Optical study of adenylyl cyclase signalling cascade in regulation of viscoelastic properties of human erythrocytes membrane

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Deformability of erythrocytes (red blood cells, RBC) is an intrinsic biomechanical property that determines their ability to pass through narrow blood vessels, which diameter is less than the linear size of the cells. Alterations of RBC deformability (RBC-D), which occur at various deceases, e.g., diabetes mellitus type I&II, arterial hypertension, sickle cell disease, etc. lead to the impairment of gas exchange processes that may result in tissue hypoxia and necrosis.

There are mechanisms of RBC-D regulation involving intercellular signalling cascades[1]. One can be an adenylyl cyclase (AC) cascade, which is directed at the synthesis of secondary messenger cAMP in response to external stimuli. An increase of the cytosolic cAMP activates protein kinases A leading to the phosphorylation of the peptide substrate. It results in the dissociation of the bonds between the cytoskeleton and the membrane protein complexes. This molecular system can be used for the fast adaptive increase of RBC-D to maintain the effectiveness of blood microcirculation.

In this work, we present our results of the optical study of the effects of adenylyl cyclase stimulation on the RBC-D using laser ektacytometry. Two different ways of the stimulation were studied: 1) stimulation via β-adrenergic receptor with epinephrine; 2) with membrane-permeable cAMP analog dibutyryl-cAMP (db-cAMP). Laser ektacytometry allows to quantitatively characterize RBC-D by analyzing the diffraction patterns obtained by illuminating a suspension of RBCs with a laser beam[2]. RBC are elongated under the shear flow in the microfluidic chamber. We observed that the RBC-D curve - the graph of RBC elongation index (EI) dependence on shear-stress in semi-log scale – located higher when AC was activated in comparison with intact RBC. The sum of the distances between points of the curves was used as a metrics for characterizing RBC-D changes: 0.337 ([epinephrine] 1 μM) and 0.361 ([db-cAMP] 2.5 μM). The results indicate that the adenylyl cyclase activation leads to the increase of RBC deformability.

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