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BIOLOGICAL SCIENCES

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ACTION OF STILBENDISULFONATE ON RED BLOOD CELL SHAPE IN SULPHATE MEDIUM

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Abstract: An initial decrease in the optical density (OD) of a suspension of red blood cells (RBCs, erythrocytes) in a sulfate medium for ~30 sec was shown, followed by a stable level of OD for ~5 min, which indicates the formation of spherical cell shapes. The presence of an anionic channel inhibitor DIDS (1-2 μmol/l) in the medium initiates a reversible retransformation of RBCs from spherical to discoid forms within 2-3 minutes and back to spherical – up to 5 minutes. At a concentration of DIDS of 5-10 μmol/l, a phase of development of cell discoidity up to 2-3 minutes is also noted, which remains stable up to ~5 minutes of the experiment, while the return phase of development of cell spheroidity is not detected. At a concentration of DIDS of 20 μmol/l, no pronounced phase of development of discoid cells was detected, which manifested itself only after the 4th minute of the experimental track of OD. The results obtained suggest that the reversible retransformation of RBCs at a DIDS concentration of 1-2 μmol/l is determined by the binding of the inhibitor in the anion channel and the modification of the electrostatic charge of the channel entrance and sections of its interfacial region upon initiation of the cell suspension mixing flow. At a DIDS concentration of 5-20 μmol/l, this charged agent begins to be adsorbed on other sites of the membrane and modifies its

electrostatics, which causes a violation of the reversible retransformation of RBCs, noted at 1-2 μmol/l DIDS.

Keywords: red blood cells, cell shape, DIDS, sulfate.

A representative of stilbendisulfonates, DIDS, is an inhibitor of the anion channel and transporter (AE1) RBCs [1, p. 1507] and, as is commonly believed, an echinocytogenic agent [2, p. 91]. At the same time, RBCs irreversibly labeled with DIDS in the anion channel are represented not only by echinocytes, but also partially by discocytes [3, p. 46968], and can also be spheroid forms, which, when stirred in saline, are retransformed into discoid forms and back into spheroids [4, c. 135]. The diversity of RBCs shapes in the flow may be determined by the fact that DIDS can be a modifier of the electrostatic structure of the interfacial regions of the membranes adjacent to AE1. Binding of DIDS in the channel neutralizes the positively charged amino acid residue Lys851 at the channel entrance and introduces two of its SO_3^- - groups, one of which neutralizes Arg730, and the second is localized at the channel exit [1, p. 1507].

In one of the hypotheses, echinocytosis of RBCs develops due to the expansion of the outer monolayer of the lipid bilayer of the membrane due to increased repulsion between the negatively charged heads of phospholipids [5, p. 629]. In this case, disruption of the electrostatic structure of membrane regions adjacent to AE1 upon binding of DIDS can lead to a change in the structure of these regions and modification of the RBCs shape. According to the position on the control of local potential by halides in the interfacial region of AE1 and the lipid bilayer, a decrease in the concentration of chloride in the medium can cause an increase in the charge in this region, an increase in sulfate adsorption, and a change in the electrostatic structure of the membrane [6, p. 99]. It is possible that the modification of the effect of DIDS on the RBCs shape in a sulfate medium can help reveal the mechanism of action of this charged agent and bring closer understanding of the role of changes in the electrostatic structure of the membrane in the mechanism of regulation of the RBCs shape.

Objective. To investigate the effect of different concentrations of stilbendisulfonate (DIDS) on the RBCs shape in a sulfate medium.

Materials and methods. We used: 4,4'-diisothiocyanato-stilbene-2,2'-disulphonic acid (DIDS, Sigma, USA); Tris, NaCl, Na_2SO_4 (chem. pure). RBCs were obtained from donated blood, as described in [4, c. 135]. Modification RBCs shape was studied by registering changes in the intensity of optical density (OD) fluctuations of a stirred cell suspension (~$3{,}0\times10^6$ cells/ml) in a spectrophotometer (SP) cuvette at a temperature of 22-24°C. Discoid cells scatter light anisotropically and produce the maximum OD fluctuation, spherical cells exhibit isotropic light scattering – no OD fluctuations are detected. Therefore, the intensity of OD fluctuations is determined by the ratio of discoid and spherical cell shapes [7, p. 1385].

Results and discussion. When RBCs are included in the sulfate medium, an initial decrease in the OD of the cell suspension is noted for ~30 sec, followed by a stable level of OD for ~5 min (Fig., track 1), which indicates the formation of spherical cell shapes. It should be noted that in a sulfate medium at pH 5.6, erythrocytes become echinocytes and retransform in the direction of echinocytes → discocytes → stomatocytes [8, p. 247]. As the authors of this work note, the change in the shape of erythrocytes is associated with Cl^-/SO_4^{2-}-exchange and redistribution of transport sites of the carrier. Probably, at pH 7.4, this redistribution of sites does not contribute to retransformation, since the development of OD-fluctuations is not observed until the 5th minute (Fig., track 1). At the same time, the presence of DIDS (1-2 μmol/l) in the medium leads to reversible cell retransformation. There is a decrease in OD with a parallel increase in OD-fluctuations for 2-3 min, followed by an increase in OD and a decrease in the intensity of OD fluctuations to ~5 min (Fig., tracks 2, 3). It should be noted that the intensity of OD fluctuations for 2-3 min is comparable to that observed when the albumin (stabilizer of discoid form RBCs) acts on erythrocytes in saline [9, p. 565]. This indicates the retransformation of a significant part of the cells into discoid forms under the action of DIDS (Fig., tracks 2, 3). With an increase in the concentration of DIDS to 5-10 μmol/l, the development

OD fluctuations of the cell suspension is also noted, but its reversibility is no longer observed (Fig., tracks 4, 5). At a concentration of this agent of 20 μmol/l, the onset of OD fluctuations and, accordingly, an increase in the proportion of discoid cells is detected only for ~4 min (Fig., track 6). The initial decrease in the OD of the RBCs suspension both in the absence and in the presence of the anion channel inhibitor DIDS (Fig., tracks 1-6) indicates that this decrease in OD is not associated with the functioning of Cl^-/SO_4^{2-}- exchange, but, probably, is determined by the modification of the electrostatic structure of the membrane and the change in the shape of the cells when transferred to a sulfate medium.

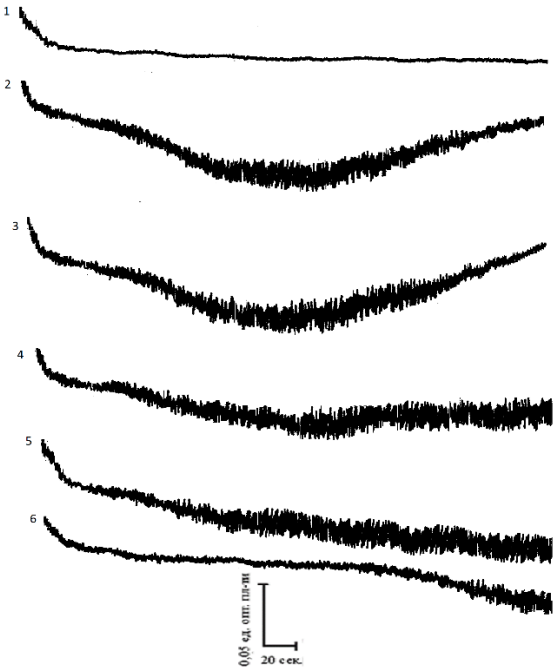

Fig. Change in the intensity of OD fluctuations in RBCs suspension (~$3{,}0\times10^6$ cells/ml)) with stirring in a spectrophotometer cuvette in a medium containing 110 mmol/l Na_2SO_4 and 10 mmol/l tris (pH 7.4). DIDS (μmol/l) was added to the medium before cell introduction: 1 – control; 2 – 1; 3 - 2; 4 - 5; 5 - 10; 6 - 20.

According to the literature, the change in RBCs shape can occur under the control of the distribution of AE1 transport sites on both sides of the membrane. In one of the hypotheses, the displacement of transport sites to the outer side of the membrane leads to cell echinocytosis [8, p. 247], while according to another hypothesis, to stomatocytosis [10, p. 343]. At the same time, there are literature data

indicating that a change in the shape and deformability of erythrocytes occurs when the conformation of the carrier is fixed with the transport site facing the outer side of the membrane with the help of an inhibitor of the anionic carrier DIDS. It is generally accepted that DIDS is an echinocytogenic agent [2, p. 91], however, DIDS-labeled erythrocytes can be represented not only by echinocytes, but also partially by discocytes [3, p, 46968], as well as spheroids, which are retransformed into discoids in saline in a flow with stirring and back to spheroids [4, c. 135]. In parallel with the echinocytogenic effect, DIDS improves the deformability of erythrocytes [2, p. 91] and membranes [3, p. 46968]. In addition, when studying the retransformation of erythrocytes in a sucrose medium in the direction of echinocytes → discocytes → stomatocytes, the inclusion of DIDS in the cell suspension at the final stage of stomatocytosis leads to the formation of discoid cell forms [10, c. 53]. The above literature data raises the question of the mechanism of regulation of RBCs shape under conditions of fixation of the conformation of the main transmembrane protein AE1.

The echinocytogenic property of DIDS when binding in the channel can be substantiated by the displacement of the carrier transport sites AE1 to the outer side of the membrane and the corresponding hypothesis [8, p. 247]. At the same time, it is not easy to explain the improvement in the deformability of erythrocyte membranes upon fixation of the DIDS conformation of the main transmembrane protein AE1. However, it can be assumed that a change in the electrostatic structure of the cell membrane at a low ionic strength of the medium or the impact of a flow can cause a change in the modifying effect of DIDS on the shape of erythrocytes. Binding of DIDS in the channel neutralizes the positively charged amino acid residue Lys851 at the entrance and introduces two of its SO_3^--groups into the channel, one of which neutralizes Arg730, and the second is localized at the exit of the channel [1, p. 1507]. DIDS has a high affinity for the anion channel; at the same time, it should be emphasized that this inhibitor, when bound in the channel, partially overlaps the transport site with a decrease in affinity for Cl^-- anions, but without disturbing their

binding [12, p. 7895]. A similar experimental situation can be observed for SO_4^{2-}-anions upon inhibition of DIDS Cl^-/SO_4^{2-}- exchange [13, p. 61].

The complete replacement of chloride in the medium with doubly charged sulfate will not only cause a change in the electrostatics of the membrane surface [6, p. 99], but will probably lead to a change in the dynamics of anions and charged amino acid residues in the incoming cavity of the channel [14, p. 100724]. This modification, upon binding of charged DIDS molecules and the action of a cell suspension agitation flow, may cause a change in the charge of the channel entry and the electrostatics of local sections of the membranes adjacent to AE1. This can affect the structure of the lipid bilayer, the shape of erythrocytes and, possibly, lead to reversible retransformation of cells due to reversible changes in the structure of these sections of the membrane. The reversible retransformation of erythrocytes observed experimentally in a sulfate medium (Fig., tracks 2, 3) is similar to that found for DIDS-labeled erythrocytes in saline [4, c. 135]. In the latter case, the treatment of cells with DIDS was carried out for 60 min without constant stirring of the incubation medium. This indicates that the binding of DIDS in the channel to the transport site predetermines the mechanism of reversible retransformation, and subsequent exposure to the flow with mixing in the experiment triggers this retransformation.

Comparison of the effects of DIDS and DNDS (4,4'-dinitrostilbene-2,2'-disulfonate) on the RBCs retransformation in sucrose medium in the direction of echinocytes → discocytes → stomatocytes can help reveal the details of the mechanism of this retransformation. The addition of DIDS at the final stage of stomatocytosis causes retransformation of cells into flattened discoid forms, while DNDS in this case does not show any effect [11, c. 53]. The absence of a modifying effect of DNDS can be explained by the fact that this inhibitor is a direct blocker of the transport site, which completely inhibits the binding of Cl^- -anions compared to DIDS [12, p. 7895]. In addition, the DNDS molecule does not contain isothiocyanate groups, as DIDS does, and therefore is not capable of neutralizing the amino groups of lysine residues. It follows from this that, in a sucrose medium, the neutralization of the Lys581 residue at the entrance to the channel during the binding of DIDS may be

a necessary condition for the retransformation of stomatocytes into discoid forms [11, c. 53]. Additional studies are needed to clarify whether DNDS in a sulfate medium can initiate reversible erythrocyte retransformation, like DIDS in our experiments. It can be assumed that the neutralization of Lys581 at the entrance to the channel during the binding of DIDS may be one of the necessary conditions for programming the reversible retransformation of erythrocytes in our experiments. At the same time, the change in the dynamics of SO_4^{2-} anion binding at the transport site in the presence of DIDS can also be the reason for the modification of the charge of the channel entry and AE1 interfacial regions under the influence of the cell suspension mixing flow.

Conclusions.

1. It has been established that the presence of DIDS in the medium at a concentration of 1-2 μmol/l initiates a reversible RBCs retransformation from spherical to discoid forms up to 2-3 min and back to spherical forms up to ~5 min.

2. At a concentration of DIDS of 5-10 μmol/l, a phase of cell discoid development up to 2-3 min is also noted, which remains stable up to ~5 min of the experiment, while the return phase of cell spheroid development is not detected.

3. It was shown that at a concentration of DIDS of 20 μmol/l, a pronounced phase of the development of cell discoidity appears only after the 4th minute of the experiment.

4. The results obtained suggest that the reversible RBCs retransformation at a DIDS concentration of 1-2 μmol/l is determined by the binding of DIDS in the anion channel and the modification of the charge of the channel inlet and sections of its interfacial region by means of the mixing flow. At a concentration of DIDS of 5-20 μmol/l, this charged agent begins to adsorb on other sites of the membrane and modify the surface electrostatics, which causes a violation of the reversible retransformation of erythrocytes, noted at a concentration of DIDS of 1-2 μmol/l. The results indicate that under physiological conditions of blood flow, the mechanism of regulation of the RBCs shape may include the modification of membrane electrostatics.

References:

1. Reithmeier R.A., Casey J.R., Kalli A.C., Sansom M.S., Alguel Y., Iwata S. 2016. Band 3, the human red cell chloride/bicarbonate anion exchanger (AE1, SLC4A1), in a structural context. Biochim Biophys Acta. V. 1858. (7 Pt A). P. 1507. doi: 10.1016/j.bbamem.2016.03.030.

2. Hoefner D.M., Blank M.E., Davis B.M., Diedrich D.F. 1994. Band 3 antagonists, p-azidobenzylphlorizin and DIDS, mediate erythrocyte shape and flexibility changes as characterized by digital image morphometry and microfiltration. J Membr Biol. V. 141. № 1. P. 91. doi: 10.1007/BF00232877.

3. Van Dort H.M., Knowless D.W., Chasis J.A., Lee G., Mohandas N., Low P.S. 2001. Analysis of integral membrane protein contributions to the deformability and stability of the human erythrocyte membrane. J. Biol. Chem. V. 276. № 50. P. 46968.

4. Рамазанов В.В., Шапкина О.А., Бондаренко В.А. 2010. Изменение формы эритроцитов под действием ингибитора анионного канала. Вісник проблем біології і медицини. № 4. С. 135.

5. Tamura A., Fujii T. 1981. Roles of charged groups on the surface of membrane lipid bilayer of human erythrocytes in induction of shape change. J Biochem (Tokyo). V. 90. № 3. P. 629.

6. Schnell K.F. 1977. Anion transport across the red blood cell membrane mediated by dielectric pores. J. Membrane Biol. V. 37. P. 99.

7. Rudenko S.V., Crowe J.H., Tablin F. 1998. Determination of time-dependent shape changes in red blood cells. Biochemistry (Mosc). V. 63. № 12. P.1385.

8. Gimsa J., Ried C. 1995. Do band 3 protein conformational changes mediate shape changes of human erythrocytes? Mol Membr Biol. V. 12. № 3. P. 247. doi: 10.3109/09687689509072424.

9. Hoffman J.F. 1987. On the mechanism and measurement of shape transformations of constant volume of human red blood cells. Blood Cells. V. 12. № 3. P. 565.

10. Wong P. 1999. A basis of echinocytosis and stomatocytosis in the disc-sphere transformations of the erythrocyte. J Theor Biol. V. 196. № 3. P. 343. doi: 10.1006/jtbi.1998.0845.

11. Руденко С.В., Румиех М.Х., Бондаренко В.А. 2007. Морфологическая реакция эритроцитов на изменение электролитного состава среды. Влияние ингибиторов анионного транспорта. Біофізичний Вісник. В. 18 (1). С. 53.

12. Falke J.J., Chan S.I. 1986. Molecular mechanisms of band 3 inhibitors. 2. Channel blockers. Biochemistry. V. 25. № 24. P. 7895. doi: 10.1021/bi00372a016.

13. Passow H. 1986. Molecular aspects of band 3 protein-mediated anion transport across the red blood cell membrane. Rev Physiol Biochem Pharmacol. V. 103. P. 61. doi: 10.1007/3540153330_2.

14. Zhekova H.R., Pushkin A., Kayık G., Kao L., Azimov R., Abuladze N., Kurtz D. 2021. Identification of multiple substrate binding sites in SLC4 transporters in the outward-facing conformation: Insights into the transport mechanism. J Biol Chem. V. 296. 100724 (Published online). doi: 10.1016/j.jbc.2021.100724.