Erythrocytes: A systems model of the control of aggregation and deformability

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Article info
Article history:
Received 10 February 2014
Received in revised form 18 March 2015
Accepted 19 March 2015
Available online 21 March 2015

Keywords:
Erythrocyte
Calcium
Calmodulin
Aggregation
Deformability

ABSTRACT

Human erythrocytes are highly specialized enucleate cells that are involved in providing efficient gas transport. Erythrocytes have been extensively studied both experimentally and by mathematical modeling in recent years. However, understanding of how aggregation and deformability are regulated is limited. These properties of the erythrocyte are essential for the physiological functioning of the cell. In this work, we propose a novel mathematical model of the molecular system that controls the aggregation and deformability of the erythrocyte. This model is based on the experimental results of previously published studies. Our model suggests fundamentally new mechanisms that regulate aggregation and deformability in a latch-like manner. The results of this work could be used as a general explanation of how the erythrocytes regulate their aggregation and deformability, and are essential in understanding erythrocyte disorders and aging.

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1. Introduction

Erythrocytes are the elements of the complicated multifunctional system, a function of which is maintaining the concentration of oxygen and carbon dioxide in tissues in a given range of values in accordance with the needs of metabolism. This is mainly provided by the intensity of ventilation, the regulation of the heart working, pre-capillary sphincters, and sphincters of arterio arteriovenous anastomoses. When autoregulating the brain’s bloodstream, maintaining the concentration is provided by control of the cerebral arterioles (Kamkin and Kamenskiij, 2004). Moreover, it has been shown that the parameters of erythrocytes can change: the ability to form rouleaux — aggregation (AG), the ability to change their shape under action of external forces — deformability (D) (Bishop et al., 2001; Cicco and Pirrelli, 1999; Das et al., 2007; Kim et al., 2005; Meiselman, 2009). This also may affect the gas transmission function.

Due to their simplicity, erythrocytes have been a subject of mathematical models in a number of works on systems biology. Early studies captured only the glycolytic pathway (Rapoport et al., 1976). Then the model was expanded to include the pentose phosphate pathway (Ataullakhanov et al., 1981). The first sophisticated models included the sodium–potassium pump and membrane transport (Brumen and Heinrich, 1984; Joshi and Palsson, 1989). Using the latter study as a basis, and introducing glutathione synthesis and export systems, a simulation analysis of glucose-6-phosphate dehydrogenase (G6PDH) deficiency has also been developed (Nanda et al., 2013). These mathematical models are good examples of attempts to model human erythrocyte metabolism, but do not cover some properties of erythrocytes, such as aggregation and deformability. In the present study, we introduce a novel mathematical model that suggests possible mechanisms of regulation of the aggregation and deformability of erythrocytes.

Based on an analysis of the mathematical model of the SS, we here suggest that the SS of the erythrocyte not only changes stationary levels of AG and D, but that this SS also switches the parameters of the erythrocyte from one steady level to another, in a latch-like
manner. In exchange capillaries there is a high level of D while AG is low; in the other parts of circulatory system there is a low level of D while AG is high. Due to this, there are additional opportunities to optimise the working of the respiratory system (Fig. 1), which allow minimizing the energy spent on moving the blood through the capillaries, minimizing the gas exchange with the walls of blood vessels, and at the same time facilitating the necessary rate of gas exchange in capillaries.

The process of combining erythrocytes into AG is sophisticated and multifactorial and has a significant impact on the main oxygen transport function of the blood (Levtov et al., 1982). AG facilitates the axial drift of the red blood cells and the formation of plasma sheet boundary level (Cokelet and Goldsmith, 1991; Goldsmith et al., 1999). The increased axial accumulation level of red blood cells reduces the local viscosity in the wall zone of the vessel (Alonso et al., 1993; Suzuki et al., 1996) and thereby modulating the activity of the vascular regulatory mechanisms (Baskurt and Meiselman, 2007) and reducing oxygen release to the vessel walls (Tateishi et al., 2001, 2002).

At the level of the exchange capillaries the efficiency of bloodstream depends on D and AG (Bishop et al., 2004, 2001; Das et al., 2007; Kim et al., 2005; Meiselman, 2009; Suzuki et al., 1996), and it increases dramatically for increasing D, and diminishing AG. Fig. 2 shows the functional scheme for the system that controls AG and D of the erythrocytes. The scheme shows that the signals from the receptors come to the SS and are then analysed. As a result the control actions are generated, which can change AG and D by phosphorylation of the membrane proteins and the protein of the cytoskeleton. Moreover, under the influence of signals, both stationary changing of AG and D and their latch-like switching may occur.

1.1. The signal system of erythrocytes

Fig. 3 illustrates the scheme of physic-chemical processes, which take place in the SS of erythrocytes that controls phosphorylation of EF1, EF2. The latter defines the level of AG, D. As mentioned before, the greater the stationary level of phosphorylation of these proteins the less the stationary levels of AG and the higher the level of D (Saldanha et al., 2007). Whether this SS can switch these parameters in latch-like manner is still unknown.

The elements of the SS are physical–chemical processes happening due to the work of certain proteins. Let us consider them in detail.

It has been shown that erythrocytes have membrane proteins AQP1 — aquaporin of type 1 — highly selective water channels (Preston and Agre, 1991). Water, oxygen, and carbon dioxide can move through these channels (Cooper et al., 2002; Endeward et al., 2006; Ivanov et al., 2007; Smith and Agre, 1991), and they are not permeable to charged molecules. When forming the complex of AQP1 with cyclic monophosphates, particularly with cGMP, AQP1 become permeable to cations. Their permeability weakly depends on the membrane potentials (Anthony et al., 2000).

There are several types of calcium channels in the membrane of erythrocytes: potential-dependent $I_{Ca}^{k}$ (Soldati et al., 1997; Bennekou et al., 2003; Kaestner, 2011) and calcium–calmodulin-dependent channels of active transport of calcium $I_{Ca}^{Ca}$ (Fidalgo da Silva et al., 2006; Zidek et al., 1992). There are also mechanoac-
Fig. 3. Schematic diagram for the SS that regulates the level of phosphorylation of the membrane proteins EF1 and the proteins of the cytoskeleton EF2 that regulate AG and D. Ca, Na, Mg, K — ions of calcium, sodium, magnesium, and potassium, respectively. CaM — calmodulin. cAMP — cyclic adenosine monophosphate. cGMP — cyclic guanidine monophosphate. NOS — molecular module that regulates concentration of NO. ACII — molecular module that regulates concentration of cAMP. sGC — molecular module that regulates concentration of cGMP. Phos (EF1, EF2) — molecular module that phosphorylates proteins EF1 and EF2, the level of phosphorylation of the latter defines AG, D.

IA (Ca) — channels of active transport of calcium. Imec (Ca) — mechanoactive cationic channels. IL (Ca) — potential-activated calcium channels. ICaM K — calcium-calmodulin dependent potassium channels. IV K — potential dependent potassium channels. AQP1 — aquaporins.

Changes in the concentration of cyclic monophosphates modulate the activity of cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG). These kinases and phosphatase PP2A define the phosphorylation level of the membrane proteins EF1 and cytoskeleton EF2. Phosphorylation of certain proteins affects the rheological properties of blood due to changes of AG and D (de Oliveira et al., 2008; Saldanha et al., 2007).

Fig. 3 illustrates the scheme for the SS that controls phosphorylation of the membrane proteins and the cytoskeleton of the erythrocyte, which in turn determines AG and D. This scheme is built using the SBGN language (Le Novere et al., 2009) taking into account its rules of representing diagrams. Let us to analyse the work of this signal system.

2. Materials and methods

We built a mathematical model based on Fig. 3 that contains only two ordinary differential equations: one for the rate of calcium concentration change in the cytoplasm of the erythrocyte $dca/dt$ and one for the rate of change of the membrane potential $dv/dt$.

We used steady-state solutions for the other variables (all reactions of complex formation, change of the concentration of NO, cAMP, cGMP) included in our model assuming that their rates of coming to the steady-state exceed the rates of coming to the steady-state for concentration of calcium $ca$ and the membrane potential $v$.

As the initial conditions, in one case, we took the values of variables in the singular points, in the other case, we took the values located on the edges of the right half of the quadrant $0.01 < ca < 1.2$, $-200 mV < v < 50 mV$.

The equations for the scheme shown on Fig. 3 can be written in dimensionless form. The rate of change of calcium concentration and membrane potential of the erythrocyte respectively:

$$
\frac{dca}{dt} = W_0 \left( im_{ca} + i_{AQP1} + i_{ATPase}\right)
$$

$$
\frac{dv}{dt} = W_1 \left( im_{ca} + i_{AQP1} + i_{ATPase} + i_{CaM} + i_{V} + i_{ATPase}'\right),
$$

where $ca = [Ca^{2+}] / h$, $h = 1 \mu M$, $[Ca^{2+}]$ — concentration of calcium, $r = t \cdot r$, $t \cdot r = 10^{-3} s^{-1}$, $i_{ion}$ — ionic currents, $v = V \cdot F \cdot z / (R \cdot K)$, $V$ — membrane potential, $F$ — Faraday constant, $T_K$ — temperature, $R$ — universal gas constant, $z$ — dimensionless charge of ion (please
see the Supplementary Materials for the derivation of the equation and full explanation of the quantities).

The rate of change of EF1 and EF2 phosphorylation levels, which define AG and D can be written as follows:

\[
\frac{da}{dt} = h_2 \left( \frac{pka(ca)}{\lambda_1 + 1 - ap} + h_20 \frac{pkg(ca)}{\lambda_2 + 1 - ap} - \frac{ap}{\lambda_2 + ap} \right)
\]  

(2)

where \( ap \) — phosphorylated proteins EF1, EF2 (please see the Supplementary Materials for the derivation of the equation and full explanation of the quantities).

We use one equation to describe the phosphorylation of the EF1 and EF2 (Goldbeter and Koshland, 1981; Sadreev et al., 2014), since both proteins are phosphorylated by the same PKA, PKG kinases and dephosphorylated by PP2A phosphatase. It can be concluded that AG decreases and D increases with the level of phosphorylation of these proteins.

For simplicity, we assume that the calcium currents, which are due to the irritation of the erythrocyte, are rapidly inactivated. The mechanotransductive and potential-dependent calcium currents \( \tilde{I}_{ca} \) have been presented as rectangular pulses of length \( dT = r(t_1 - t_0) \) and of dimensionless magnitude \( A \), being the change in conductance of the erythrocyte membrane for calcium ions:

\[
\tilde{I}_{ca} = \tilde{I}_{0} = \begin{cases} 
\tilde{I}_{0} & t_0 < t < t_1 \\
0 & \text{otherwise}
\end{cases}
\]  

(3)

where \( \tilde{I}_{0} = v - (1/2) \cdot \ln \left( \frac{[q_{out}]}{[q_{in}]} \right) \) — concentrations of the ion \( q \) inside and outside the erythrocyte, respectively, \( A = \frac{\tilde{I}_{0} \cdot \tilde{I}_{0}}{\tilde{I}_{0} \cdot \tilde{I}_{0}} \) (all details can be found in the Supplementary Materials). This function also includes the constant \( A_{con} = \frac{\tilde{I}_{0} \cdot \tilde{I}_{0}}{\tilde{I}_{0} \cdot \tilde{I}_{0}} \), which denotes the change in conductance for calcium ions.

Our model contains parameters, whose values can be found in Tables 1–4 of the Supplementary Materials. We have found the values of the parameters that make our system work in a latch-like manner with two steady-state calcium concentrations: the first under 0.1 \( \mu \)M, the other under 1 \( \mu \)M. The membrane potential is about –50 mV. The switching from one steady-state to the other and back is induced by similar calcium impulses.

3. Results

3.1. The analysis of the mathematical model

When varying the parameters in a wide range of values, Eq. (1) gives three fundamentally different types of phase portraits:

1. one singular point (stable node) for calcium concentration \( \sim 0.1 \mu\)M (ca \( \sim 0.1 \) (Fig. 4A));
2. three singular points (two stable nodes): the first singular point for calcium concentration \( \sim 0.1 \mu\)M (ca \( \sim 0.1 \)), the second singular point for \( \sim 1 \mu\)M (ca \( \sim 1 \)) (Fig. 4B);
3. one singular point (stable node) for calcium concentration \( \sim 1 \mu\)M (ca \( \sim 1 \)) (Fig. 4C).

We analyze next the work of the SS for the parameters that give three singular points a, b, and c. It can be shown in this case that switching from the a to c and back may occur by rectangular impulse of calcium current of certain magnitude A and duration dT.

Fig. 5 illustrates the family of phase portraits of system (1) for different values of \( A_{con} \). The values of the other parameters are shown in Tables 1, 2, 3, 4 (Supplementary Materials).

It can be seen that for some critical value of parameter \( A_{con} \) the change in the number of singular points occurs. When \( A_{con} \geq 0.011 \), system (1) has only the one singular point c. For lower values of \( A_{con} \) there are three singular points. If initially the system is in a (for \( A_{con} = 0 \)), then for \( A_{con} \geq 0.011 \) the representative point moves to the only one singular point c.

Depending on the impulse duration dT, the behavior of the representative point may be different. Fig. 6 shows phase trajectories of model (1) in response to rectangular impulses of a magnitude A and duration dT.

The switching from a singular point a to c is caused by the rectangular impulse of the magnitude \( A = A_{con} \), at which system (1) has only one singular point c (Fig. 4C) and the duration of the impulse is in a certain range. In this case the representative point, which initially was in a, under the action of impulse of magnitude A, starts to move to a singular point c. (This phase trajectory is marked by pink colour). Then, depending on the impulse duration, the representative point may come back to the singular point a (Figs. 6A and 7) or switch to a singular point c (Figs. 6A and 7A, \( A = 0.011 \), dT \( \geq 9 \)), or pass the singular point c and return to a singular point a (Figs. 6A and 7A, \( A = 0.06 \), dT \( \geq 55 \)).

The switching from a singular point c to a is caused by the rectangular impulse of the magnitude \( A = A_{con} \), at which the representative point of system (1) by the end of action of the impulse, is in the phase trajectory (Figs. 6B and 7B, \( A = 0.1 \), dT \( \geq 10 \)), which moves to a singular point a.
Fig. 5. (A) Zero-isoclines of the system (1) for different values of the parameter $A_{con}$. $V$ — the membrane potential, $Ca$ — calcium concentration in erythrocyte. (B) Stationary concentrations of calcium $Ca$ (a, b, c) as a function of $A_{con}$, stationary membrane permeability for calcium. The values of other parameters are presented in Tables S1, S2, S3, S4 (Supplementary Materials).

Fig. 6. The phase trajectories of the system (1) in response to rectangular impulse of calcium current of magnitude $A$ and duration $dT$. (A) Initial values in state a. Phase trajectories in response to rectangular impulse of magnitude $A = 0.011$, and durations $dT = 8, 9$; magnitude $A = 0.06$, and durations $dT = 30, 55$. The green line represents phase trajectory for $A = 0, A_{con} = 0.06$ that passes through a and singular point $c_1$. (B) Initial values in point c. Phase trajectories in response to stimulation by rectangular impulse of magnitude $A = 0.1$ and durations $dT = 3, 5, 9, 10$. The green line represents phase trajectory for $A = 0, A_{con} = 0.1$ that passes through c and singular point $c_1$. $V$ — the membrane potential, $Ca$ — calcium concentration in erythrocyte. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In response to the rectangular impulse of certain magnitude $A$ and duration $dT$, our model switches from a steady-state a to a steady-state c and vice versa. Fig. 8 illustrates the threshold characteristics of our system. If the parameters of the impulse are in the red field, then the erythrocyte switches from a singular point a to c, and from c to a.

Fig. 7. The concentration of calcium ($Ca$) as a function of time when the cell is stimulated by the rectangular impulse of magnitude $A$ and duration $dT$. The impulse is turned on when $t_0 = 0$. The duration of the impulse is denoted by dash lines. (A) Initial values in singular point a. The cell undergoes impulses of magnitude $A = 0.011$, and durations $dT = 8, 9$; magnitude $A = 0.06$, and durations $dT = 30, 55$. (B) Initial values in singular point c. The cell undergoes impulses of magnitude $A = 0.1$ and durations $dT = 3, 5, 9, 10$. 

Fig. 9 illustrates calcium concentration in the erythrocyte as a function of time when stimulating the cell by the impulses, the
parameters of which are in different fields. There are three blue points A, B, C belonging to a certain field (Fig. 8).

So what determines the amplitude and duration of calcium impulses, which are run by mechanoreceptors? On the one hand the speed of erythrocytes movement when entering the pre-capillary area does it. This speed determines time, during which the erythrocyte is being deformed (impulse duration). On the other hand the diameter of pre-capillaries, which determines how much erythrocytes are deformed (magnitude of the impulse). If we take into account potential-dependent channels then the dependence of parameters of calcium impulse will also be determined by the characteristics of the channels.

We have found the parameters (Table S3 in Supplementary Materials), at which system (1) has three singular points a, b, c, located on the axis of potential in the area −50 mV, on the axis of calcium concentration a (Ca=0.1 μM), c (Ca=1 μM). Moreover, we have found the parameters, at which the system switches from a to c and vice versa in a switch-like manner (the threshold characteristics for a and c are almost equal). From our point of view these parameter values of the SS of erythrocyte may be determined as one of the normal variants.

However, it is worth to note that all the parameters of real erythrocytes (channel density, total concentration of ferments etc.) are continuously changed.

Erythrocyte does not have nucleus, endoplasmic reticulum and therefore system of protein regeneration. Different proteins have different t_{1/2}: from several hours to several months. Thus, real erythrocytes have a discrepancy of all parameters, which lead to different steady-state calcium concentrations and thresholds. How does this affect their dynamic behavior depends on parameters of the discrepancy.

Under the action of various pathologies, aging and physiologically active compounds the changes in the parameters of the SS take place. The situations are possible where there is only one singular point — either a or c. In this case, the system responds only by pulse change of its characteristics in response to calcium pulse stimulation.

A special case of pathology is such changes of the parameters of our system, in which there are three singular points, but at the same time there are great differences between thresholds of switching from a to c and from c to a, which may lead to high discrepancy of steady states of erythrocytes under various conditions (for various speeds of bloodstream).
3.2. Protein phosphorylation

PKA and PKG kinases phosphorylate EF1 and EF2 proteins, which are responsible for the AG and D, while PP2A dephosphorylates them. Fig. 10 illustrates the level of non-phosphorylated proteins are responsible for the AG and D, while PP2A dephosphorylates for the following parameters $\mu_{\text{AMP}}=1.45, 2, 3$. It can be seen from the figures that $\mu_{\text{AMP}}$ decreases when the activity of ACII increases. At the same time, the aggregation decreases and the stationary calcium concentration increases.

4. Discussion and conclusion

In this work, we propose a mathematical model of the signal system that regulates properties of the erythrocyte such as aggregation and deformability. The proposed model is consistent with experimental studies of erythrocytes. The results we obtain show that the parameters of erythrocyte aggregation and deformability can switch in a latch-like manner.

The presence of molecules (Fig. 3) and links between them, which are included into the SS of erythrocyte, have been shown experimentally. In addition to these molecules, which could be included into the SS, there are others that we have not yet considered. However, not all of the properties of these molecules are revealed in experiments. Total protein concentrations, specific gravities of channels and their characteristics, enzymatic reactions rate constants, Michaelis constants, levels of calcium-calmodulin independent activities of enzymes and ion channels have been evaluated theoretically. Our theoretical evaluations are within the physiological range. The model predicts that the erythrocyte is able to switch in a latch-like manner not only for the used set of parameters. Many parameters are interrelated, when a change in one may be compensated by changes in another. Finding the limits of how the parameters may change with regard to their mutual influence, in which erythrocytes are normally functioning, requires a new study.

Beside the fact that there are many proteins forming the SS in erythrocyte, which can work in a latch-like manner, there are some other facts that support the existence of the proposed SS. Erythrocytes have mechanoreceptors that are able to increase the permeability for calcium and other cations under mechanistic stimulation. In the work (Brain et al., 2004) it was shown that initially the concentration of calcium in all erythrocytes is approximately 0.1 $\mu$M. When the erythrocyte is moving through the capillary, the diameter of which is 3–5 $\mu$M (less than the diameter of erythrocyte), 30% of erythrocytes have calcium concentration approximately 1 $\mu$M while the others 0.1 $\mu$M. Our mathematical model suggests that dysfunctions in the proposed SS may lead to this effect. It is currently not clear how to explain the increase of calcium concentration in order in only 30% of erythrocytes.

If we assume that the SS (Fig. 3) exists, then this result can be interpreted in such a way that, upon entry into a capillary, the concentration of calcium in many of the erythrocytes will increase up to 1 $\mu$M; however, on leaving the capillary and undergoing repeated mechanical stimulation, the calcium level returns to its initial value in only 70% of erythrocytes in the blood sample (Brain et al., 2004).

In other words, 70% of erythrocytes have such values of parameters and the rate of moving through the capillary that they move from the steady-state a to c at the first mechanical stimulation, and return back at the second stimulation (Fig. 9). It should be noted here that 30% of erythrocytes after moving to steady-state c under the action of the second pulse remain in c. This fact is considered as an indirect evidence of the existence of the SS discussed in this study.

It was shown that the aging of erythrocytes is accompanied by the increasing of their calcium concentration (Aiken et al., 1992). For young erythrocytes it is in average 0.062 $\mu$M, while for the olds it is approximately 0.2 $\mu$M, but this all is for the field of the steady-state singular point a (Fig. 4B). Therefore it can be assumed that during the aging of the erythrocyte the system of calcium homeostasis deteriorates.

It has been shown that both the condition of certain membrane proteins and the concentration of certain plasma proteins covering the cell surface affect the aggregation of erythrocytes.

We assume here that the spread of erythrocytes over the states with different levels of phosphorylation of protein EFx (in singular points a and c) also affects the aggregation. The erythrocytes that are in c facilitate stopping of growth of the rouleaux since there is a low aggregation in this state. The more erythrocytes are in state c, where they are moving through large capillaries, the less is the average length of the rouleaux. When all the erythrocytes are in c both the rouleaux and erythrocyte sedimentation rate are the smallest.

This study suggests new mechanisms for the regulation of aggregation and deformability of the erythrocyte. The analysis of the proposed model demonstrates the conditions and parameters values under which the erythrocyte switches its aggregation and deformability from one steady state to another, in a latch-like manner with approximately the same thresholds. The proposed mathematical model might be useful in understanding the mechanisms of erythrocyte diseases and aging.

Acknowledgements

This work was supported by Russian Foundation for Basic Research. We are grateful to Dr. Najl Valeyev for many helpful discussions. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biosystems.2015.03.003.

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