Available online at www.sciencedirect.com
ScienceDirect
Thermochimica Acta 456 (2007) 7–12
thermochimica acta
www.elsevier.com/locate/tca

Role of membrane proteins in thermal damage and necrosis of red blood cells

Ivan T. Ivanov [a,*], Myriam Brähler [b], Radostina Georgieva [c], Hans Bäumler [b]

[a] *Department of Physics and Biophysics, Medical Faculty of Thracian University, Stara Zagora 6000, Bulgaria*
[b] *Institute of Transfusion Medicine, Charité-Universitätsmedizin Berlin, Schumannstr. 20/21, 10117 Berlin, Germany*
[c] *Max-Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Golm/Potsdam, Germany*

Received 27 August 2006; received in revised form 12 January 2007; accepted 17 January 2007
Available online 24 January 2007

Abstract

This study presents evidence for the role of spectrin and the anion exchanger in the temperature activation of ion permeability, hyperthermic permeability transition (HPT), involved in thermal necrosis of RBCs. Membranes, undergoing HTP at conditions sparing the structure of spectrin (39 °C, 18%, v/v ethanol, 3 min), became irreversibly permeabilized for NaCl but retained impermeability for sucrose. SDS-PAGE and thermal gel analysis did not detect denaturation of tertiary structure of membrane proteins. The denaturation temperature of spectrin did not change, whereas that of the anion exchanger, band 3 protein, decreased by 2.5 °C compared to that of control. Specific inhibition of the anion exchanger with 4,4′-diiso-thiocyanato stilbene-2,2′-disulfonic acid (DIDS) prior to or after permeabilization increased the denaturation temperature to 76.5 °C, which was still lower by 3.5 °C than that of inhibited intact membranes. The results suggest initially reversible, predenaturational transition in the anion exchanger was involved in the activation of ion permeability.

Keywords: Red blood cell membrane; Thermal necrosis; Basal ion permeability; Thermal stability of membranes; Sparing permeabilization; Glass transition

1. Introduction

Medical application of blood pumps, lasers, ultrasound, radiation and electric current all lead to rise in temperature, and thermal necrosis of cells motivated this study of the effects of high temperatures on red blood cells (RBCs).

On continuous heating, thermal hemolysis is negligible below 62 °C and rises sharply above that temperature [1]. Others have found the activation energy (E_a) of 300 ± 20 kJ/mol for thermal hemolysis [2–4]. Based on this high E_a some investigators [3–5] suggested that denaturation of a single protein or a group of similar proteins is the prime target of heat. Proteins proposed as involved include spectrin [6], hemoglobin [7] and the anion exchanger [8]. The bulk of experimental evidence definitively rejects, however, spectrin as well as hemoglobin as the prime target of heat in thermal hemolysis [1,9,10–12].

The participation of the anion exchanger in hemolysis is controversial. The resistance of RBCs against thermal hemolysis is reduced by photochemical modification of the anion exchanger, allowing the conclusion that heat denaturation of the anion exchanger is involved in thermal hemolysis [8]. By contrast, Lepock et al. [5] concluded that, although membrane proteins are implicated in the thermal hemolysis, the heat denaturation of the anion exchanger is not involved. In an effort to resolve the problem, Lepock [13] later proposed that several different proteins are involved, and thermal hemolysis occurs when a critical portion (between 5 and 17%) denatures.

Another point of view arises from studies of the passive movement of ions in whole RBCs and their isolated, resealed membranes during transient heating [10] and at fixed temperatures [11,14]. Above 46 °C the prelytic interval included a lag period and a time interval during which ion leakage increased to a constant rate that corresponded to a constant permeability P [8,11]. With increasing temperature, the lag period shortened and P increased. Earlier kinetic studies on whole RBCs and their resealed ghosts carried out within 50–58 °C interval found an

Abbreviations: RBCs, red blood cells; RBCM, red blood cell membrane; P, basal permeability for ions (K^+; Na^+; Cl^-) in red blood cells; HPT, hyperthermic permeability transition; DSC, differential scanning microcalorimetry; DIDS, 4,4′-diiso-thiocyanato stilbene-2,2′-disulfonic acid; DTT, dithiothreitol

* Corresponding author.
E-mail address: ivanov_it@gbg.bg (I.T. Ivanov).

0040-6031/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2007.01.020

inducing temperature of 61–62 °C for this transition [11]. It was demonstrated that *P* was activated by a single mechanism with a high value of E_a (250 kJ/mol). In another study on the K^+ leakage from human RBCs heated within the 46–54 °C interval, Prinsze et al. [8] came to the same conclusion and determined the same value of E_a (246 kJ/mol). Based on this very high E_a it could be concluded that a direct effect of temperature on the conformation of membrane proteins, is responsible for the permeability increase.

This study presents new evidence for the role of spectrin and the anion exchanger in the temperature activation of ion permeability involved in thermal necrosis of RBCs.

2. Materials and methods

2.1. Materials

4,4′-Diiso-thiocyanato stilbene-2,2′-disulfonic acid (DIDS), EGTA, adenosine, sodium dodecylesulfate (SDS) and the SDS-PAGE chemicals were purchased from Sigma Chemicals Co., St. Louis, MO, USA.

2.2. Treatment of RBCs and membrane preparation

Human blood samples were obtained from donor centers (Stara Zagora, Bulgaria and Charité, Berlin, Germany). RBCs were isolated by centrifugation and washed thrice in physiological buffered saline. Each sample was divided into equal portions. One portion served as control with no further treatment. The other portions were treated as described below:

2.3. DIDS treatment [15]

Washed RBCs were suspended at hematocrit of 0.05 in physiological buffered saline and treated with 30 μM DIDS at dark and room temperature for 15 min. The treatment of cells previously subjected to sparing permeabilization was performed following the same protocol in isotonic NaCl/sucrose solution.

2.4. Preparation of white ghost RBCs [16]

Briefly, packed RBCs (intact, subjected to sparing permeabilization, and DIDS-treated) were diluted 20 times with a 4 °C-cold hypotonic solution (1 mM $MgCl_2$, 0.5 mM EGTA, 5 mM phosphate buffer, pH 8.0). The membranes were isolated by centrifugation at 15,000 × *g* and washed thrice in the same medium to white color.

2.5. Sparing permeabilization [17]

After the washing procedure the RBCs were incubated at 39.5 ± 0.1 °C in an isotonic solution containing 50 mM NaCl, sucrose and 18% (v/v) ethanol (final concentration) for 3 min. Then the suspension was rapidly diluted (1:1) with cold isotonic solution of 50 mM NaCl and sucrose and kept for several minutes at 4 ° in order to reduce the heat damage of membranes. Then RBCs were washed thrice in excess volume of isotonic 50 mM NaCl/sucrose solution to remove the ethanol. The final hemolysis was less than 0.5%. The mechanism of hemolysis induced by ethanol at such concentrations has been described elsewhere [18]. The 3 min incubation at the inducing temperature of hyperthermic permeability transition (HTP) is sufficient to alter about half the number of protein copies but too short to induce a change in membrane structure and composition such as lipid solvation and vesiculation [19].

2.6. Differential scanning calorimetry

DSC measurements were performed with a MSC Microcalorimeter, MicroCal, MA, USA. The volume of the measuring cell was 1.442 ml. For the measurements the membranes were suspended in 100 mM NaCl, 10 mM phosphate buffer, pH 8 at a volume ratio of approximately 1:0.3 (membranes: solution). The reference cell was filled with the same solution. The scanning was carried out at 0.7 °C/min from 20 to 90 °C. Only two portions of each sample were scanned if there was no substantial difference between the thermograms obtained.

2.7. Derivative thermal analysis of the electrical impedance of RBC and ghost suspension [10]

Intact RBCs or resealed ghosts (inside content usually 150 mM NaCl) were suspended (hematocrit 0.07) in an isotonic 50 mM NaCl/sucrose medium, thus imposing an outward ion concentration gradient across the membranes. The suspension was heated with constant heating rate and the suspension impedance was continuously measured at 7 kHz. The output signal U_s of conductometer was fed into a differentiating amplifier and its output voltage *U* was recorded (derivative impedance thermogram).

The dependence of U_s on the temperature, *T*, is closely linear: $U_s = U_{so}(1 + K_s \Delta T)$, where K_s is the temperature coefficient of U_s. When *T* increases at a steady-rate, *V*, *U* could be expressed as $U = 1/(RC)\mathrm{d}U_s/\mathrm{d}t = 1/(RC)$. $U_{so}K_sV$, where 1/(RC) is the amplification coefficient. During the heating, only K_s is allowed to change, which sensed the possible change in suspension impedance. The time differentiation was applied in order to compensate for the strong Boltzmann dependence of the suspension conductivity on temperature. At a steady-rate heating, the thermogram appeared as a horizontal line, unless the suspension conductivity or capacitance changed, producing a sharp peak around the inducing temperature T_m. At a heating rate of 2.0 °C/min, the reproducibility for obtaining T_m was within ± 0.3 °C.

2.8. Thermal gel analysis of membrane proteins [20]

RBC membranes heated to a given temperature were analyzed by SDS-PAGE electrophoresis using gels with 5% acrylamide and acrylamide /bis-acrylamide ratio of 40:1 [21]. The membrane proteins were solubilized in 2% SDS for 5 min at 100 °C with or without 40 mM dithiothreitol as a reducing agent taking into account the following considerations. The heat

denaturation of a single membrane protein causes aggregation of its copies by intermolecular bisulfide bridges. The SDS-PAGE electrophoretic band of this protein will be absent or present depending on whether or not a disulfide reducing reagent is used during the membrane solubilization. Thus, thermal gel analysis could indicate the occurrence of thermal denaturation of specific membrane proteins.

3. Results

During heating of RBC and ghost suspensions, the suspension impedance underwent sharp changes about 52 and 66 °C [10]. These changes were detected as positive peaks by recording the first derivative of suspension impedance (Fig. 1). The threshold changes in electric properties of membranes revealed thermally induced conformation changes in two different portions of membrane proteins, peripheral and intrinsic ones, respectively [22]. The peak at 52 °C (peak A) corresponded to a decrease in electric capacity of membranes [23] associated with the denaturation of spectrin [22] which takes place at 49.5 °C [24]. The peak at 66 °C (peak G) corresponds to the collapse of the ion concentration gradient due to thermal activation of basal ion permeability at 62 °C [11].

The shift of both peaks toward temperatures higher than the inducing temperatures of corresponding membrane events is due to the heating rate applied in the presented measurement.

Lower heating rates caused a decrease in the peak temperature, approaching the denaturation temperature (49.5 or 62 °C). The method is not sensitive to changes in the shape of cells during heating [23].

DIDS is a highly specific covalent inhibitor of the anion transport of RBCs that thermally stabilizes the anion exchanger [25]. Binding of DIDS to the anion exchanger of cell membranes did not affect the peak temperature and half-width of peak G (Fig. 1). Hence, heat denaturation of the anion exchanger was apparently not needed and not involved in hyperthermic activation of ion permeability (HPT) and thermal hemolysis.

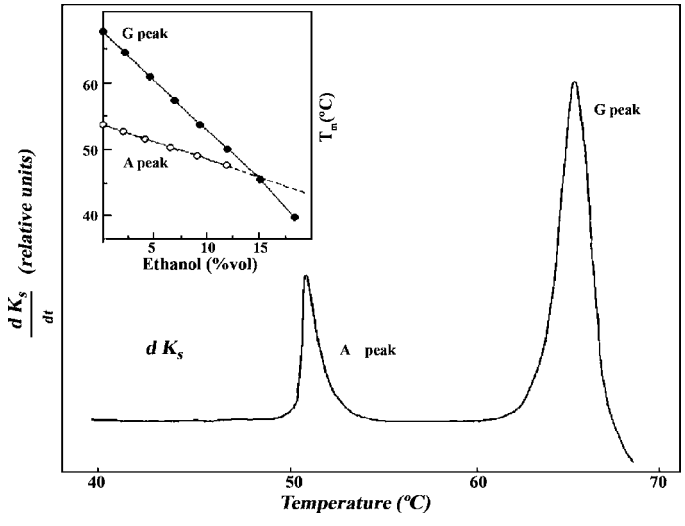

Fig. 1. Derivative thermogram of the electric impedance of RBC ghost suspension. The resealed membranes contained 150 mM NaCl and were suspended in isotonic solution of 50 mM NaCl and sucrose, pH 7.0. Hematocrit and heating rate were 0.07 and 2.0 °C/min, respectively. The thermograms of intact RBCs and DIDS-inhibited RBCs had the same form as that of RBC ghosts. Insert shows the dependence of peak temperatures on ethanol present in the suspension media at isotonicity.

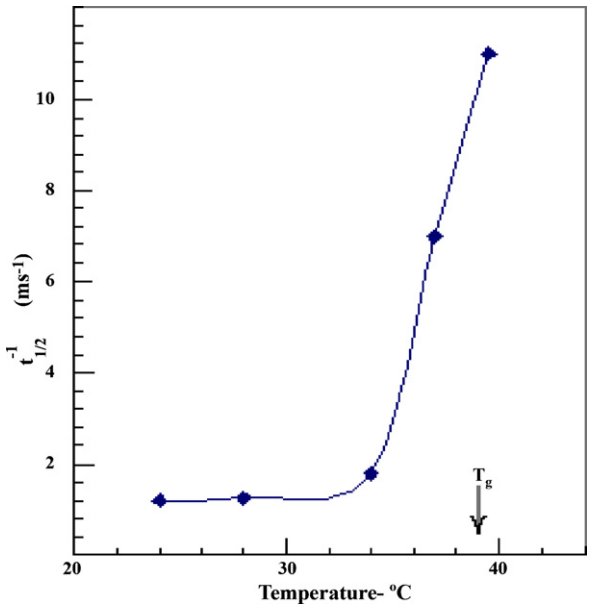

Fig. 2. Sparing permeabilization of human RBCs produced by short-time incubation close to T_g. The cells were incubated in isotonic medium containing sucrose, 50 mM NaCl and 18% (v/v) ethanol and exposed to the indicated temperature for 3 min. The permeabilization obtained is quantified by the inverse of the time $t_{1/2}$ needed for 50% hemolysis of these cells in 150 mM NaCl saline.

In a direct study of HPT, it is desirable to avoid spectrin denaturation which does not participate in thermal hemolysis but should alter membrane structure and dynamics. A set of conditions has been found where HPT precedes thermal denaturation of spectrin. The temperatures of both spectrin denaturation (T_A) and HPT (T_g) were linearly lowered by ethanol, the latter displaying 3.5 times greater sensitivity (Fig. 1, insert). At ethanol concentration of 18% (v/v), the HPT occurred at 39.5 °C while spectrin denatured at about 45 °C, which allowed the independent study of HPT. This transition was induced by exposing RBCs to 39.5 °C isotonic medium that contained 18% ethanol for 3 min.

RBCs, permeabilized by exposure to T_g at such conditions, retained their permeability for ions and impermeability for sucrose after the withdrawal of ethanol and hyperthermia. The latter cells are referred to as selectively permeabilized cells. These cells were stable in isotonic 50 mM NaCl/sucrose solution over a long period of time but lysed completely within 5 min in isotonic NaCl solution (Fig. 2). Addition of albumin (1 mg/ml), restored their discoid shape, proving the spectrin/actin cytoskeleton network was intact. Incubation of cells at the same ethanol concentration at temperatures higher than 43 °C led to irreversible permeabilization for sucrose, while incubation at 34 °C and lower temperatures induced reversible permeabilization for ions (Fig. 2).

SDS-PAGE shows the polypeptides of RBCMs avoided denaturation during heat exposure up to T_g (Fig. 3). This is shown by the absence of aggregates produced by disulfide bond

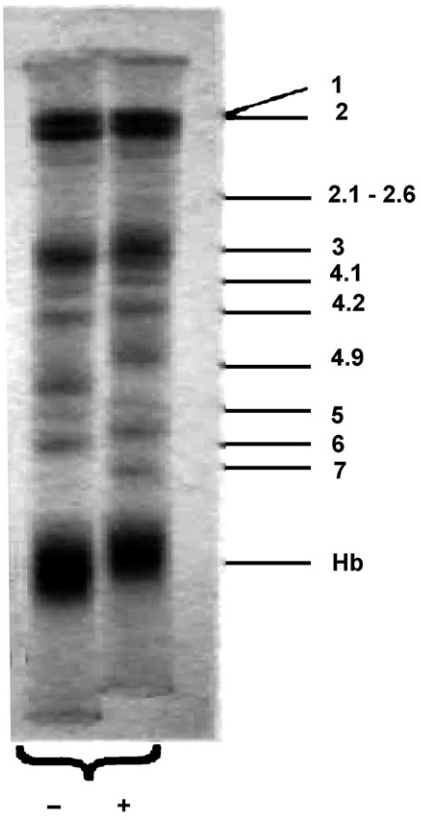

Fig. 3. SDS-PAGE electrophoresis of membrane of one-step resealed ghosts or intact RBCs subjected to sparing permeabilization at T_g. The cells (ghosts) were heated in the presence of 18% (v/v) ethanol for 3 min at 39.5 °C. After permeabilization, the membranes were hypotonically separated, washed in 5 mM phosphate buffer, pH 8.0, and subjected to electrophoresis. The solubilization of the ghosts was carried out with (+DTT) or without (−DTT) 40 mM dithiothreitol. The protein bands are indicated according to [21].

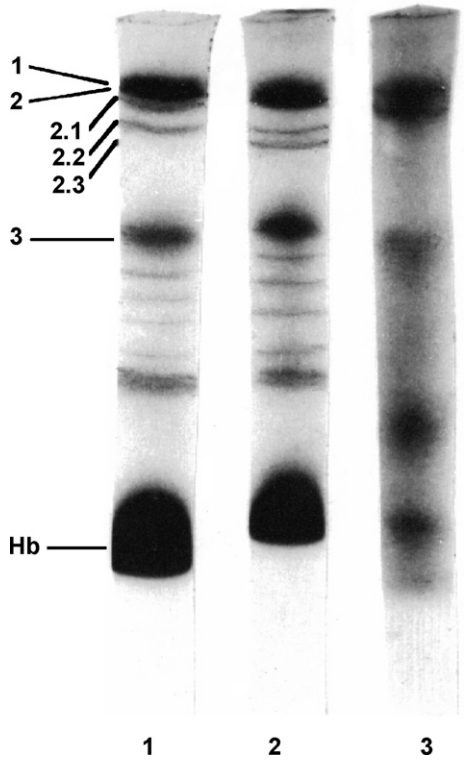

Fig. 4. Thermal gel analysis of the polypeptides of RBCMs subjected to sparing permeabilization around T_g. The RBCs (ghosts) were incubated in the presence of 18% (v/v) ethanol for 3 min at the indicated temperatures: 25 °C (1), 39.5 °C (2) and 45 °C (3). After permeabilization, the membranes were hypotonically separated, washed in 5 mM phosphate buffer, pH 8.0, and subjected to electrophoresis.

formation following the heat denaturation. In support of this conclusion, the thermal gel analysis showed that exposure to temperatures above T_g (45 °C) produced significant diminution of polypeptide bands, especially those of integral proteins and spectrin (Figs. 3 and 4). In conclusion, temperature activation of ion permeability at the stage of reversibility (34 °C) and even irreversibility (39.5 °C) was not accompanied by heat denaturation of membrane proteins, including spectrin and the anion exchanger. The induction of sucrose permeability was, however, concomitant with significant protein denaturation (45 °C).

Fig. 5 (curve 1) shows DSC profiles of RBCMs that were irreversibly permeabilized by exposure to T_g. This demonstrates the generally intact structure of the major membrane proteins; spectrin, actin and tropomyosin (representing about 35% of the membrane protein content) and especially the anion exchanger (about 30% of membrane proteins), according to [26]. These results are supported by the electrophoretic profile of RBCMs subjected to sparing permeabilization by heat (Figs. 3 and 4). The lack of protein aggregates in this profile and the appearance of the same bands as those in intact RBCMs confirmed that HPT was not accompanied by detectable thermal denaturation.

That the anion exchanger of permeabilized membranes was intact was further tested with DIDS. In agreement with published

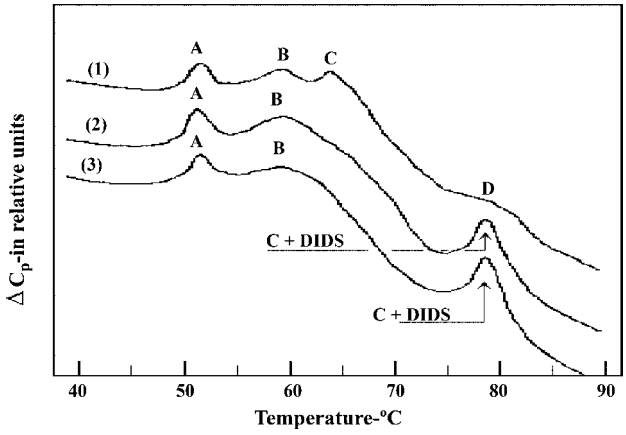

Fig. 5. DSC thermograms of permeabilized RBCMs: (1) membranes isolated from permeabilized cells; (2) membranes isolated from DIDS-treated and subsequently permeabilized cells; (3) membranes from permeabilized and subsequently DIDS-treated cells. To permeabilize cells, they were exposed to the HPT inducing temperature (T_g) in the presence of ethanol (18 v/v%) for 3 min only. Letters and arrows indicate the heat denaturations of specific portions of membrane proteins: major peripheral protein spectrin (A), minor fraction of peripheral proteins and the cytoplasmic part of the anion exchanger (B), transmembrane portion of the anion exchanger (C) and presumably the peripheral protein tropomyosin (D), according to [24]. Other details as for Fig. 3.

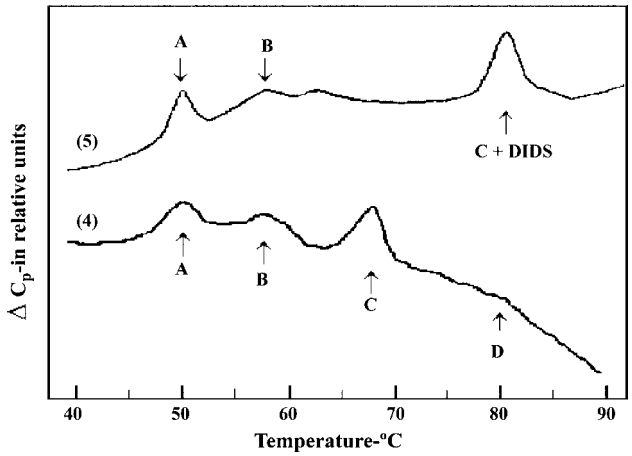

Fig. 6. Thermograms of intact RBCMs. (4) membranes isolated from intact cells; (5) membranes isolated from DIDS-treated intact cells.

data [25], binding of DIDS increased the denaturation temperature of the transmembrane part of the anion exchanger by 13 °C (Fig. 6). Induction of HPT in permeabilized cells did not affect the ability of DIDS to stabilize the anion exchanger (Fig. 5, curve 2). Moreover, similar stabilization was observed when DIDS was allowed to bind the anion exchanger prior to or after the permeabilization (Fig. 5, curves 2 and 3). This finding also supports the conclusion that the majority of the anion exchanger molecules preserved their tertiary structure after exposure to T_g and permeabilization.

Thus, data obtained by DSC, electrophoresis and thermal analysis of suspension impedance all lead to a conclusion that heat denaturation of the anion exchanger was not involved and not needed for the HPT, thermal hemolysis and the activation of ion permeability at supraoptimal temperatures. While the denaturation temperature of spectrin was preserved in permeabilized membranes (Fig. 5, curves 1, 2 and 3) that of the anion exchanger was lower by 2.5 °C (Fig. 5, curve 1, Fig. 6). In addition, the stabilization of the anion exchanger of permeabilized membranes by DIDS was by 9.5 °C only instead of 13 °C known for the control membranes (Fig. 5, curves 2 and 3). The latter findings all indicate, in contrast to spectrin, a small change in the tertiary structure of the anion exchanger accompanied membrane permeabilization by heat.

4. Discussion

Heat changes erythrocytes, leading to increase in ion permeability and hemolysis [8,10,11,14,27]. Studies on whole and reconstructed erythrocytes have clearly showed thermal hemolysis has colloid-osmotic nature at its earliest stage [10,11] thus possessing the major requisites of the heat-induced necrosis of animal cells. The rise in permeability responsible for hemolysis was related to a thermally induced change, HPT, in integral proteins with inducing temperature T_g of 61–62 °C and E_a 250 kJ/mol [10,11].

Human RBCs do not lose their discoid shape up to 49 °C [6,28]. At 46–51 °C, spectrin irreversibly denatures which induces spherization [6], partial fragmentation and vesiculization [6], increase in membrane shear elasticity and bending rigidity [28], loss of membrane deformability and RBC aggregability [29,30] without significant hemolysis and loss of electrolytes. Studies on human RBCs with various spectrin mutants confirm the involvement of spectrin denaturation in all these events [31–33]. In addition, spectrin is found involved in barrier function [34].

Nevertheless, the denaturation of spectrin is a major factor in the heat-induced changes of erythrocyte morphology, it was proved not the prime target of heat in thermal hemolysis [9,13] and permeability activation [22]. Based on that, in this study we induced HPT at conditions, sparing the denaturation of spectrin, and studied membrane proteins. According to the data of SDS-PAGE electrophoresis (Fig. 3), thermal gel analysis (Fig. 4) and DSC (Figs. 5 and 6), although erythrocyte membranes were exposed to T_g and incurred severe permeabilization to ions, no traces of thermal denaturation in tertiary structure of major membrane proteins, including spectrin and the anion exchanger, were detected.

This conclusion should be yet more convincing in respect to thermal hemolysis at temperatures lower than T_g. According to the data in Fig. 2, supported by experiments of [8], the induced permeability had reversible character below T_g and, hence, the respective changes in membrane proteins should be reversible as well. The reversible character of the permeability below T_g excludes spectrin denaturation once more.

At this stage, the presented data does not allow final insight into latter changes. Close to T_g, however, DSC recorded slight, although well detectable change (2.5–3.5 °C) in thermal stability of tertiary structure of the anion exchanger (Fig. 5). These findings are in line with earlier data [10,14,22] that integral proteins are the major participant in HPT, permeability activation and hemolysis.

Based on the initial reversibility, several lines of events could lead to this predenaturational change in the conformation of the anion exchanger; dissociation of multimeric proteins, vertical displacement, dynamic transition. Additional experiments, that are underway, will elucidate the nature of this important membrane change.

References

[1] W.T. Coakley, A.J. Bater, L.A. Crum, J.O.T. Deeley, Morphological changes, haemolysis and microvesiculation of heated human erythrocytes, J. Therm. Biol. 4 (1979) 85–95.
[2] D.A. Juckett, B. Rosenberg, The kinetics and thermodynamics of lysis of young and old sheep red blood cells, Mech. Ageing Dev. 18 (1) (1982) 33–45.
[3] R.E. Gershfeld, M. Murayama, Thermal instability of red blood cell membrane bilayers: temperature dependence of thermohemolysis, J. Membr. Biol. 101 (1988) 67–72.
[4] E.A. Chernitskii, I. Iamaikina, Thermohaemolysis of erythrocytes, Biofizika 33 (1988) 319–322 (article in Russian).
[5] J.R. Lepock, H.E. Frey, H. Bayne, J. Markus, Relationship of hyperthermia-induced hemolysis of human erythrocytes to the thermal denaturation of membrane proteins, Biochim. Biophys. Acta 980 (1989) 191–201.
[6] W.T. Coakley, J. Owen, T. Deeley, Effects of ionic strength, serum protein and surface charge on membrane movements and vesicle production in heated erythrocytes, Biochim. Biophys. Acta 602 (1980) 355–375.

[7] I.V. Iamaikina, E.A. Chernitskii, Denaturation of hemoglobin—the first stage of erythrocyte thermohemolysis, Biofizika 34 (1989) 656–659 (article in Russian).
[8] C. Prinsze, K. Tijssen, T.M. Dubbelman, J. Van Stevenick, Potentiation of hyperthermia-induced haemolysis of human erythrocytes by photodynamic treatment. Evidence for the involvement of the anion transporter in this synergistic interaction, Biochem. J. 277 (1991) 183–188.
[9] I.V. Iamaikina, E.A. Chernitskii, Possible role of spectrin in thermohemolysis of erythrocytes, Biofizika 33 (1988) 626–628 (article in Russian).
[10] I.T. Ivanov, L.C. Benov, Thermohemolysis of human erythrocytes in isotonic NaCl/sucrose media during transient heating, J. Therm. Biol. 17 (1992) 381–389.
[11] I.T. Ivanov, Thermohaemolysis of human erythrocytes in sucrose containing isotonic media, J. Therm. Biol. 17 (1992) 375–379.
[12] A.B. Borisova, A.S. Goryunov, Thermal hemolysis of red blood cells differing in affinity of hemoglobin to oxygen, J. Evol. Biochem. Physiol. 33 (1997) 126–130.
[13] J.R. Lepock, Cellular effects of hyperthermia: relevance to the minimum dose for thermal damage, Int. J. Hyperthermia 19 (2003) 252–266.
[14] I.T. Ivanov, Thermohaemolysis of mammalian erythrocytes, J. Therm. Biol. 18 (1993) 177–183.
[15] J.W. Snow, J.F. Brandts, P.S. Low, The effects of anion transport inhibitors on the structural transitions in erythrocyte membranes, Biochim. Biophys. Acta 512 (1978) 579–591.
[16] J.T. Dodge, C. Mitchell, D.J. Hanahan, The preparation and chemical characteristics of hemoglobin-free ghosts of erythrocytes, Arch. Biochem. Biophys. 100 (1963) 119–130.
[17] I.T. Ivanov, R. Todorova, I. Zlatanov, Spectrofluorometric and microcalorimetric study of the thermal poration relevant to the mechanism of thermohemolysis, Int. J. Hyperthermia 15 (1999) 29–43.
[18] L.M. Li, W.G. Wu, Mechanism of hemolysis of red blood cells mediated by ethanol, Biochim. Biophys. Acta 1062 (1991) 46–50.
[19] H. Hägerstrand, B. Isomaa, Lipid and protein composition of exovesicles released from human erythrocytes following treatment with amphiphiles, Biochim. Biophys. Acta 1190 (1994) 409–415.
[20] K. Lysko, R. Carlson, R. Taverna, J. Snow, J.F. Brandts, Protein involvement in the structural transitions of erythrocyte ghosts, Biochemistry 20 (1981) 5570–5576.
[21] G. Fairbanks, T. Steck, D. Wallach, Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane, Biochemistry 10 (1971) 2606–2617.
[22] I.T. Ivanov, Involvement of erythrocyte membrane proteins in temperature-induced disturbances of the permeability barrier, Membr. Cell Biol. 11 (1997) 45–56.
[23] I.T. Ivanov, Changes in passive electric parameters of human erythrocyte membrane during hyperthermia: role of spectrin phosphorylation, Gen. Physiol. Biophys. 18 (1999) 165–180.
[24] J.F. Brandts, L. Erickson, K. Lysko, A.T. Schwartz, R.D. Taverna, Calorimetric studies of the structural transitions of the human erythrocyte membrane. The involvement of spectrin in the A transition, Biochemistry 16 (1977) 3450–3454.
[25] S.R. Davio, Ph.S. Low, Characterization of the calorimetric C-transition of the human erythrocyte membrane, Biochemistry 21 (1982) 3575–3582.
[26] R. Wagner, G. Zimmer, L. Lacko, An interspecies approach to the investigation of the red cell membrane glucose transporter, Biochim. Biophys. Acta 771 (1984) 99–102.
[27] W. Wilbrandt, Osmotische Natur sogenannter nicht-osmotischer Hemolysen. (Kolloidosmotische Hemolyse), Arch. Ges. Physiol. (Pflagers) 245 (1941) 22–52.
[28] A.L. Rakow, R.M. Hochmuth, Effect of heat treatment on the elasticity of human erythrocyte membrane, Biophys. J. 15 (1975) 1095–1100.
[29] P. Snabre, H. Bäumler, P. Mills, Adhessiveness and aggregation kinetics of human erythrocytes after heat treatment, Biorheology 20 (1983) 427.
[30] D. Lerche, H. Bäumler, Moderate heat treatment of only red blood cells (RBC) slows down the rate of RBC-RBC aggregation in plasma, Biorheology 21 (1984) 393–403.
[31] J.L. Vives-Corrons, I. Besson, M. Aymerich, S. Ayala, N. Alloisio, J. Delaunay, I. Gonzalez, E. Manrubia, Hereditary xerocytosis: a report of six unrelated Spanish families with leaky red cell syndrome and increased heat stability of the erythrocyte membrane, Br. J. Haematol. 90 (4) (1995) 817–822.
[32] H.S. Zarkowsky, Heat-induced erythrocyte fragmentation in neonatal elliptocytosis, Br. J. Haematol. 41 (4) (1979) 515–518.
[33] K. Chang, J.R. Williamson, H.S. Zarkowsky, Effect of heat on the circular dichroism of spectrin in hereditary pyropoikilocytosis, J. Clin. Invest. 64 (1) (1979) 326–328.
[34] S. Klonk, B. Deuticke, Involvement of cytoskeletal proteins in the barrier function of the human erythrocyte membrane. II: Formation of membrane leaks in ghost membranes after limited proteolysis of skeletal proteins by trypsin, Biochim. Biophys. Acta 1106 (1992) 137–142.