Biochimica et Biophysica Acta, 946 (1988) 429–430
Elsevier

BBA 70588

BBA Report

Elastic properties of the erythrocyte membrane and the critical cell volume of erythrocytes

Marian Mosior

Institute of Physics, Technical University of Wrocław, Wrocław (Poland)

(Received 9 May 1988)

Key words: Osmotic fragility; Critical cell volume; Membrane elasticity; (Human erythrocyte)

The results on elastic membrane area extension during hemolysis, reported by Richieri and Mel (Richieri, G.V. and Mel, H.C. (1985) Biochim. Biophys. Acta 813, 41–50), are discussed. Careful analysis of their data leads to the conclusion, that the differences in osmolarity, as found in the experiment, were insufficient to cause the reported values of elastic changes in erythrocyte volume (17–22%) and of membrane area extension (11–14%). The recalculated values of the elastic extensions of membrane area are not different from those measured by the micropipet method (i.e. 3–4%).

The role of elastic properties of erythrocyte membrane in hemolytic phenomena has been discussed for several decades [1–4]. In the seventies Evans and co-workers reported that the elastic area compressibility modulus of the membrane of human erythrocyte, measured by a micropipet method, was 0.45 N/m, while the maximal fractional area expansion of the membrane of intact cells was 2–4% [4,5]. Comparable results were obtained by a cell poking method [6]. Recently, Richieri and Mel [1], using resistive pulse spectroscopy, confirmed previous reports [7,8] on hemolysis as a two-phase process. The red blood cells, placed in hypotonic medium, reached spherical shape with a volume of 140 μm^3, and then, for a short time, elastically increased their membrane area. Dependent on the external tonicity the cells lysed, or returned to the steady-state volume of 140 μm^3. Assuming the hemolytic volumes of erythrocytes to be equal to the ghost volumes, measured after hemolysis, Richieri and Mel [1] reported that the elastic membrane area expansion was temperature dependent. They found an increase of at least 14% in membrane area, without hemolysis at 40 °C – much more than the 2–4% membrane extension at 25 °C, reported previously by Evans et al. [5].

However, the data of Richieri and Mel allow us to calculate the critical cell volume of erythrocyte, V_c, from van't Hoff's law modified for red blood cells [3],

$$V_c = \frac{\varphi \cdot n}{\pi_h} + b$$

in which φ is the mean osmotic coefficient of internal solutes, n is the amount of internal solutes, π_h is the mean osmolarity of solutions in which hemolysis took place, and b is the osmotically non-active volume of the cell. If the distribution function of osmotic fragility were symmetrical the mean osmolarity of the solution in which erythrocytes hemolyzed, π_h, would be equal to the osmolarity of the solution in which 50% cells hemolyzed, π_{50}. From the data of Richieri and

Correspondence: M. Mosior, Institute of Physics, Technical University of Wrocław, Wybrzeże St. Wyspiańskiego 27, 50-370 Wrocław, Poland.

Mel (Ref. 1, Figs. 1 and 7) it follows that at 40 °C $b = 40$ μm^3, $\pi_{50} = 111$ mosM and $\varphi \cdot n = 11.3$ mosM $\cdot$ μm^3. Thus the critical cell volume, calculated from van't Hoff's law, assuming that the hemolysis protective potassium efflux [8,9] was negligible and the elastic area compressibility modulus $K = 0$, was 158 μm^3, i.e. 113% of the steady-state volume. However, in high-KCl hypotonic solutions, where the hemolysis-protective potassium efflux was limited, Richieri and Mel found an increase of hemolysis from 23 to 49%; equivalent to a shift of the hemolysis curve to the more fragile region by 5 mosM. The compressibility area modulus K equals 0.45 N/m [4], thus the difference in osmolarity between both sides of erythrocyte membrane, required e.g. for an 4% area expansion, is 2.3 mosM. The maximal cell volume which the red cell should reach in a 111 mosM solution, calculated from van't Hoff's law taking into account the hemolysis-protective potassium efflux and the membrane tension, is 150 μm^3 i.e. 107% of the steady-state volume. The 4% membrane area extension is equivalent to a 6% increase of cell volume, which is in good agreement with the 7% increase calculated from the data of Richieri and Mel, but significantly less than their reported value of 17% (11% increase of membrane area).

Also, the reported increase of erythrocyte volume above 170 μm^3 in a 120 mosM solution at 40 °C is difficult to understand. The maximal volume, which red cells should reach in such a case, is 149 μm^3 (assuming that $K = 0$). It is equivalent to a 3–4% extension of membrane area–also significantly less than the reported value of 14%. It thus seems that the elastic expansion of membrane area of the erythrocyte during hemolysis does not differ from that measured by the micropipet method [5].

Since in the experiment discussed the van't Hoff's law was satisfied (as follows from Fig. 7), the above considerations lead to the conclusion that the volume of ghosts after the erythrocyte hemolysis was not equal to the hemolytic volume of the cells. The larger volume of the ghosts, and its dependence on temperature, may be explained by temperature-dependent changes of the diameter of the hole occurring in the erythrocyte membrane during hemolysis [10,11]. The dependence of the maximal area expansion on temperature, evident also for the calculated critical cell volume, may result from the well known temperature dependence of membrane fluidity. At low temperatures a more rigid membrane may be ruptured by a smaller membrane extension.

Erythrocytes would have reached the volume of 170 μm^3 in a 120 mosM solution, as reported by Richieri and Mel, if the amount of internal solutes had increased during cell swelling. This would have been possible, if the stretched membrane of erythrocyte had had a selective permeability for sodium ions. The influx of sodium ions would have then resulted in a water influx and the cell would have reached a larger volume than that calculated using van't Hoff's law. However, only net potassium efflux during erythrocyte swelling and hemolysis [8,9,12] has been reported. So, a large increase in volume of the spherical state of erythrocyte swelling should have been visible during direct microscopic observations of the hemolysis; this, however, does not seem to have been observed [8].

References

1 Richieri, G.V. and Mel, H.C. (1985) Biochim. Biophys. Acta 813, 41–50.
2 Ponder, E. (1948) in Hemolysis and Related Phenomena, pp. 50–114, Churchill, London.
3 Dick, D.A.T. (1959) Int. Rev. Cytol. 8, 387–448.
4 Evans, E.A. and Skalak, R. (1980) in Mechanics and Thermodynamics of Biomembranes, pp. 173–233, CRC Press, Inc., Boca Raton, FL.
5 Evans, E.A., Waugh, R. and Melnik, L. (1976) Biophys. J. 16, 585–596.
6 Daily, B., Elson, E.L. and Zahalak, G.I. (1984) Biophys. J. 45, 671–82.
7 Saari, J.T. and Beck, J.S. (1975) J. Membr. Biol. 23, 213–226.
8 Jay, A.W.L. and Rowlands, S. (1975) J. Physiol. 252, 817–832.
9 Seeman, P., Sauks, T., Argent, W. and Kwant, W.O. (1969) Biochim. Biophys. Acta 183, 476–489.
10 Lieber, M.R. and Steck, T.L. (1982) J. Biol. Chem. 257, 11651–11659.
11 Lieber, M.R. and Steck, T.L. (1982) J. Biol. Chem. 257, 11660–11666.
12 Eskelinen, S. (1983) Biomed. Biochim. Acta 42, 97–101.