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Effect of the level of ATP and of the state of spectrin on osmotic properties of bovine erythrocytes

Marian Mosior [1], Małgorzata Bobrowska [2] and Jan Gomułkiewicz [1]

[1] *Institute of Physics, Technical University of Wrocław, Wrocław and* [2] *Department of Biophysics, Medical Academy of Wrocław, Wrocław (Poland)*

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The effect of the intracellular level of ATP and of the state of spectrin on the critical cell volume of bovine erythrocyte was studied. The state of spectrin was changed by thermal denaturation, which for the bovine red cell took place at similar temperature as for the human erythrocyte. The increase of the ATP level and the spectrin denaturation increased the critical cell volume, while metabolic starvation decreased it. The changes of the ATP level did not influence the critical volume after the denaturation of spectrin. The results suggest that the ATP-dependent effect on the critical cell volume was caused by an alteration of the membrane extensibility due to the change of the membrane skeleton–lipid bilayer interaction(s).

1. Introduction

The osmotic fragility of erythrocytes depends on: their surface area/isoosmotic volume ratio, the amount of internal solutes, the osmotically non-active volume [1,2], the hemolysis-protective potassium efflux [3–5], and the membrane extensibility [6,7]. From the data on the membrane extensibility [6,8] and the modulus of surface compressibility of the erythrocyte membrane [7] one can evaluate the influence of mechanical properties of the membrane on osmotic fragility as 4–12%.

For 25 years now alterations of erythrocyte shape and deformability have been observed upon metabolic starvation of the cells [9–12]. It is generally accepted today that these ATP-dependent phenomena result from the membrane skeleton–lipid bilayer interactions, regulated by the process of phosphorylation [12–15], though the mechanisms of these interactions remain obscure. The phosphorylation of phosphatidylinositol [12,16,17], of spectrin [18–21], and of other membrane skeletal proteins [15,22–25] have been postulated as the mechanism of regulation.

The method of metabolic starvation of erythrocytes was utilized most frequently to study the influence of the ATP level on the properties of erythrocyte membrane [9–12,22,23]. In the present study the effect of the activity of glycolysis on the osmotic properties of erythrocyte was examined by an activation of the Embden-Meyerhof pathway of glycolysis by phosphate at high concentration [28,29]. The measure of the activity of glycolysis was the intracellular level of the ATP. Preliminary experiments indicated an increase of the critical cell volume and a decrease of osmotic fragility upon an activation of glycolysis [30]. With a supposition that the membrane skeleton participates in the regulation of the critical cell volume of erythrocyte we have attempted to check whether there exists any effect of the state of spectrin, the main constituent of the membrane skeleton, altered by thermal denaturation at 49 °C, on the glycolysis dependent change of the critical cell volume.

2. Materials and Methods

2.1. Erythrocytes

Fresh, heparinized, bovine blood was centrifuged at $2700 \times g$ for 10 min at 4 °C, plasma and buffy coat were discarded and the red cells were washed thrice with isotonic (310 mosM) solution A: 138 mM NaCl, 5

Abbreviations: ATP, adenosine 5′-triphosphate; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DIDS; 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid.

Correspondence: J. Gomułkiewicz, Institute of Physics, Technical University of Wrocław, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland.

mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 7.4). The chemicals were of analytical grade.

2.2. Cells treatment

(i) Denaturation of spectrin. Glas tubes containing the red blood cells suspension of 25% hematocrit were heated in a water bath at 49.0 ± 0.1° C for 10 min [31]. The temperature equilibrium was reached in less than 2 min. The samples were then cooled down to room temperature by swirling the tubes in a beaker of water.

(ii) Activation of glycolysis. The cells suspension of 5% hematocrit was incubated at 37° C for 2 h in solution B: 109 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 20 mM Na_2HPO_4/NaH_2PO_4, 10 mM glucose (pH 7.4).

(iii) Metabolic starvation. The erythrocytes suspension of 5% hematocrit was incubated at 37° C for 4 h in solution C: 114 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 20 mM Na_2HPO_4/NaH_2PO_4 (pH 7.4).

(iv) Inhibition of anion exchange. The suspension of red blood cells of 25% hematocrit was incubated at 37° C for 1 h in solution D: 143 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 20 μM DIDS, 5 mM Hepes (pH 7.4) [32]. DIDS was obtained from Sigma. All samples were washed twice after incubation with 10 volumes of the solution A.

2.3. Critical cell volume

The critical cell volume was calculated from van't Hoff's law modified for erythrocytes [2]. The osmotically non-active volume and the amount of intracellular solutes were obtained by a mathematically modified microhematocrit method described in detail in previous papers [30,33]. The measure of osmotic fragility was the mean osmolarity of solutes in which hemolysis occurred [30]. Hemolysis was carried out in hypotonic NaCl or mixed in the ratio 3:1 NaCl/KCl solutions buffered with 5 mM phosphate (pH 7.4). The relative isoosmotic volume of erythrocyte was determined from the compared absorbances of hemoglobin released from control and from erythrocytes in suspensions with identical hematocrits [30]. The volume of the solution trapped between packed cells was estimated by the hemoglobin method described elsewhere [33].

2.4. Concentration of ATP

The erythrocyte suspension was mixed with equal volume of trichloroacetic acid (12% w/w), left for 15 min and then centrifuged to clarity. The supernatant was assayed for ATP by the phosphoglyceric phosphokinase/ glyceraldehydephosphate dehydrogenase method using a standard kit from Sigma.

2.5. Extraction of spectrin

Erythrocyte ghosts were isolated from fresh bovine red blood cells, according to Dodge et al. [34]. Spectrin was extracted from erythrocyte ghosts at 37° C for 30 min with 0.1 mM EDTA in 0.3 mM phosphate buffer (pH 7.2) containing 20 μg/ml of phenylmethylsulfonyl fluoride. The concentrated extract was chromatographed on Sepharose CL-4B column (55 × 1.6 cm) equilibrated with 5 mM sodium phosphate buffer (pH 7.2) containing 50 mM NaCl, 0.1 mM 2-mercaptoethanol and 0.1 mM EDTA. The second peak consisting of spectrin dimer was collected, pooled and used for the fluorescence measurements. Purity of spectrin was tested in (0.1%) SDS (5.6%)-polyacrylamide gel electrophoresis [35]. Protein concentration was determined according to Mejbaum-Katzenellenbogen [36].

2.6. Fluorescence measurements

Fluorescence measurements were performed with a Perkin-Elmer MPF-3L spectrofluorimeter equipped with a temperature controlling device. The temperature of the sample was measured with a digital thermometer with a platinum resistance sensor. The excitation wavelength was 290 nm and the emission maximum was found to be 337 nm.

3. Results

3.1. Denaturation of spectrin

The change of the intensity of fluorescence of spectrin heated to 65° C at the rate of 2 C°/min was registered. The intensity of fluorescence decreased with the temperature, but at about 50° C it rapidly increased, and then it decreased again. That rapid increase of the intensity of fluorescence was not observed when the sample was heated again in the same way, to 65° C. The fluorescence intensity shift occurred within 8 min, also when the heating was stopped at 48–49° C. This was not seen at lower temperatures.

3.2. Intracellular concentration of ATP

The relative concentrations of ATP after different cell treatments are shown in Table I.

The incubation at 49° C for 10 min decreased the ATP level by 13% with respect to the control sample. A still larger decrease was observed in both metabolically starved erythrocytes, preincubated at 49° C, as well as in the non-heated but starved control. The ATP level after the starvation was lower in the preheated cells. However, the difference of the intracellular concentrations of ATP in both samples was then smaller than the initial 13%.

The incubation of red blood cells at 37° C for 2 h in a solution containing 20 mM phosphate and 10 mM glucose increased the intracellular concentration of ATP in the cells preheated at 49° C as well as in those non-heated. The ATP level after the activation of glycolysis was higher in the preheated cells. The mean values of the ATP level, relative with respect to the control, overlap due to the spread of values of the ATP

TABLE I

Effect of cell modifications on intracellular concentration of ATP and critical cell volume

All values are relative in respect to the control parameters (concentration of ATP, 310 ± 35 μM ($n = 6$); critical cell volume, 143.5 ± 4.8% ($n = 16$) of isoosmotic cell volume; mean ± S.D.). The numbers shown are confidence limits at $1 - P = 0.95$ level. The average values of the critical cell volume and their changes after the modifications of erythrocytes, measured in six samples with known ATP levels, did not differ statistically significantly from that value measured for $n = 16$ samples.

Modification	%	
	relative ATP level	relative critical cell volume
Denaturation of spectrin	87 ± 8	102.1 ± 0.8
Activation of glycolysis	124 ± 13	102.6 ± 1.1
Starvation	60 ± 23	98.9 ± 0.6
Denaturation of spectrin + activation of glycolysis	134 ± 15	101.9 ± 0.9
Denaturation of spectrin + starvation	54 ± 10	102.1 ± 0.9
Starvation + denaturation of spectrin	51 ± 12	101.7 ± 0.8

concentration, associated with the individual animal whose blood was studied. However, the differences between the ATP levels in the control and in the modified cells from the same animal were statistically significant at $P < 0.01$ in the paired t-test.

3.3 The critical cell volume

The changes of the critical cell volume after spectrin denaturation, an activation of glycolysis, and a metabolic starvation are shown in Table I as relative values with respect to the control. The values of all parameters used for the calculations, are shown in Table II in relative values to the corresponding parameters for the control cells. The values of the critical and of the osmotically non-active volumes were also multiplied by the relative isoosmotic volume of the modified cells, to allow a direct comparison of the data. The incubation of erythrocytes in a high-phosphate medium with glucose, or heating to 49 °C, led to an increase of the critical volume by about 2–2.5%. A metabolic starvation of erythrocytes led to a decrease of the critical cell volume. Neither the activation of glycolysis, nor the metabolic starvation of red cells preheated to 49 °C caused any further change of the critical cell volume. The increase of the critical cell volume of erythrocytes first starved and then heated was similar to that of the cells first preheated and then starved (Table I). The differences of both the critical cell volumes and the osmotic fragilities of the respective samples were small but usually significant in the paired t-test at $1 - P = 0.95$ (Table III).

To test whether the experimental procedures alter the hemolysis-protective potassium efflux [3,4], we mea-

TABLE III

Comparison of the alterations of critical cell volume and osmotic fragility

The differences between the parameters in all pairs of the control and the modified cells from the same animal were checked in the paired t-test, $P < 0.05$, $n = 16$; +, a significant difference; n.s., non-significant. Results for osmotic fragility above diagonal, for critical cell volume-beneath. Mean vlaues of critical cell volume and of osmotic fragility are shown in Tables I and II. 1, control; 2, upon spectrin denaturation; 3, upon glycolysis activation; 4, upon spectrin denaturation and glycolysis activation; 5, upon metabolic starvation; 6, upon spectrin denaturation and metabolic starvation.

	1	2	3	4	5	6
Osmotic fragility						
1	−	n.s.	+	+	+	+
2	+	−	+	+	+	+
3	+	+	−	+	+	+
4	+	n.s.	+	−	n.s.	+
5	+	+	+	+	−	+
6	+	n.s.	+	n.s.	+	−
Critical cell volume						

TABLE II

Effect of the state of spectrin and of glycolysis on osmotic properties of bovine erythrocyte

All values are relative in respect to the control parameters ($b = 44.8 \pm 2.1\%$ V_i; $\pi_h = 56.1 \pm 2.7\%$ (100% = 310 mosM); mean ± S.D., $n = 16$). Since the osmolarities and the cell volumes are in relative units, the amount of intracellular solutes equals $1 - b$ (for the control cells). The numbers shown are confidence limits at $1 - P = 0.95$ level, $n = 16$.

Modification	%			
	isoosmotic volume, V_i	amount of internal solutes, c	osmotically non-active volume, b	osmotic fragility, π_h
Denaturation of spectrin	101.7 ± 0.5	103.3 ± 2.5	99.4 ± 2.9	100.2 ± 0.6
Activation of glycolysis	98.6 ± 0.7	100.8 ± 3.0	96.4 ± 4.2	95.6 ± 1.6
Metabolic starvation	97.9 ± 0.5	90.3 ± 0.7	107.0 ± 1.8	94.8 ± 1.5
Denaturation of spectrin + activation of glycolysis	98.7 ± 0.7	96.2 ± 1.2	102.0 ± 1.6	94.6 ± 1.8
Denaturation of spectrin + metabolic starvation	98.2 ± 0.5	94.9 ± 1.5	101.8 ± 2.7	93.6 ± 1.4
Metabolic starvation + denaturation of spectrin	100.6 ± 0.6	95.9 ± 1.7	107.8 ± 2.1	97.5 ± 1.5

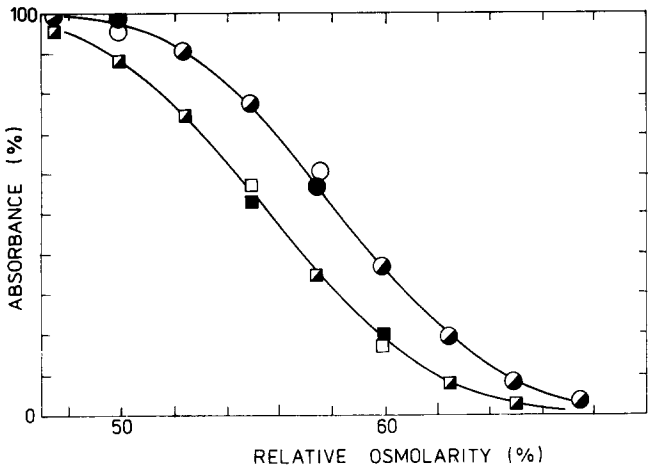

Fig. 1. Effect of cation composition of hypotonic solutions on osmotic fragility of bovine erythrocytes. Data for control sample (circles) and for sample with denatured spectrin and activated glycolysis (squares). Filled symbols, 100% Na^+; open symbols, Na^+/K^+ in the ratio of 3:1 in hypotonic solutions (in the intracellular solution of bovine erythrocytes potassium ions constitute no more than 25% of all cations [37]).

sured the osmotic fragilities of all samples in hypotonic NaCl and NaCl/KCl solutions [5]. The hemolysis curves did not depend on the cationic composition of hypotonic solutions. Since the shifts of the hemolysis curves, due to cell modifications, were similar, only one is shown as an example (Fig. 1).

The volumes of trapped extrcellular solution, which could influence the calculations of the osmotic parameters of erythrocyte, did not change significantly in all samples after the modifications, being within 2.2–2.8% of the isoosmotic cell volume.

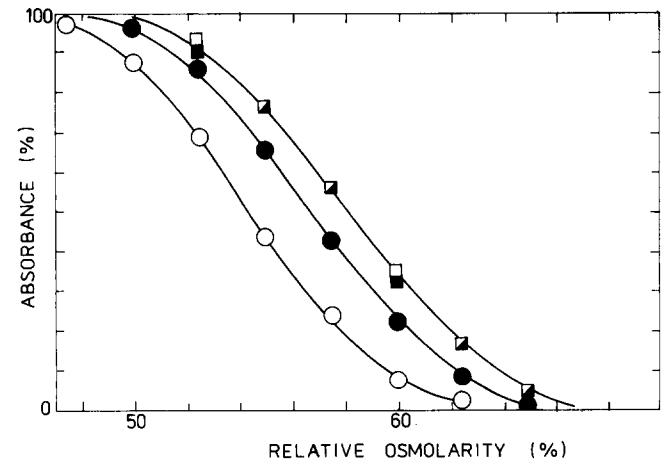

Fig. 2. Effect of the inhibition of anion exchange on the osmotic fragility shift caused by incubation at high phospahte concentration. The anion exchange was inhibited irreversibly by incubation of the cells at 37 °C for 1 h in solution containing 20 μM DIDS. Circles, erythrocytes with normal anion exchange; squares, erythrocytes with inhibited anion exchange. Filled symbols, incubation in solution buffered by 10 mM Hepes-NaOH; open symbols, incubation in solutions buffered by 20 mM phosphate.

The isoosmotic cell volume of erythrocytes has changed during incubation (Table II). The isoosmotic volume and the osmotic fragility of erythrocytes with anion exchange inhibited by DIDS were not changed upon incubation at 37 °C in a solution buffered with 20 mM phosphate in contrast to the DIDS non-treated red blood cells (Fig. 2).

4. Discussion

4.1. Denaturation of spectrin

Incubation of human erythrocytes at temperatures above 48 °C leads to a massive, irreversible thermal unfolding of spectrin [38,39] followed by an increase of the elastic shear modulus of the membrane [31,40], and of the membrane viscosity [40]. The rapid increase of the intensity of fluorescence, typical for protein denaturation [41], observed in this study for spectrin extracted from the bovine erythrocyte membrane, indicates that the denaturation of this protein occurs in a similar range of temperatures as for the spectrin from the human red cell. Bovine red blood cell heated under these conditions changed its shape from discocyte to stomatocyte but without any visible fragmentation or vesiculation of its membrane (as observed under the phase-contrast microscope), similar to the human erythrocyte [31].

4.2. Activation of glycolysis and metabolic starvation

The activation of glycolysis with phosphate ions increased the intracellular concentration of ATP, while the metabolic starvation decreased it. The increase of the ATP level after an activation of glycolysis in the cells preheated at 49 °C indicates that the heating did not lead to denaturation of glycolytic enzymes of bovine red blood cells. It agrees with the observation that the heating at 50 °C for 15 min did not change the activity of the studied glycolytic enzymes of bovine red blood cells [42]. The larger increase of the ATP level after an activation of glycolysis, and its smaller decrease after the metabolic starvation in the cells preheated at 49 °C point out that the utilization of the ATP is slower in these cells. The decrease of ATP utilization may be caused by the absence of phosphorylation of spectrin after its denaturation.

4.3. Critical cell volume

The calculation of the critical cell volume from the van't Hoff's law modified for erythrocytes [2], whose validity was proven up to a spherical shape of erythrocyte [5], required the measurements of the osmotically non-active volume, of the amount of intracellular solutes and of the osmotic fragility. The first two parameters were measured by the mathematically modified microhematocrit method [30,33]. Since their values are average for about 10^9 cells measured the measure of the

osmotic fragility was also computed as the mean relative osmolarity of the solutions in which the hemolysis took place [30]. The accuracy of the isoosmotic cell volume and of the osmotic fragility was 0.7%, while that of the measurement of the amount of internal solutes, and of the osmotically non-active volume, was 1%. However, an overestimation of the isoosmotic cell volume leads to the overestimation of the osmotic fragility, while an overestimation of the amount of internal solutes is, on the contrary, correlated with the underestimation of the osmotically non-active volume. Therefore, the standard error of the critical cell volume calculated from these parameters equals only 0.6%, since the erros caused by the parameters reduce each other. A computer simulation yielded the same estimation of the accuracies.

The hemolysis protective potassium efflux, observed in human erythrocytes [3–5], seems to be, in the light of our results (Fig. 1), nonexistent or unmeasurably low in normal and experimentally modified bovine red blood cells. The observed, statistically non-significant, changes ofo the volume of the trapped solution between packed cells could result in a 0.25% change of the critical cell volume at most [33].

The isoosmotic volume of the cells incubated in high-phosphate media decreased (Table II). A lack of such change for the cells with the anion exchange inhibited by DIDS suggests that the decrease of the isoosmotic cell volume due to the incubation in high phosphate media may be caused by an exchange of divalent phosphate for monovalent chloride ions, followed by water efflux. The increase of the isoosmotic volume of the cells heated at 49 °C was probably a result of a higher permeability of the erythrocyte membrane for sodium than for potassium ions. An elevated influx of ions at the raised temperature, followed by water influx, may cause the increase in both the amount of internal solutes and the isoosmotic volume (Table II).

The osmotically non-active volume of erythrocyts depends on the intracellular pH [1]. The opposite effects of an activation of glycolysis and of metabolic starvation on that volume may, therefore, be a consequence of the different pH of the intracellular solution at different metabolic states of the cell. A lack of such dependence for the preheated cells might be caused by an influence of heating on the osmotic properties of hemoglobin.

4.4. Mechanism of the change of the critical cell volume

The consideration of the influence of the amount of intracellular solutes, and of both the osmotically non-active and the isoosmotic volumes, on the calculated critical cell volume allows us to conclude that the observed changes of this last parameter in the experimentally modified cells was a membrane-linked phenomenon. The reported changes of the critical cell volume of 1–3%, small in comparison with the critical volume itself, seem now to be large when compared with the increase of the cell volume due to the elastic extensibility of the membrane which is 4–9% of the spherical volume of erythrocyte [6–8].

A weaker interaction of the denatured spectrin with the lipid protein matrix [43] may lead, according to the Evans-Skalak model of the erythrocyte membrane [7], to its larger extensibility and, therefore, to a larger critical cell volume, too. The effect of spectrin denaturation on the critical cell volume did not depend on the metabolic state of the erythrocyte (Table I).

It is interesting, whether and how the changes of the intracellular ATP concentration may influence the membrane extensibility. The lack of any further effects of the ATP level on the critical cell volume of the erythrocyte with denatured spectrin suggests that the interaction(s) between the membrane skeleton and the lipid-protein matrix may also be involved in this case. Phosphorylation of some proteins of membrane skeleton may provide a mechanism for the creation of a relaxed, flexible structure of the membrane skeleton [15,23–25] interacting more weakly with the lipid-protein matrix.

The effect of the intracellular concentraiton of ATP on the lipid mobility of bovine erythrocyte membrane has been observed [44]. However, it seems necessary to find out whether the lipid mobility may affect directly the membrane extensibility.

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References

1 Ponder, E. (1948) in Hemolysis and Related Phenomena, pp. 53–170, Churchill, London.
2 Dick, D.A.T. (1959) Int. Rev. Cytol. 8, 387–448.
3 Seeman, P., Sauks, T., Argent, W. and Kwant, W.O. (1969) Biochim. Biophys. Acta 183, 476–489.
4 Jay, A.W.L. and Rowlands, S. (1975) J. Physiol. 252, 817–832.
5 Richieri, G.V. and Mel, H.C. (1985) Biochim. Biophys. Acta 813, 41–50.
6 Evans, E.A., Waugh, R. and Melnik, L. (1976) Biophys. J. 16, 585–595.
7 Evans, E.A. and Skalak, R. (1980) in Mechanics and Thermodynamics of Biomembranes, pp. 173–233, CRC Press, Boca Raton, FL.
8 Massaldi, H.A., Fuchs, A. and Borzi, C.H. (1983) J. Biomech. 16, 103–107.
9 Nakao, M., Nakao, T. and Yamazoe, S. (1960) Nature 187, 945–946.
10 Weed, B.I., LaCelle, P.L. and Merril, E.W. (1969) J. Clin. Invest. 48, 795–809.
11 LaCelle, P.L. (1969) Transfusion 9, 238–245.
12 Backman, L. (1986) J. Cell Sci. 80, 281–298.
13 Jinbu, Y., Nakao, M., Otsuka, M. and Sato, S. (1983) Biochem. Biophys. Res. Commun. 112, 384–390.
14 Jinbu, Y., Sato, S., Nakao, M. and Tsukita, S. (1984) Expt. Cell Res. 151, 160–170.

15 Cianci, C.D., Giorgi, M. and Morrow, J.S. (1988) J. Cell. Biochem. 37, 301–315.
16 Anderson, R.A. and Marchesi, V.T. (1985) Nature 318, 295–298.
17 Ferrel, J.E. and Huestis, W.H. (1984) J. Cell Biol. 98, 1992–1998.
18 Birchmeier, W. and Singer, S.J. (1977) J. Cell Biol. 73, 647–659.
19 Sheetz, M.P. and Singer, S.J. (1977) J. Cell Biol. 73, 638–646.
20 Pinder, J.C., Dhermy, D., Baines, A.J., Lux, S.E. and Gratzer, W.B. (1983) Br. J. Haematol. 55, 455–463.
21 Lutz, H.V., Stringaro-Wipf, G. and Maretzki, D. (1986) J. Cardiovasc. Pharmacol. 8, S76–S79.
22 Fowler, V.M. (1986) J. Cell. Biochem. 31, 1–9.
23 Cohen, C.M. and Foley, S.F. (1986) in Membrane Skeletons and Cytoskeletal-Membrane Associations, pp. 211–222, Alan R. Liss, New York.
24 Eder, P.S., Soong, C.-J. and Tao, M. (1986) Biochemistry 25, 1764–1770.
25 Lu, P.W., Soong, C.-J. and Tao, M. (1985) J. Biol. Chem. 260, 14958–14964.
26 Haest, C.W.M. and Deuticke, B. (1975) Biochim. Biophys. Acta 401, 468–480.
27 Bütikofer, P. and Ott, P. (1985) Biochim. Biophys. Acta 821, 91–96.
28 Rose, A.I., Warms, J.V.B. and O'Connel, E.L. (1964) Biochem. Biophys. Res. Commun. 15, 33–37.
29 Wang, X.-L., Gallagher, C.H., McClure, T.J., Reeve, V.E. and Canfield, P.J. (1985) Res. Vet. Sci. 39, 333–339.
30 Mosior, M. and Gomułkiewicz, J. (1985) Stud. Biophys. 107, 169–178.
31 Rakow, A.L. and Hochmuth, R.M. (1975) Biophys. J. 15, 1095–1100.
32 Cabantchik, Z.I. and Rothstein, A. (1972) J. Membr. Biol. 10, 311–330.
33 Mosior, M., Białas, W.A. and Gomułkiewicz, J. (1988) Biochim. Biophys. Acta 945, 51–55.
34 Dodge, J.T., Mitchell, J.T. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119–130.
35 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2616.
36 Mejbaum-Katzenellenbogen, W. (1955) Acta Biochim. Polon. 2, 279–294.
37 Bartoz, G., Świerczyński, B. and Gondko, K. (1981) Experientia 37, 323.
38 Brandts, J.F., Erickson, L., Lysko, K., Schwartz, A.T. and Taverna, R.D. (1977) Biochemistry 16, 3450–3454.
39 Minetti, M., Ceccarini, M., Di Stasi, A.M.M., Petrucci, T.C. and Marchesi, V.T. (1986) J. Cell. Biochem. 30, 361–370.
40 Kucera, W., Meier, W. and Lerche, D. (1986) Biomed. Biochim. Acta 45, 353–358.
41 Freifelder, D. (1980) in Physical Biochemistry. Applications to Biochemistry and Molecular Biology, pp. 423–427, Mir, Moskva (in Russian).
42 Bartosz, G. (1980) Mech. Ageing Dev. 13, 379–385.
43 Voshino, H. and Minari, O. (1987) Biochim. Biophys. Acta 905, 100–108.
44 Mosior, M., Mikołajczak, A. and Gumułkiewicz, J. (1990) Biochim. Biophys. Acta 1022, 361–364.