Bioelectrochemistry 73 (2008) 117–122

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Effects of charged amphiphiles in depolarising solutions on potassium efflux and the osmotic fragility of human erythrocytes

Anna Wróbel *

Institute of Physics, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

ARTICLE INFO

Article history:
Received 1 June 2007
Received in revised form 18 March 2008
Accepted 4 April 2008
Available online 11 April 2008

Keywords:
Human erythrocyte
Low ionic strength
Charged amphiphile
Potassium efflux
Osmotic fragility

ABSTRACT

The effect of the presence of charged amphiphiles during the incubation of human erythrocytes in a sucrose-substituted low-Cl^- solution on the shift of the osmotic resistance profile and the net K^+ efflux was investigated. Osmotic fragility was determined by fitting the complementary error function to the haemolysis resistance curve. K^+ efflux was calculated from the increase in the K^+ concentration in supernatant measured by inductively coupled plasma atomic emission spectrometry (ICP-AES). The cationic amphiphile hexadecyltrimethylammonium bromide (CTAB) at 14 μM decreases, whereas the anionic amphiphile sodium dodecyl sulfate (SDS) at 50 μM increases the shift of the haemolysis resistance curve of erythrocytes incubated in isotonic sucrose by 0.069 and 0.079 %NaCl, respectively. Both the positively and the negatively charged amphiphile caused a significant change in the K^+ efflux into isotonic sucrose solution: CTAB decreased and SDS increased K^+ efflux by about 40%. In view of the lack of effect of the investigated compounds on the haemolysis resistance curve and K^+ efflux from human erythrocytes incubated in isotonic NaCl solution, these results suggest that the insertion of charged amphiphiles into the erythrocyte membrane modulates the properties of the K^+ transport pathway which is activated under low ionic strength (LIS) conditions.

1. Introduction

It has long been known that suspending human erythrocytes in a sucrose-substituted low-Cl^- solution leads to a significant increase in net K^+ efflux. This phenomenon, originally observed by Davson [1], was later studied by a number of authors [2–5]. Because the replacement of NaCl by sucrose results in both a change in ionic strength and a change in transmembrane potential (in physiological solution this being the Nernst potential for chloride), attempts have been made to explain the increased K^+ efflux with regard to both these factors. Many responsible pathways for this transport have been proposed [2,6–8]. Based on literature published in recent years, two seem to be involved in the increased K^+ efflux from human erythrocytes in solutions of low ionic strength (LIS): the voltage-dependent non-selective cation (NSVDC) channel [4,5,7,9–12] and the $K^+(Na^+)/H^+$ exchanger [3,8,13,14]. The electroneutral $K^+(Na^+)/H^+$ exchanger operates independently of membrane potential and is activated by the reduction of ionic strength of the extracellular medium and subsequent increase in the cation concentration near the ion-binding site of the carrier [3,8,13]. The NSVDC channel is activated by a positive membrane potential, as has been demonstrated by the patch clamp technique [12,15], but the molecular identity of this channel has not been identified. The importance of the parts played by these two mechanisms, described as causing the observed increase in K^+ efflux in an LIS solution, is still under discussion [12,13] and the possibility that both pathways are based on the same transporter has been taken into account. However, the recently published results reported differential effect of HOE642 on $K^+(Na^+)/H^+$ exchanger and NSVDC channel provide evidence that at least two independent ion transport pathways are involved in this phenomenon [16]. Significant enhancement of K^+ efflux from erythrocytes suspended in sucrose-substituted low-Cl^- solution leads to cellular dehydration and shrinkage, causing a time-dependent shift of the haemolysis resistance curve to lower osmolarities [5]. This shift of the haemolysis curve can be reduced by the chloride conductance blocker NS 1652 and completely abolished by preincubating the erythrocytes with *N*-ethyl-maleimide (NEM) [5]. Because the pathway for K^+ efflux is activated by decreasing the concentration of Cl^- in the extracellular medium, there is a possibility that it might be activated when erythrocytes are incubated in lytic hypotonic solutions, and this could be an important factor limiting hypotonic haemolysis [6]. Charged amphiphiles at sublytic concentrations are known to be potent inhibitors of hypotonic haemolysis [17]. On the other hand, these compounds have been shown to affect erythrocyte membrane permeability [17] and to modulate voltage-dependent cation pathways in other cells [18,19]. In view of these facts, the aim of this study was to determine whether charged amphiphiles are capable of modulating the shift of the haemolysis curve caused by the incubation of erythrocytes in a sucrose-substituted low-Cl^- solution and if this effect is related to the change in properties of the K^+ transport pathway activated under low ionic strength conditions.

* Tel.: +48 71 3203235; fax: +48 71 3283696.
E-mail address: anna.wrobel@pwr.wroc.pl.

1567-5394/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2008.04.001

2. Materials and methods

2.1. Erythrocytes and solutions

Blood from 22 healthy donors was drawn into vacuum tubes containing EDTA as anticoagulant and was kept at 4 °C until use. All experiments were performed within 24 h after taking the blood. Erythrocytes were separated by centrifugation (2000 ×g, 5 min, 4 °C). Plasma and buffy coat were aspirated and the cells were washed three times in unbuffered isotonic NaCl solution (154 mM NaCl, 2 mM KCl). After washing, the final haematocrit of the cell suspension was in the range 60 to 93%. Before measuring osmotic fragility and K^+ efflux (see below), the erythrocytes were resuspended in isotonic NaCl or isotonic sucrose (264 mM sucrose, 2 mM KCl) solutions to a final haematocrit of 9% and incubated at 37 °C (15, 30, and 60 min) in the presence or absence of the investigated amphiphilic compounds at sublytic concentrations known to cause maximal antihaemolytic effect (14 μM CTAB and 50 μM SDS). To check the effect of the size of hydrophobic moiety the erythrocytes from three donors were incubated in the presence of alkyltrimethylammonium bromides (14 μM) of varying alkyl chain length (12, 14 and 16 carbon atoms). To check the effect of other depolarising solutions on K^+ efflux, erythrocytes from two donors were incubated in isotonic sorbitol (259 mM sorbitol, 3 mM KCl), isotonic mannitol (250 mM mannitol, 3 mM KCl), and isotonic Na-gluconate (150 mM Na-gluconate, 3 mM KCl).

2.2. Measurement of the osmotic fragility

After incubation the erythrocytes were withdrawn, mixed gently, and 30 μl of the cell suspension was transferred to 1.5-ml Eppendorf tubes containing 1.2 ml of NaCl solutions varying in concentration from 0 to 0.9% (w/v). Following 30 min of incubation at room temperature, the samples were centrifuged (2000×g, 3 min) and the absorbance of the supernatant was measured at λ=540 nm. Osmotic fragility was determined numerically by fitting the complementary error function to the experimental haemolysis resistance curve (for details, see the section "Numerical calculations and statistical analysis").

2.3. Measurement of net K^+ efflux

Following incubation of the erythrocytes in the isotonic solutions (containing 3 mM KCl), the erythrocytes were withdrawn, centrifuged (2000×g, 2 min), and 300 μl of supernatant were mixed with 2700 μl of distilled water and kept at −20 °C until measurement. The net K^+ efflux was calculated from the increase in the K^+ concentration in the supernatant measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) at λ=766.46 nm, with calibration by a K^+ standard in the range of 1–20 ppm.

2.4. Reagents

Inorganic salts, sucrose, sorbitol, and mannitol were of analytical grade. Na-gluconate, hexadecyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), dodecyltrimethylammonium bromide, and tetradecyltrimethylammonium bromide were obtained from SIGMA.

2.5. Numerical calculations and statistical analysis

Osmotic fragility was determined according to Orcutt [20] by fitting the dependence of the fraction haemolysed (FH) vs. the NaCl concentration with the equation:

$$\mathrm{FH} = p_3 \,\mathrm{erfc}\left(\frac{[\mathrm{NaCl}] - p_1}{p_2}\right) \quad (1)$$

where erfc is complementary error function, p_1 is the NaCl concentration (in %w/v) corresponding to 50% haemolysis (osmotic fragility), p_2 is the haemolytic dispersion (the range of NaCl concentrations over which haemolysis occurs around the p_1 point) and p_3 is the half value of the fraction haemolysed at total haemolysis (p_3 equals to 0.5 by definition). Eq. (1) was fitted to the data using the method of nonlinear least-squares minimization. The standard deviation of the fit (STD_{fit}) of Eq. (1) to the haemolysis curve data was calculated using the sum of the squares of the residuals from the nonlinear least least-squares minimization.

The final results are presented as the mean±SE of n separate experiments, each carried out on blood of different donor. Differences between means were evaluated by Student's t-test. The values were taken as significantly different when $p<0.05$. Mean values of p_1 were calculated from the group of samples incubated in isotonic sucrose with the chloride concentration in the range of 5±1 mM. The parameter Δp_1 was used to show the difference between p_1 of the control sample (incubated in isotonic NaCl) and the sample incubated in isotonic sucrose, whereas $\Delta p_{1\mathrm{amph}}$ was used to show the difference between p_1 of samples incubated in isotonic sucrose in the absence and in the presence of the investigated amphiphilic compounds.

3. Results

Incubation of human erythrocytes in sucrose-substituted low-Cl^- solution leads to a shift of the haemolysis resistance profile to lower osmolarities caused by cellular dehydration [5]. This effect was checked in erythrocytes from different blood donors and it has been found that the magnitude of the shift in the haemolysis curve in isotonic sucrose is donor dependent (Fig. 1).

A comparison of the haemolysis curves for erythrocytes from two different donors (incubated in isotonic sucrose containing 5 mM of Cl^-) clearly indicates a difference in response of erythrocytes to the LIS condition. Because the concentration of Cl^- during incubation varied slightly from sample to sample (depending on the haematocrit of the cell suspension after washing), the change in osmotic fragility caused by the incubation of the erythrocytes in isotonic sucrose as a function of Cl^- concentration is shown in Fig. 2 for all investigated erythrocyte samples. Each point in Fig. 2 represents Δp_1 calculated for an individual blood donor. There is a significant negative correlation ($R^2=0.58$) between Δp_1 and Cl^- concentration in the whole investigated population, as should be expected knowing the inhibitory potency of Cl^- ions on net K^+ efflux [1,2,3], but analysis of Δp_1 for samples incubated under exactly the same conditions (e.g. with Cl^- equal to 5 mM) demonstrates that the shift in the haemolysis curve may differ even by

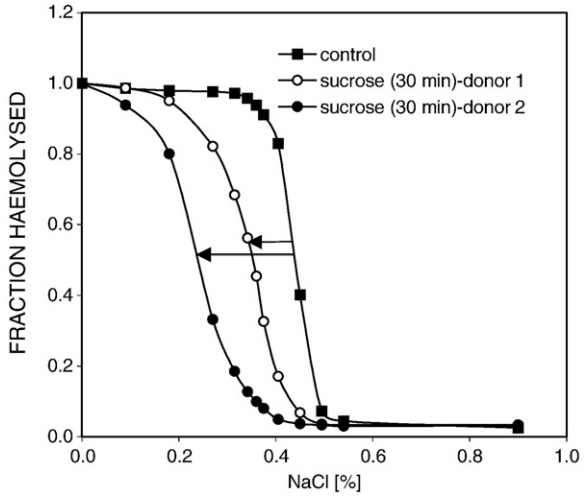

Fig. 1. The effect of incubating human erythrocytes in isotonic sucrose (30 min) on the normalised haemolysis curve (lines: guides for the eye). The arrows show the shift of osmotic resistance profile for two different blood donors.

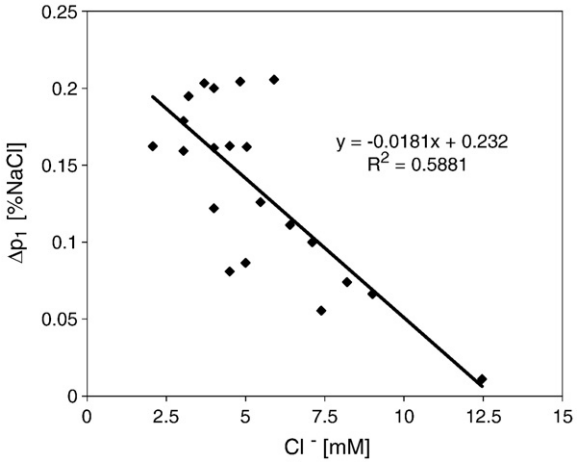

Fig. 2. The change in osmotic fragility of human erythrocytes incubated in isotonic sucrose solution as a function of extracellular chloride concentration. Each point in the figure represents Δp_1 calculated for an individual blood donor.

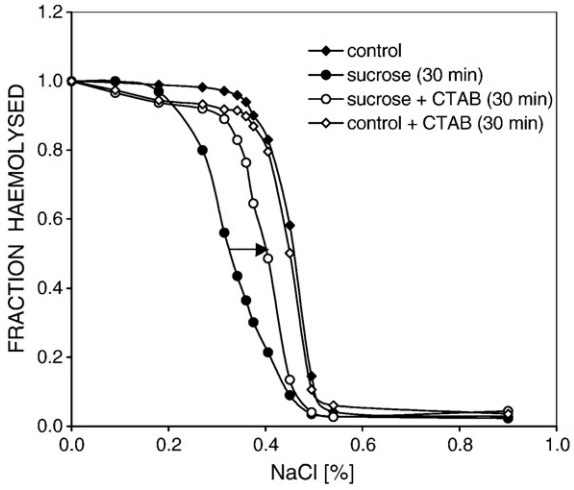

Fig. 3. The effect of CTAB (14 μM) on the shift of osmotic resistance profile of human erythrocytes incubated in isotonic sucrose.

a factor of 2.5 from donor to donor. The statistical comparison of the parameters p_1 and p_2 derived from the fit of Eq. (1) to the experimental haemolysis curve is given in Table 1 (rows 1–2). Comparison of the p_1 values for the control and the erythrocytes incubated in isotonic sucrose demonstrates a significant decrease in osmotic fragility after incubation under LIS conditions. The decrease in p_1 is accompanied by an increase in the dispersion of the NaCl concentration producing haemolysis as reflected by the increased value of p_2. This indicates that under experimental conditions used in this study the population of erythrocytes responded differently to incubation in isotonic sucrose.

To check if the haemolysis resistance profile after incubation of erythrocytes in isotonic sucrose may be modulated by inserting charged compounds into the erythrocyte membrane the incubation was carried out in the presence of charged amphiphiles (CTAB and SDS). The concentrations of the amphiphiles were equal to these reported causing maximal antihaemolytic effect during incubation in hypotonic media [17]. In the Figs. 3 and 4 the haemolysis profiles of erythrocytes incubated in isotonic sucrose in the presence and in the absence of the investigated amphiphiles are shown. For cells incubated in the presence of the positively charged CTAB (Fig. 3), the haemolysis curve moves towards the right with respect to the curve for cells incubated in the absence of this compound. This means that CTAB decreases the shift in the haemolysis resistance profile caused by incubating erythrocytes in isotonic sucrose. The opposite effect was observed for the cells incubated in the presence of the negatively charged SDS (Fig. 4). In this case the haemolysis curve moves leftwards with respect to the curve for cells incubated without SDS, indicating that this compound increases the shift in the haemolysis curve caused by LIS medium. A statistical comparison of the parameters p_1 and p_2 derived from the fit of Eq. (1) to the experimental haemolysis curve for erythrocytes incubated in the presence of amphiphilic compounds is shown in Table 1 (rows 3–4). Cationic CTAB causes an increase in osmotic fragility, whereas anionic SDS causes a decrease in p_1 compared with the values obtained after incubating erythrocytes in isotonic sucrose without amphiphiles. The Δp_{1amph} after 30 min of incubating erythrocytes in the presence of cationic CTAB in isotonic sucrose was (−0.069±0.019) %NaCl, whereas Δp_{1amph} after 30 min of incubating erythrocytes in the presence of anionic SDS was (0.079±0.017) %NaCl. In view of the fact that both the investigated compounds did not change the haemolysis resistance profile during incubation in isotonic NaCl, the observed changes suggest that charged amphiphiles influence the transport pathway(s) activated in LIS media. Comparison of the p_2 values leads to the conclusion that the positively charged CTAB abolishes the increase in p_2 caused by incubating erythrocytes in isotonic sucrose, that is it makes the population more homogeneous. In the case of SDS, p_2 is not different from the value in isotonic sucrose. The length of the alkyl chains of the cationic amphiphiles seems not to have a significant effect on the shift in the haemolysis curve caused by incubating erythrocytes in isotonic sucrose because similar results for Δp_{1amph}

Table 1
Statistical comparison of the parameters p_1 and p_2 determined from fitting Eq. (1) to the experimental haemolysis curve for erythrocytes incubated in isotonic NaCl solution (control) and isotonic sucrose solution in the absence and in the presence of the charged amphiphilic compounds (CTAB and SDS) for 30 min

Isotonic solution	p_1 (%NaCl)	p_2 (%NaCl)	STD_{fit}	n
NaCl (control)	0.455±0.004	0.058±0.004	0.037±0.003	11
Sucrose	0.303±0.014***	0.135±0.007***	0.033±0.004	11
Sucrose+CTAB	0.426±0.012**	0.055±0.005**	0.040±0.004	3
Sucrose+SDS	0.263±0.048*	0.111±0.020	0.036±0.006	3

STD_{fit}: Standard deviation of the fit, p_1: osmotic fragility, p_2: haemolytic dispersion. Data are given as means±SE. * — $p<0.05$, ** — $p<0.01$, *** — $p<0.001$, (sucrose vs. control, sucrose+amphiphile vs. sucrose), n is the number of separate experiments carried out on blood of different donors.

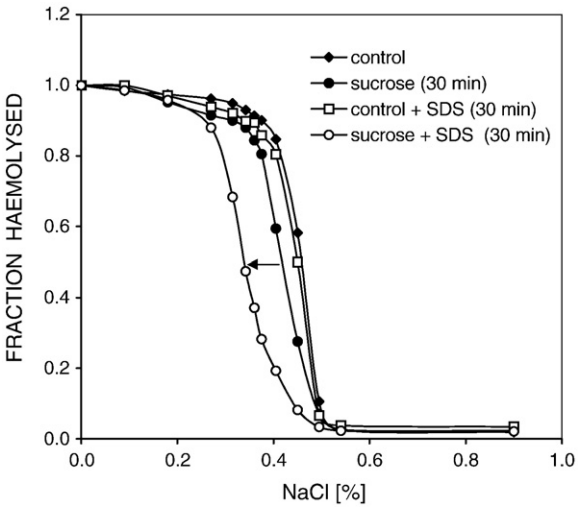

Fig. 4. The effect of SDS (50 μM) on the shift of osmotic resistance profile of human erythrocytes incubated in isotonic sucrose.

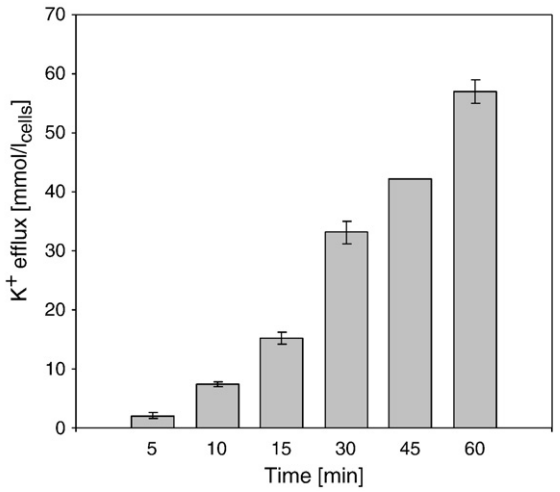

Fig. 5. The net K^+ efflux from human erythrocytes into isotonic sucrose vs. the time of incubation. Bars represent the mean±SE for n different blood donors (n=2, 4, 15, 15, 1 and 10 for times 5, 10, 15, 30, 45 and 60 min, respectively).

were obtained with alkyltrimethylammonium bromides (14 μM) with 12, 14, and 16 carbon atoms in the chain (data not shown).

To check if the modulation of the haemolysis resistance profile caused by the charged amphiphiles during incubation of erythrocytes in isotonic sucrose is caused by the effect of these compounds on the transport pathway involved in K^+ efflux activated under LIS conditions, the net K^+ efflux from erythrocytes was measured in the presence and in the absence of CTAB and SDS. The time dependence of K^+ efflux into isotonic sucrose containing 3 mM of KCl is shown in Fig. 5. The magnitude of K^+ efflux was donor dependent and varied by factor 2.5 after 15 min of incubation. After 1 h of incubation the mean K^+ efflux was equal to (57±2) mmol/l_{cells} what is comparable to values reported previously [3]. In Fig. 6 the effect of CTAB and SDS on K^+ efflux into isotonic sucrose is given. Both the positively and the negatively charged amphiphile led to significant changes in K^+ efflux: CTAB decreased, whereas SDS increased K^+ efflux by about 40%. The changes in K^+ efflux caused by charged amphiphiles agree with the observed changes in p_1, which confirms the thesis that the modulation of the shift in the haemolysis curve is caused by the influence of the investigated amphiphiles on the K^+ pathway activated under LIS conditions.

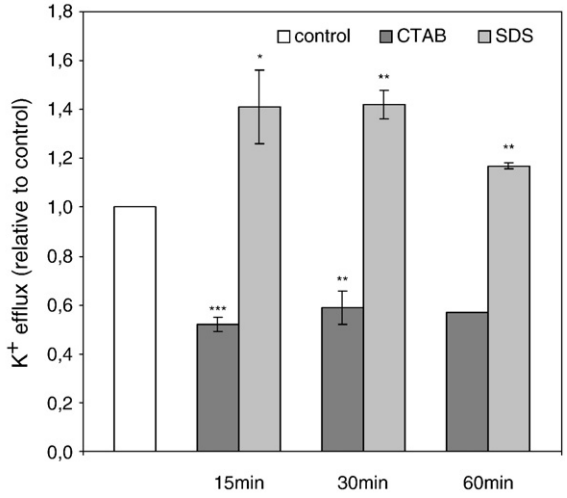

Fig. 6. The effect of CTAB and SDS on the net K^+ efflux from human erythrocytes into isotonic sucrose (related to control). Bars represent the mean±SE for n different blood donors. CTAB: n=8, 4 and 2 for times 15, 30 and 60 min, respectively. SDS: n=7, 3 and 4 for times 15, 30 and 60 min, respectively. * — $p<0.05$, * * — $p<0.01$, *** — $p<0.001$.

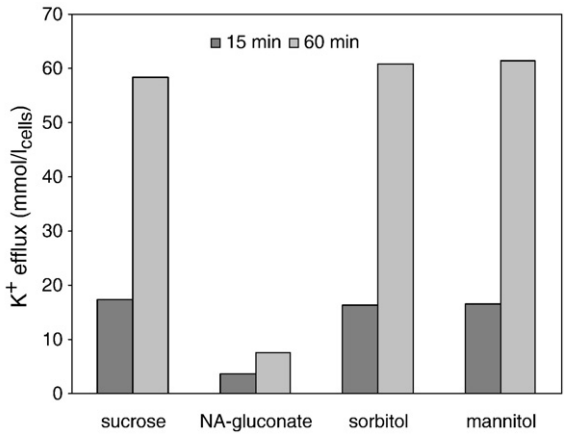

Fig. 7. The effect of incubating human erythrocytes in isotonic depolarising solutions on the net K^+ efflux. Bars represent the mean of two different blood donors.

To check if the modulatory effect of charged amphiphiles in isotonic sucrose will also occur in other depolarising media, a set of measurements of K^+ efflux from erythrocytes suspended in isotonic sorbitol, isotonic mannitol, and isotonic Na-gluconate was carried out. The values of K^+ efflux from erythrocytes incubated in various depolarising solutions in the absence of the charged amphiphiles are summarized in Fig. 7. In isotonic sucrose, sorbitol, and mannitol the K^+ effluxes were equal after 15 and 60 min of incubation, whereas in isotonic Na-gluconate K^+ efflux was significantly lower. Both cationic CTAB and anionic SDS were shown to modulate K^+ efflux into isotonic sorbitol and mannitol, similarly to that observed in isotonic sucrose (data not shown).

4. Discussion

The shift of the haemolysis resistance profile to lower osmolarities observed after incubation of erythrocytes in isotonic sucrose is a manifestation of cellular dehydration caused by dramatic K^+ efflux in LIS solutions [5]. The determination of osmotic fragility and haemolytic dispersion of the erythrocyte population from the haemolysis curve is a simple and low-cost experiment and the shift of the haemolysis resistance profile may be used as a first rough test to investigate the properties of the K^+ transport pathway activated under LIS conditions. At present, two transport pathways are suggested to be causative of the observed K^+ efflux in LIS media: the NSVDC channel [4,5,7,9–12] and the $K^+(Na^+)/H^+$ exchanger [3,8,13,14]. In this study the osmotic haemolysis test was used to check if the shift in the haemolysis resistance profile observed during incubation of erythrocytes in isotonic sucrose may be modulated by charged amphiphilic compounds. Comparing the change in osmotic fragility caused by incubation in isotonic sucrose in the absence of amphipiles, it has been found that Δp_1 might differ even by factor 2.5 for different blood donors (Figs. 1 and 2). This effect might be caused both by differences in the number of channels/carriers between individual donors and/or by differences in the activation of these pathways under non-physiological LIS conditions. The significant increase in haemolytic dispersion in erythrocytes incubated in isotonic sucrose for 30 min (Table 1) indicates that under experimental conditions used in this study the population of erythrocytes responded differently to the LIS condition. This fact demonstrates that the pathway activated under these conditions is not present in all cells or is not activated identically. The difference between these results and those obtained by Bennekou et al. [5], who showed no differences in haemolytic dispersion after 15 and 30 min of incubation of erythrocytes in isotonic sucrose, might be caused by differences in the time after blood taking (it has been shown that K^+ fluxes in LIS media were changed in stored red blood cells [21]) or by the differences in chloride concentration during experiments.

Because the pathway for K^+ efflux is activated when the membrane potential is made positive by decreasing the concentration of Cl^- in the incubation medium, Halperin et al. [6] suggested that it might be activated when erythrocytes are incubated in lytic hypotonic solutions. In this situation, the net salt and water loss induced by activation of this pathway could be an important factor reducing hypotonic haemolysis [6]. In view of the great effectiveness of different amphiphilic compounds in diminishing haemolysis in hypotonic solutions [17] and taking into account reports about the ability of charged amphiphiles to alter the properties of voltage-dependent cation pathways in other cells [18,19] it has been decided to check if the charged amphiphiles will modulate the shift of the haemolysis curve in isotonic sucrose and if this effect is related to the change in the properties of the K^+ efflux pathway activated under LIS conditions. Two differently charged amphiphiles, i.e. cationic CTAB and anionic SDS, were checked at concentrations reported as causing maximal antihaemolytic effect in hypotonic solutions [17]. The results indicate that positively charged CTAB increases, whereas negatively charged SDS decreases the shift of the haemolysis curve caused by incubating erythrocytes in isotonic sucrose (Figs. 3 and 4, Table 1). This stresses the role of the electrical properties of the membrane in the observed phenomenon. Cationic CTAB reduces the change in osmotic fragility and eliminates the increase in haemolytic dispersion caused by incubation in isotonic sucrose, that is it makes erythrocytes more homogeneous in response to the LIS condition. In contrast, the anionic SDS enhances the effect of LIS, which is seen in the increased value of Δp_1. The lack of significant effect of the alkyl chain length (12, 14 and 16 carbon atoms) of alkyltrimethylammonium bromides on Δp_{1amph} with regard to the large differences in their partition coefficient leads to the conclusion that the membrane concentration of amphiphiles needed for the modulation of osmotic fragility in isotonic sucrose is rather low.

From the comparison of the effects of charged amphiphiles on the haemolysis resistance profile and the net K^+ efflux from erythrocytes under LIS conditions (Figs. 3, 4, and 6), it is clear that the modulation of osmotic fragility is related to the modulation of K^+ efflux. The significant decrease in net K^+ efflux (by 40%) in the presence of cationic CTAB and the opposite effect caused by anionic SDS (increase in K^+ efflux by 40%) indicate that the charged amphiphiles dramatically changed the properties of the K^+ transport pathway activated in LIS media. The surface potential of the erythrocyte membrane is more negative under LIS conditions than under physiological conditions, which leads to an increase in cation concentration and a decrease in anion concentration near the cell surface. The insertion of charged amphiphiles into the cell membrane changes the surface charge density and, consequently, the surface potential. During 30 min of incubation in physiological isotonic solution, both the investigated amphiphiles caused erythrocyte shape changes, from discocytes to echinocytes, which suggests that they stay in the outer leaflet of the bilayer [22]. It is not known whether the intercalation process differs under LIS conditions. It has been reported that the inward translocation (flip) of anionic phospholipids in erythrocyte membranes depends on the membrane potential and is increased in low-Cl^- media [23]. Assuming that the same mechanism may cause faster movement of anionic SDS into the inner monolayer and taking into account the large influence of the surface potential on the K^+ transport pathway activated under LIS conditions, a decreased effect of SDS on K^+ efflux during the incubation time should be detected, which was in fact observed (Fig. 6). In contrast, the effect of cationic CTAB on K^+ efflux was constant during the 60 min of incubation.

The changes in erythrocyte surface potential may be used to explain the role of both transport pathways suggested to cause the observed increase in K^+ efflux under LIS conditions: the $K^+(Na^+)/H^+$ exchanger [3,8,13] and NSVDC channel. Since the surface potential of the erythrocyte membrane is more negative under LIS conditions, ion transport via the $K^+(Na^+)/H^+$ exchanger is increased because of the rise in the cation concentration near the membrane surface, which enhances the carrier-mediated flux [3,8,13]. The insertion of a positively charged compound causes a decrease in the negative surface potential, a decrease in the cation concentration near the cell surface, and consequently a decrease in transport. The opposite effect (increased transport) should be expected in the case of negatively charged compounds. This explanation was used by Richter et al. [8] to describe the effect of dodecyltrimethylammonium bromide and SDS on "leak" K^+ influx under LIS conditions. The reported decrease in K^+ efflux after reduction of the electrical charge of the erythrocyte membrane by neuraminidase [24] may also indicate the important role of the cation concentration near the membrane surface in K^+ efflux under LIS conditions. On the other hand, the alternative explanation of the effect of altered surface charge on K^+ efflux under LIS conditions, a preferred NSVDC pathway, is likely. The NSVDC channel is activated if the membrane potential changes from −9 mV (under physiological conditions) to 30 mV [12]. A strongly positive potential is achieved by reducing the extracellular Cl^- concentration under LIS conditions. The conductance of an ion channel varies with the concentration of permeating ions. The changes in surface charge, caused for example, by the insertion of charged compounds, may cause changes in the cation concentration near the mouth of the channel and in this way alter the channel's conductance. The surface potential may also have a direct effect on voltage gating of the ion channel because a channel's gating charge "feels" the effective potential across the membrane. This effective potential is equal to the sum of the membrane potential measured in the bulk solutions inside and outside of erythrocyte membrane and the difference between the inner and outer surface potentials. In this way the insertion of charged amphiphilic compounds by altering surface potential may change the electric field acting on the NSVDC pathway. Positively charged CTAB, by decreasing the negativity of the outer surface potential, causes partial hyperpolarisation of the effective voltage difference across the membrane and therefore a decrease in the open state probability of the channel and, consequently, reduced K^+ efflux under LIS conditions. The opposite effect should be expected after insertion of anionic SDS, which will subsequently cause increased K^+ efflux. The importance of the surface potential in the modulation of voltage-dependent ionic currents has long been recognised. It has been reported that charged amphiphiles (dodecyltrimethylammonium bromide and SDS) altered the amplitude and gating of the voltage-depended sodium, potassium and calcium currents in excitable cells [18,19]. It has been suggested that the observed effects are consequences of the insertion of the amphiphiles in the lipid environment surrounding the Na^+ and K^+ channels, but direct channel protein–amphiphile interactions are also likely [19]. A similar mechanism governing the behaviour of the NSVDC channel under LIS conditions cannot be excluded. On the other hand, Bernhardt et al. [21] suggested that LIS-induced cation fluxes are not dependent on transmembrane potential and isotonic sucrose medium rather acts by lowering ionic strength. The results presented in this study showing the values of K^+ efflux from erythrocytes incubated in various depolarising solutions (Fig. 7) confirm this thesis because in isotonic Na-gluconate the K^+ efflux was significantly lower than in the other isotonic sugar solutions. The reduction of the observed K^+ efflux in Na-gluconate compared with that in isotonic sucrose agrees with the values reported previously for K^+ influx [21]. Because Na-gluconate solution causes the same membrane depolarisation as sucrose solution, maintained at the same time erythrocytes at physiological ionic strength, this observation leads to the conclusion that to activate the transport pathway for K^+ efflux in isotonic sucrose, probably both factors, the positive membrane potential and low ionic strength, are required.

5. Conclusion

It has been demonstrated in this study that the charged amphiphiles cationic CTAB and anionic SDS are potent modulators of the K^+ efflux pathway which is activated in human erythrocytes under low

ionic strength conditions. The modulation of the net K^+ efflux caused by the intercalation of these amphiphiles into the cell membrane manifests as a shift change in the haemolysis resistance curve of erythrocytes incubated in isotonic sucrose. Both the magnitude of the shift in the haemolysis curve and the net K^+ efflux from erythrocytes incubated under low ionic strength conditions are donor dependent.

Acknowledgements

I thank Agata Dryjańska, Marta Sierańska, and Joanna Połukord for their invaluable technical assistance. I am indebted to Professor Jan Gomułkiewicz and Professor Adam Jezierski for stimulating discussion.

References

[1] H. Davson, Studies on the permeability of erythrocytes. VI. The effect of reducing the salt content of the medium surrounding the cell, Biochem. J. 33 (1939) 389–401.
[2] G.S. Jones, P.A. Knauf, Mechanism of the increase in cation permeability of human erythrocytes in low-chloride media. Involvement of the anion transport protein capnophorin, J. Gen. Physiol. 86 (1985) 721–738.
[3] D. Kummerow, J. Hamann, J.A. Browning, R. Wilkins, J.C. Ellory, I. Bernhardt, Variations of intracellular pH in human erythrocytes via $K^+(Na^+)/H^+$exchange under low ionic strength conditions, J. Membr. Biol. 176 (2000) 207–216.
[4] P. Bennekou, B.I. Kristensen, P. Christophersen, The human red cell voltage-regulated cation channel. The interplay with the chloride conductance, the Ca^{2+}-activated K^+ channel and the Ca^{2+} pump, J. Membr. Biol. 195 (2003) 1–8.
[5] P. Bennekou, T.L. Barksmann, P. Christophersen, B.I. Kristensen, The human red cell voltage-dependent cation channel. Part III: distribution homogeneity and pH dependence, Blood Cells Mol. Diseases 36 (2006) 10–14.
[6] J.A. Halperin, C. Brugnara, M.T. Tosteson, T. Van Ha, D.C. Tosteson, Voltage-activated cation transport in human erythrocytes, Am. J. Physiol. 257 (1989) C986–C996.
[7] P. Christophersen, P. Bennekou, Evidence for a voltage-gated, non-selective cation channel in the human red cell membrane, Biochim. Biophys. Acta 1065 (1991) 103–106.
[8] S. Richter, J. Hamann, D. Kummerow, I. Bernhardt, The monovalent cation "leak" transport in human erythrocytes: an electroneutral exchange process, Biophys. J. 73 (1997) 733–745.
[9] P. Bennekou, T.L. Barksmann, L.R. Jensen, B.I. Kristensen, P. Christophersen, Voltage activation and hysteresis of the non-selective voltage-dependent channel in the intact human red cell, Bioelectrochemistry 62 (2004) 181–185.
[10] T.L. Barksmann, B.I. Kristensen, P. Christophersen, P. Bennekou, Pharmacology of the human red cell voltage-dependent cation channel. Part I. Activation by clotrimazole and analogues, Blood Cells Mol. Diseases 32 (2004) 384–388.
[11] P. Bennekou, T.L. Barksmann, B.I. Kristensen, L.R. Jensen, P. Christophersen, Pharmacology of the human red cell voltage-dependent cation channel. Part II: inactivation and blocking, Blood Cells Mol. Diseases 33 (2004) 356–361.
[12] L. Kaestner, C. Bollensdorff, I. Bernhardt, Non-selective voltage-activated cation channel in the human red blood cell membrane, Biochim. Biophys. Acta 1417 (1999) 9–15.
[13] I. Bernhardt, D. Kummerow, E. Weiss, $K^+(Na^+)/H^+$ exchange in human erythrocytes activated under low ionic strength conditions, Blood Cells Mol. Diseases 27 (2001) 108–111.
[14] E. Weiss, H.-J. Lang, I. Bernhardt, Inhibitors of $K^+(Na^+)/H^+$ Exchanger of human red blood cells, Bioelectrochemistry 62 (2004) 135–140.
[15] L. Kaestner, P. Christophersen, I. Bernhardt, P. Bennekou, The non-selective voltage-activated cation channel in the human red blood cell membrane: reconciliation between two conflicting reports and further characterisation, Bioelectrochemistry 52 (2000) 117–125.
[16] I. Bernhardt, E. Weiss, H.C. Robinson, R. Wilkins, P. Bennekou, Differential effect of HOE642 on two separate monovalent cation transporters in the human red cell membrane, Cell Physiol. Biochem. 20 (2007) 601–606.
[17] B. Isomaa, H. Hägerstrand, G. Paatero, A.C. Engblom, Permeability alterations and antihaemolysis induced by amphiphiles in human erythrocytes, Biochim. Biophys. Acta 860 (1986) 510–524.
[18] J.A. Post, S. Ji, K.S. Leonards, G.A. Langer, Effects of charged amphiphiles on cardiac cell contractility are mediated via effects on Ca^{2+} current, Am. J. Physiol. 260 (1991) 759–769.
[19] S. Ji, J.N. Weiss, G.A. Langer, Modulation of voltage-dependent sodium and potassium currents by charge amphiphiles in cardiac ventricular myocytes. Effects via modification of surface potential, J. Gen. Physiol. 101 (1993) 355–375.
[20] R.H. Orcutt, T.S. Thurmond, K.E. Ferslew, Mathematical modeling of the osmotic fragility of rabbit red blood cells, J. Pharmacol. Toxicol. Methods 34 (1995) 169–174.
[21] I. Bernhardt, A.C. Hall, J.C. Ellory, Effects of low ionic strength media on passive human red cell monovalent cation transport, J. Physiol. 434 (1991) 489–506.
[22] B. Isomaa, H. Hägerstrand, G. Paatero, Shape transformations induced by amphiphiles in erythrocytes, Biochim. Biophys. Acta 899 (1987) 93–103.
[23] C.W. Haest, A. Oslender, D. Kamp, Nonmediated flip-flop of anionic phospholipids and long-chain amphiphiles in the erythrocyte membrane depends on membrane potential, Biochemistry 36 (1997) 10885–10891.
[24] I. Bernhardt, E. Donath, R. Glaser, Influence of surface charge and transmembrane potential on rubidium-86 efflux of human red blood cells, J. Membr. Biol. 78 (1984) 249–255.