38 Effect of membrane permeability to anions on the swelling rate of erythrocytes treated with amphotericin b or gramicidin d

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Introduction

The most important erythrocyte function within an organism is to transport oxygen from lungs to tissues. One of the main factors that determine the functional integrity of erythrocytes is their ability to circulate. The ability of an erythrocyte to circulate is largely determined by its capacity to be deformed when passing through narrow tissue capillaries. In turn, erythrocyte deformability depends on the surface area-to-volume ratio, which is about 1.6–1.7 μm^{-1} in normal erythrocytes [1]. A change in this ratio, whether decrease or increase, leads to a decrease in erythrocyte deformability. In fact, as the erythrocyte surface area is fixed, an increase in volume results in the formation of a sphere that cannot be deformed because of geometric limitations. A decrease in volume will cause an increase in the intracellular hemoglobin concentration and the corresponding increase in the erythrocyte content viscosity. Increased viscosity adversely affects the erythrocyte deformability. Therefore, the erythrocyte must keep a certain optimum value of its volume.

Mature human erythrocytes lack such ion transport systems as K^+-Cl^- and Na^+,K^+-$2Cl^-$ co-transport which are involved in the volume stabilization in a variety of cells under the osmotic changes in the medium [2, 3]. This may indicate that as plasma osmolarity does not appreciably vary in the circulation, the mechanisms that allow erythrocytes to cope with changes in plasma osmolarity are not necessary. However, circulating erythrocytes are exposed to high oxygen concentrations. Oxidation of cell membrane components may increase the membrane permeability to cations [4]. The significant (up to several-fold) increase

in cell membrane permeability is observed in some pathologies [3, 5]. Impaired membrane permeability will result in osmotic imbalance between the cell and the medium and, hence, in an increase in the cell volume. To provide the normal ability to circulate, the erythrocyte must have special mechanisms for stabilizing its volume against changes in the cell membrane permeability. Studying the relationship between erythrocyte membrane permeability and volume is of significant interest from the standpoint of elucidating the mechanisms responsible for functional integrity and viability of these cells. In this study, we examined the *in vitro* responses of human erythrocytes to a nonselective increase in cell membrane permeability to univalent cations.

Methods

Experimental procedure Erythrocytes were incubated at 37°C in glucose-containing phosphate-buffered saline. In all experiments, ouabain was added to the erythrocytes to inhibit their Na,K-ATPase. Antibiotics amphotericin B or gramicidin D were used to increase the cell membrane permeability. Extracellular and intracellular Na^+ and K^+ were determined using flame photometry; Cl^- was determined colorimetrically with mercury chloranilate or mercury thiocyanate in perchloric extracts. The erythrocytes were separated from the medium by centrifuging through a dibutyl phthalate layer. Ion concentrations in erythrocytes were expressed as millimoles per liter cell water, assuming that cell water volume accounts for 0.7 of the total cell [6]. The extent of hemolysis was defined as the extracellular to total hemoglobin ratio in suspension. The erythrocyte volume change was determined from the change in hematocrit of a suspension, corrected for hemolysis.

Mathematical model The experimental results were analyzed by means of the mathematical model describing erythrocyte volume change under *in vitro* conditions in suspension. The model includes differential equations describing Na^+, K^+, H^+, and Cl^- transmembrane fluxes, equations for electroneutrality and osmotic balance between the medium and the cell, with the allowance for the concentration dependence of the osmotic coefficient of hemoglobin, and the closing relationships resulting from the constancy of the suspension volume and the constant contents of osmotically active species in the suspension. The DBSolve software [7], which was kindly provided by Dr. I. Goryanin, was used to analyze the model.

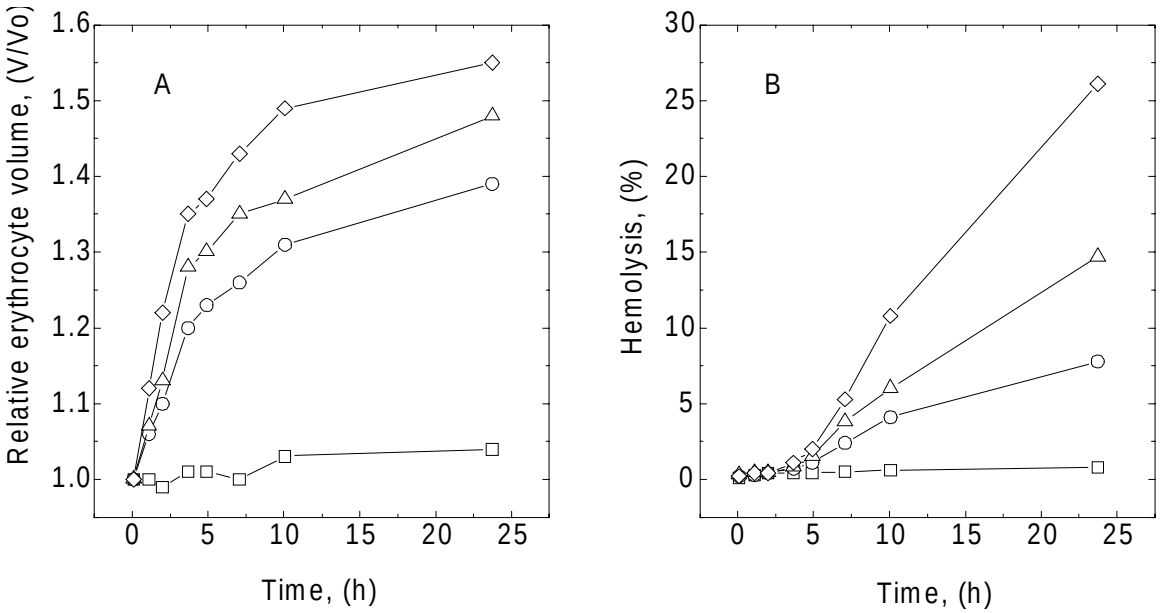

Fig. 38.1 Kinetics of (A) erythrocyte swelling and (B) hemolysis induced in 30% erythrocyte suspension by amphotericin B added to a concentration of (circles) 6, (triangles) 8, or (diamonds) 10 mg/l suspension. Control erythrocytes are depicted by squares.

Results and discussion

Both amphotericin B (in the concentration range of 5–10 mg/l suspension) and gramicidin D (in the concentration range of 10–100 μg/l suspension) caused K^+ to rapidly leak from and Na^+ to accumulate within the erythrocyte. In our experiments, the interval needed to reach the equilibrium between the intracellular and extracellular ion concentrations ranged from 30 min to several hours, depending on the antibiotic concentration. The intracellular ion concentrations changed causing the suspension hematocrit value to increase; this was accompanied by an appreciable hemolysis. These data allowed us to obtain the kinetics of cell volume changes (Fig. 38.1).

It is of interest that in spite of a rapid disappearance of Na^+ and K^+ transmembrane gradients, cell volume changes were relatively slow. Even 24 h after the membrane permeability was changed, the volume of most erythrocytes did not increase to the lytic values (about 1.6 times the normal volume). This is not consistent with the current concepts of erythrocyte volume regulation, according to which the volume is maintained owing to nonequilibrium distribution of cations between the cell and its environment. Membrane permeability to Cl^- is thought to be sufficiently high to ensure the equilibrium distribution of this anion between the cell and the medium. If it were true, the equalization of extra- and intracellular Na^+ and K^+ concentrations would immediately increase the erythrocyte volume, its sphericity, and eventually cause cell lysis.

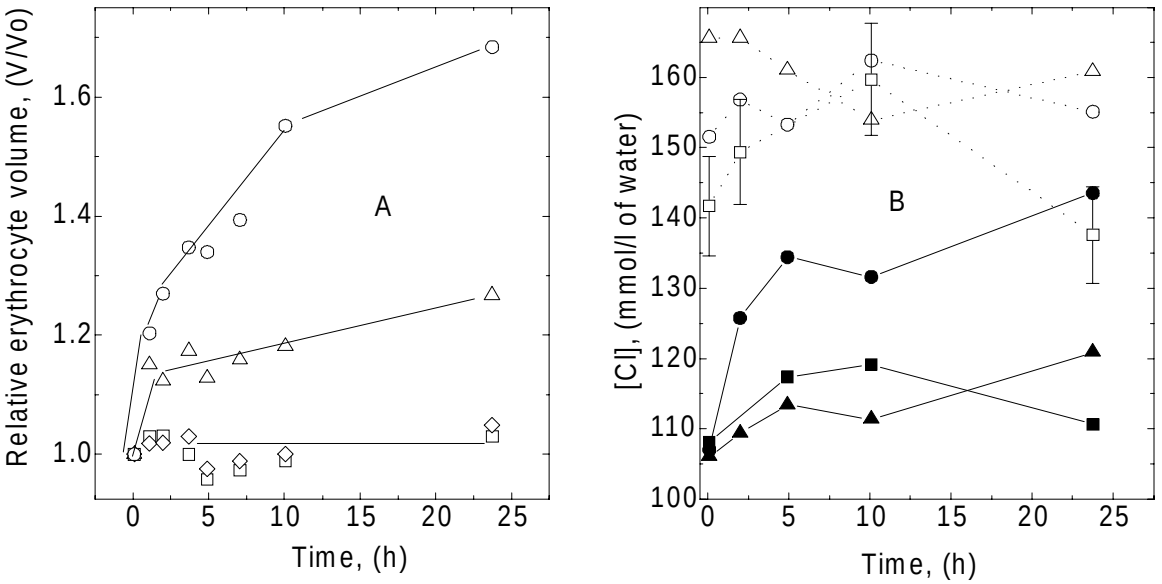

Fig. 38.2 Effect of DIDS, an inhibitor of anion transport in erythrocytes, on (A) erythrocyte swelling and (B) the transmembrane Cl^- gradient in the presence of amphotericin B: (squares) control erythrocytes, (diamonds) 20 μmol/l suspension DIDS, (circles) 8 mg/l suspension amphotericin B, and (triangles) 8 mg/l suspension amphotericin B and 20 μmol/l suspension DIDS. Intracellular and extracellular Cl^- concentrations are indicated by black and open symbols, respectively. The initial hematocrit value was 25 %.

We proposed that the slow increase in erythrocyte volume observed in our experiments might be accounted for by the slow equilibration of Cl^- distribution between the cell and the medium due to relatively low cell membrane permeability to Cl^-. Our experimental data suggest that, after the transmembrane Na^+ and K^+ gradients rapidly disappeared, the transmembrane Cl^- gradient still persisted, slowly decreasing proportionately with increasing erythrocyte volume. DIDS, a specific inhibitor of anion transport, while producing no effect on the transmembrane Na^+ and K^+ fluxes induced by the antibiotics, significantly inhibited the decrease in the transmembrane Cl^- gradient and the increase in erythrocyte volume (Fig. 38.2). On the other hand, furosemide, the specific inhibitor of K^+-Cl^- co-transport did not influence the erythrocyte swelling in our experiments.

Analysis of the mathematical model showed that, if the membrane permeability to anions was assumed to be infinite (i.e., the equilibrium Cl^- distribution between the cell and the medium was achieved instantaneously), the erythrocyte volume increased proportionately with decreasing Na^+ and K^+ transmembrane gradients, and reached the critical lytic value as soon as the cation distribution between the cells and the medium became equilibrium. According to the data available from the literature, cell membrane permeability to Cl^- is some 100 times greater than to Na^+ or K^+ [8]. Under these circumstances a significant increase in

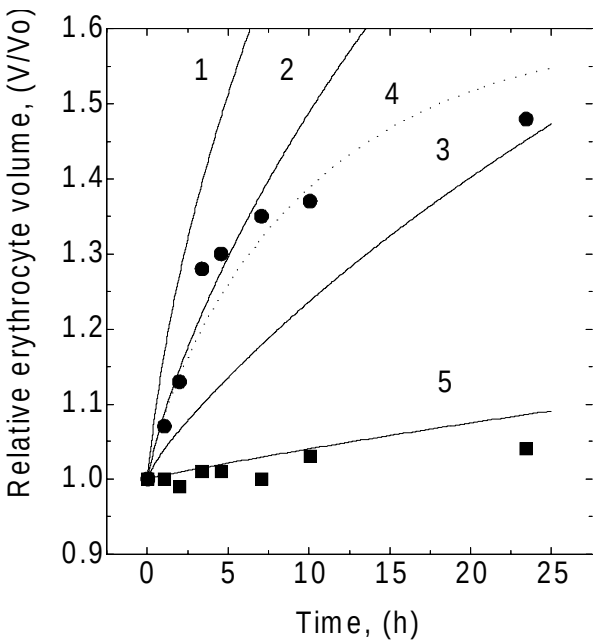

Fig. 38.3 Kinetics of erythrocyte swelling in response to an abrupt increase in the membrane permeability to cations. Symbols are experimental data points: (■) control erythrocytes, and (•) 8 mg/l suspension amphotericin B. The initial hematocrit value was 30%. Lines depict the model results for (5) control erythrocytes and (1–4) cells whose membrane permeability to cations was increased 50 fold at time $t = 0$: the rate constant for transmembrane Cl^- transport (P_{Cl}) was (1) 100 h^{-1}, (2) and (5) 1 h^{-1}, (3) 0.25 h^{-1}, and (4) $P_{Cl} = 1 - 1.67 \times ((V/V_0) - 1)$ h^{-1}. Here, V and V_0 are the current and normal values of erythrocyte volume, respectively.

the membrane permeability to cations in the model led to the relatively slow increase in erythrocyte volume, so that its lytic value was achieved several hours after the moment when both Na^+ and K^+ had reached equilibrium. The slow cell volume increase in the model was accounted for by the slow decrease in the transmembrane Cl^- gradient.

Nevertheless, our model failed to satisfactorily describe the experimental kinetics of erythrocyte swelling in response to large increases in the membrane permeability to univalent cations if its permeability to Cl^- was set to be constant (Fig. 38.3). The satisfactory description of this kinetics could be achieved by assuming that the membrane permeability to anions did not remain constant, e.g., it decreased with increasing erythrocyte volume (Fig. 38.3). The membrane permeability to anions might change with increasing erythrocyte volume if it were dependent on the membrane potential [9] or other cell parameters.

The results obtained demonstrate that human erythrocyte membrane permeability to Cl^- can control the cell swelling. In this way, transmembrane anion transport may be considered to be a component of the mechanism responsible for

the erythrocyte volume stabilization, because a significant decrease in the swelling rate allows the erythrocytes with damaged membranes to activate a relatively slow (metabolic) mechanisms of cell volume stabilization and/or repair their damaged membranes.

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