fig. 3). This indicates that the variation of incubation pH can influence both the shape and the size of a RBC.

3.2. Effect of pH on the surface charge density of the RBC membrane

It is shown in Fig. 4 that the surface charge density of the human RBC membrane increases from 5.93 ± 0.76 mC/m^2 at pH 5.6 to 18.25 ± 1.31 mC/m^2 at pH 9.0. The charge density first increases quickly in the pH range from 5.6 to 8.0, and then almost keeps constant in the pH interval of 8.0–9.0.

3.3. Effect of pH on the elastic moduli of the RBC membrane

The deformability of a RBC depends mainly on its membrane elastic properties. The membrane bending elastic modulus, k_c, and the membrane shear elastic modulus, μ_c, are the two parameters representing the membrane elastic properties. The smaller are k_c and μ_c, the better is the deformability of the RBC membrane. Fig. 5 shows how the k_c and μ_c of a young RBC change with incubation pH. We can see that the two elastic moduli have minimum values of 1.56×10^{-19} J and 4.00×10^{-6} N/m at pH 7.4, respectively. This is consistent with those measured by micropipette aspiration and optical tweezers [8,13]. However, both the moduli increase significantly when the pH value increases or decreases from 7.4. This indicates that the RBC membrane has the best deformability at pH 7.4.

4. Discussion

The surface negative charges on the membrane of a RBC mainly locate on the sialic acid residues in the polar macromolecular groups, like glycocalyx. They can be altered if the membrane structure or its outside environment changes, especially when there are changes in the thickness and structure of the charged protein layer [14] or adsorption of some ions from the environmental solution to the membrane. On the other hand, the surface