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Diffusional Water Permeability of Bovine Erythrocytes: A Pulse Nuclear Magnetic Resonance Study*

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A pulse nuclear magnetic resonance (NMR) technique is employed to study the temperature dependence of the diffusional water exchange time τ_{exch} for normal and *p*-hydroxymercuribenzoate (*p*-MB) treated bovine erythrocytes. The method is based on measurements of the proton NMR transverse relaxation function of water exchange between the diamagnetic intracellular space and the paramagnetically doped extracellular solution of the erythrocyte suspension. The semi-log plots of τ_{exch} *versus* inverse absolute temperature for both normal and mercurial treated erythrocytes are straight lines, implying that one activation energy (E_a) determines the rate of transmembrane diffusional water transport in the whole temperature range studied (5–40 °C). For normal erythrocytes, the value E_a of 20.4 kJ/mol is similar to that for selfdiffusion of water (E_a = 19.3–20.1 kJ/mol). The value τ_{exch} = 12.5 ms, obtained for normal bovine erythrocytes at 20 °C, corresponds to the cell membrane diffusional water permeability coefficient P_d of 3.6×10^{-3} cm/s. This is similar to the range of room temperature P_d between 3.3 and 4.7×10^{-3} cm/s, deduced recently for fresh normal human erythrocytes. The data for *p*-MB treated erythrocytes display a lengthening of τ_{exch} and increased E_a of 29.0 kJ/mol. This E_a value and a permeability coefficient P_d of 2.6×10^{-3} cm/s at 20 °C, if compared to the corresponding permeability data for artificial lipid bilayer membranes, indicate either only partial inhibition of the mercurial sensitive part of the membrane diffusional water permeability, possibly as a result of poor accessibility of the functionally important transmembrane protein SH-groups to *p*-MB, or »complete« inhibition plus new leaks.

INTRODUCTION

Transport properties of biological membranes have been subject to great research activity for many years. One aspect of this research area refers to water balance in living systems. Consideration of this topic raised discussion of how water permeates

* Dedicated to Professor Dušan Hadži on the occasion of his seventieth birthday.

the cell membrane but no conclusive evidence for the mechanism of water transport across a membrane has been put forward as yet. A mosaic model[1] of the plasma membrane emphasizes the existence of at least two parallel permeation pathways, namely across the lipid bilayer portion of the membrane and through the polar channels that span the membrane, which are provided by the presence of integral proteins. Regarding water exchange across the lipid bilayer, Träuble[2] proposed that water permeates by transient pockets of free volume that are formed by the thermal motion of the hydrocarbon chains. The diffusion coefficient of these mobile pockets (called »kinks«) was calculated to be of the order of 10^{-5} cm^2 s^{-1}. The corresponding theoretical value for the room temperature water permeability coefficient[2] was $P = 1.3 \times 10^{-3}$ cm s^{-1}. This theoretical water permeability is systematically lower than the values found experimentally.[3] Namely, though attractive, Träuble's hypothesis anticipates that the hydrocarbon phase of the bilayer lipids is much more ordered than it now appears to be the case. This observation implies that one does not know to what extent kinks actually cross the lipid bilayer and whether water molecules may hop from one kink to another.[3]

In plasma membranes, however, in addition to this nonspecific water permeation across the lipid bilayer, there exists a parallel hydrophilic pathway of water transport through the polar channels or pores that span the membrane. Functional evidence for the existence of such channels – called water channels – has emerged primarily from the characteristic differences in water permeability properties between the erythrocyte and pure phospholipid membranes.[4,5] Red blood cells are preferably chosen for such comparative studies because of their availability, relatively simple structure lacking internal membrane systems and the small number of their integral membrane proteins. Thus, the measured activation energy E_a of 17–25 kJ mol^{-1} for the water diffusional (P_d) and osmotic (P_f) permeabilities of human and dog erythrocyte membranes[6] is similar to the value $E_a = 19.3$ kJ mol^{-1} for self-diffusion of water[7] but much lower than the activation energies $E_a \approx 46$–60 kJ mol^{-1} for permeation of water through lipid bilayers.[3] The P_f values of red blood cells are an order of magnitude higher than those for lipid bilayers despite the high degree of variability for bilayers of different compositions. In erythrocytes P_f is several times higher than P_d,[8] while in artificial bilayers $P_f = P_d$.[9] The osmotic or filtration permeability P_f is defined by the net transmembrane water flow, induced by an osmotic gradient across the membrane or by application of hydrostatic pressure. P_f is usually determined from the kinetics of cell or vesicle volume change in response to an imposed transmembrane osmotic gradient. Volume change is conveniently followed *via* the time course of scattered or transmitted light intensity.[10] P_d is defined by transmembrane diffusional water exchange under stationary conditions. Determination of P_d is more demanding because of very short water exchange times (~10 ms) in erythrocytes and in small liposomes. Diffusional water exchange through the erythrocyte membrane is measured either by the radioactive tracer (THO) diffusion methods[8,6,11] (*e.g.*, the diffusional efflux of tritium-labeled water counterbalanced by an influx of nonradioactive water), or by the doping nuclear magnetic resonance (NMR) techniques.[12-14] (Paramagnetic doping of the erythrocyte extracellular suspension with Mn^{2+} ions at concentrations at least up to 19 mM was shown[11] not to change diffusional water permeability).

Valuable information about water transport processes in the erythrocyte membrane has come from the evaluation of the effects of various membrane reagents that chemically modify water channels. Unique among these chemical probes are mer-

curial sulfhydryl reagents, including inorganic Hg (*e.g.*, $HgCl_2$), which appear to be the only potent inhibitors of water transport.[15] Among them, for instance, *p*-chloromercuribenzene sulfonate (*p*-CMBS) at saturating doses (≥2 mM) decreases the osmotic water permeability P_f of human red blood cells by an order of magnitude, increases the activation energy E_a for water permeation from ~20 kJ mol^{-1} to ~48 kJ mol^{-1}, and decreases the ratio P_f/P_d to unity,[16] so that the water transport properties of *p*-CMBS treated erythrocytes become practically indistinguishable from those of lipid bilayers. A simple explanation of this observation is that organic mercurials block water channels by binding to sulfhydryl (–SH) groups in integral membrane proteins associated with water channels, leaving the primitive lipid bilayer matrix as the only alternative route for water transport.[4] This view favours the specificity of water channels (transporting water and very little else), while an opposite unitary pore hypothesis[17] attempts to confine transport pathways of all small polar permeants, including water, anions, cations and nonelectrolytes, to the same transport pathway in the form of an aqueous pore ~9 Å in diameter that passes between the two monomers of band 3 membrane integral protein. There are arguments[4] against this hypothesis, *e.g.*, that a dramatic inhibition of erythrocyte water permeability by *p*-CMBS has no effect on transmembrane anion exchange while cation permeability is even increased by at least an order of magnitude, or that the anion exchange capacity in erythrocytes of diverse origin (human, chicken, duck, amphiuma) is found similar in all four types while water and urea permeabilities are both high in human cells, both low in chicken cells, high and low in duck cells, and low and high in amphiuma cells, respectively. Thus, a fully functional band 3 anion transport system can exist independently of the other solute or water transport systems. Nevertheless, water permeation inhibition experiments with a radioactively (^{203}Hg) labelled SH-reactive mercurial *p*-CMBS[18] point to the major integral membrane proteins band 3 and band 4.5 as being involved in water permeation; the third major erythrocyte transmembrane protein glycophorin could not be associated with the water channel because it lacks SH groups. On the other hand, the importance of the integral membrane proteins for water transport above the ground (lipid-bilayer-like) permeability was not revealed (*i.e.*, P_d and P_f were not changed) by treating the erythrocyte membranes with protein fixatives[11] that induce extensive conformational changes by forming cross-links in all the erythrocyte proteins. As a result, the intermediate phase between integral membrane proteins and their surrounding lipids is suggested as a possible location of water channels.[11]

These introductory remarks are intended to illustrate some of the open problems of transmembrane water transport. Measurement of water permeability under various physico- chemical conditions proves to be an important source of information, essential in clarifying various aspects of erythrocyte membrane behaviour. In this report, pulse nuclear magnetic resonance (NMR) is employed to study the temperature dependence of the diffusional water exchange time τ_{exch} in normal and in mercurial (*p*-hydroxymercuribenzoate, *p*-MB) treated bovine erythrocytes.

THEORETICAL CONSIDERATIONS

Several pulse NMR techniques have been employed for the determination of water diffusional transport across the erythrocyte membrane. Information about the membrane water permeation rate has been obtained from the analysis of measured NMR quantities which are inherently modified by the transmembrane diffusional water exchange. This information is contained in the proton NMR transverse (T_2) and

longitudinal (T_1) relaxation function of water exchanging across the erythrocytes in suspensions doped extracellularly with certain (small) amounts of paramagentic ions (*e.g.*, Mn^{2+}),[12-14] in the ^{17}O NMR longitudinal (T_1) relaxation curve of the erythrocyte suspension enriched with $H_2{}^{17}O$,[19] or in restricted diffusion data of water molecules within the erythrocytes,[20] using a pulsed field gradient NMR method.

The use of pulse NMR techniques to determine the rate of water diffusional exchange across the erythrocyte membrane is based on the following principle.[12] When water molecules are placed in a magnetic field, their proton spins become oriented. Application of a radiofrequency pulse reorients the spins and this reorientation serves as a label that can be detected by NMR. Following the application of the pulse, this label relatively slowly decays both in the intracellular space and in the extracellular solution of the erythrocyte suspension. However, if impermeable paramagnetic ions are added into the extracellular space, the label in this compartment is very quickly quenched (say, within 1 ms). Application of a radiofrequency pulse has actually the role of labelling both intra- and extracellular water. However, the label in the extracellular water disappears very soon, because of the quenching action of added paramagnetic ions, and only cellular water remains labelled. Therefore, the decay of label from the total sample is almost entirely due to the transmembrane water exchange process. Thus, water leaving the cells loses label almost immediately after contacting the extracellular solution, while water entering the cells is mostly unlabeled. In other words, the time constant of the decay of label from the cell suspension is effectively determined by the mean life time of water molecules in the cells.

In this paper the T_2-method[14] is used, in which the water proton NMR transverse relaxation function of a paramagnetically doped erythrocyte suspension is measured by the Carr-Purcell-Meilboom-Gill (CPMG) pulse sequence.[21,22] Water exchange between the diamagnetic erythrocyte interior (compartment *a*) and the paramagnetic extracellular solution (compartment *b*) represents a typical two- site exchange problem, giving rise to a double exponential proton NMR transverse relaxation curve $M(t)$ of the form[23]

$$M(t) = P'_a \exp(-t/T'_{2a}) + P'_b \exp(-t/T'_{2b}), \tag{1}$$

where the sum of the coefficients is

$$P'_a + P'_b = 1 \tag{2}$$

and coefficient P'_b is given by

$$P'_b = \frac{1}{2} - \frac{1}{4}\left[(P_b - P_a)(1/T_{2a} - 1/T_{2b}) + 1/\tau_a + 1/\tau_b\right]/C_2 \tag{3}$$

and where

$$1/T'_{2a} = C_1 - C_2, \tag{4}$$

$$1/T'_{2b} = C_1 + C_2, \tag{5}$$

$$C_1 = \frac{1}{2}(1/T_{2a} + 1/T_{2b} + 1/\tau_a + 1/\tau_b), \tag{6}$$

$$C_2 = \frac{1}{2}\left[(1/T_{2b} - 1/T_{2a} + 1/\tau_b - 1/\tau_a)^2 + 4/(\tau_a\tau_b)\right]^{1/2} \quad (7)$$

Here, P_a and P_b are the fractions of the total water proton signal in the two components, and τ_a and τ_b are the mean lifetimes of the water molecule in each compartment. Because of detailed balancing, we also have

$$P_a + P_b = 1 \quad (8)$$

and

$$P_a/\tau_a = P_b/\tau_b \quad (9)$$

The diffusional water exchange time τ_{exch} is characterized by the mean life time τ_a of water in the red blood cell:

$$\tau_{exch} = \tau_a \quad (10)$$

The water exchange time τ_{exch} was shown[24] to be independent of hematocrit. The diffusional water permeability P_d of the erythrocyte membrane ir related to τ_{exch} by

$$P_d = (V/A)(1/\tau_{exch}), \quad (11)$$

where V is the intracellular solvent volume and A the cell membrane area of the erythrocyte, respectively. V represents[11,23] some 70% to 80% of the total erythrocyte volume V_c, while the remaining fraction of V_c is inaccessible to water because of the presence of intracellular proteins (mostly hemoglobin). The experimentally determined transverse NMR relaxation curve yields three independent quantities (T_{2a}', T_{2b}' and P_a' or P_b'), but these, in turn, contain four parameters to fit theoretically the curve (T_{2a}, T_{2b}, P_a or P_b, and τ_a or τ_b) . Thus, one of these four parameters has to be measured independently. In practice, this is T_{2a} and is measured on a sample of densely packed red blood cells (without extracellularly added paramagnetic Mn^{2+}).

EXPERIMENTAL

The water proton NMR transverse relaxation curves have been measured on isolated normal and *p*- hydroxymercuribenzoate (*p*-MB) treated bovine erythrocytes suspended in isotonic buffered solutions (0.13 M NaCl, 0.02 M Tris. HCl, pH 7.4), containing 19.2 mM $MnCl_2$. The mercurial treated samples contained 3 mM *p*-MB; treatment with *p*-MB at room temperature induced maximum inhibition of the erythrocyte membrane diffusional water permeability 15 to 30 minutes after addition of the mercurial.[25] The red blood cells were prepared from fresh titrated blood by standard methods. The volume fraction of the red cells in the samples was 30%. Relaxation measurements were performed on a Bruker B-KR 322 s pulsed NMR spectrometer at the proton resonance frequency of 56.7 MHz for two values of the 180°-pulse separation in the CPMG sequence (0.6 ms and 1 ms, respectively). Measurements were performed at the temperature interval between 5 and 40 °C. The temperature was varied by streaming thermostated nitrogen over a 7 mm O.D. glass tube containing ~0.3 ml of the sample. In order to prevent natural aggregation of red cells into rouleaux, which is shown[23] to cause most of the variation among results from NMR experiments, the sample was carefully stirred immediately ($\leq$10 seconds) before each recording of the NMR relaxation curve. The intracellular water proton transverse relaxation time T_{2a} was measured separately between 5 and 40 °C on a sample of packed cells[26,27] (with no added $MnCl_2$), in which case the 180°-pulse separation in CPMG se-

quence was 6 ms. The experimental NMR transverse relaxation curves $M(t)$ were analyzed in terms of the theoretical expression (1) on a VAX-11/750 computer by the method of minimization of a sum of squares.

RESULTS AND DISCUSSION

A typical example of the computer fit to the measured $M(t)$ data in terms of Eq. (1) is shown in Figure 1. The temperature dependence of the diffusional water exchange time τ_{exch} obtained by this analysis for normal and *p*-MB treated bovine erythrocytes are presented as the Arrhenius plots in Figure 2. In the limits of experimental error these plots are straight lines and, hence, in both cases characterized by one activation energy E_a that determines the rate of transmembrane diffusional water permeation in the whole temperature range studied. For normal erythrocytes, the value E_a = 20.4±1.3 kJ mol^{-1} is of comparable magnitude as the activation energy of self-diffusion of water[7] (E_a = 19.3-20.1 kJ mol^{-1}), meaning that water molecules on their way through the membrane channels either interact only little with the channel components or the activation energy of the corresponding interaction has a value comparable to that of self-diffusion of water.[4,11] On the other hand, the data of Figure 2 for *p*-MB treated erythrocytes display an overall lengthening of τ_{exch} with respect to the corresponding values for untreated cells, and, in particular, an increased activation energy E_a of 29.0±1.9 kJ mol^{-1}. Yet, this E_a value indicates that only partial inhibition of the membrane diffusional water permeability was obtained. Namely, maximal inhibition of transmembrane water transport would be obtained if the activation energy E_a (as derived from the temperature dependence of τ_{exch}) were similar to the theoreti-

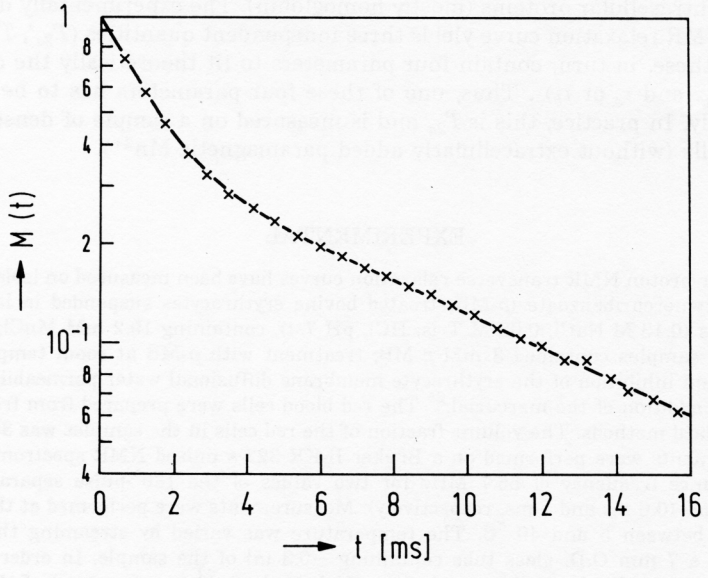

Figure 1. An example of the computer fit (line) to the measured $M(t)$ curve as determined by the echo amplitudes (crosses) in the Carr-Purcell-Meiboom-Gill pulse sequence applied to a paramagnetic suspension (19.2 mM $MnCl_2$) of normal bovine erythrocytes (30% hematocrit, at 39 °C).

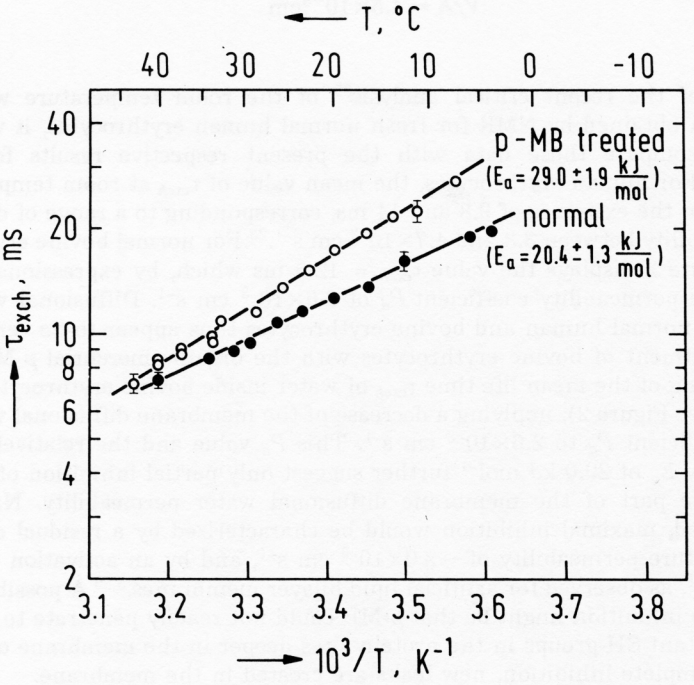

Figure 2. Temperature dependence of the diffusional water exchange time constant τ_{exch} for normal and mercurial (*p*-hydroxymercuribenzoate, *p*-MB) treated bovine erythrocytes.

cally[28] and experimentally[29] verified value of 50–58 kJ mol^{-1} for water diffusion through the hydrocarbon phase of a lipid bilayer. In this latter case, the organic mercurial *p*-MB would appear to completely block water channels by its binding to SH-groups in integral membrane proteins associated with water channels, leaving a residual permeability due to the lipid bilayer portion of the membrane.[4] Permeability inhibition experiments with the mercurial *p*-CMBS on erythrocyte ghosts have shown that the maximal inhibition is obtained if *p*-CMBS is added before resealing the erythrocytes.[11] In this way, both sides of the membrane were exposed to *p*-CMBS. Thus, addition of the mercurial to the inside of the membrane appears to facilitate inhibition as a result of better access of *p*-CMBS to the functionally important population of SH-groups, probably located deeper in the membrane.

The exchange time τ_{exch} is connected to the diffusional water permeability coefficient P_d of the erythrocyte membrane by relation (11) as $P_d = (V/A)(1/\tau_{exch})$ where (V/A) is the ratio between the intracellular solvent volume (V) and the cell membrane area (A). For bovine erythrocytes, the average values of the cell volume V_c and of the cell membrane area A at room temperature are $V_c = 0.48\times10^{-10}$ cm^3 and $A = 0.76\times10^{-6}$ cm^2, respectively.[30] Taking[11] that about 30% of the isotonic cell volume is occupied by intracellular protein components (mostly hemoglobin), the cell water fraction is $V \approx 0.7\ V_c = 0.34\times10^{-10}$ cm^3, so that the ratio V/A for bovine erythrocytes is estimated as:

$$V/A \approx 4.5\times10^{-5}\text{cm}. \quad (12)$$

In view of the recent critical analysis[23] of the room temperature water permeability data obtained by NMR for fresh normal human erythrocytes, it will be instructive to compare these data with the present respective results for bovine erythrocytes. For human erythrocytes, the mean value of τ_{exch} at room temperature is placed between the extremes of 9.8 and 14 ms, corresponding to a range of diffusional water permeability between 3.3 and 4.7×10^{-3} cm s^{-1}.[23] For normal bovine erythrocytes at 20 °C, Figure 2 displays the value τ_{exch} = 12.5 ms which, by expressions (11) and (12), gives the permeability coefficient P_d of 3.6×10^{-3} cm s^{-1}. Diffusional water permeabilities of normal human and bovine erythrocytes thus appear to be very similar. However, treatment of bovine erythrocytes with the organic mercurial *p*-MB results in a lengthening of the mean life time τ_{exch} of water inside bovine erythrocytes to 17.3 ms at 20 °C (see Figure 2), implying a decrease of the membrane diffusional water permeability coefficient P_d to 2.6×10^{-3} cm s^{-1}. This P_d value and the relatively low activation energy E_a of 29.0 kJ mol^{-1} further suggest only partial inhibition of the mercurial sensitive part of the membrane diffusional water permeability. Namely, as already stressed, maximal inhibition would be characterized by a residual or ground room temperature permeability of $\sim2.0\times10^{-3}$ cm s^{-1}, and by an activation energy of 50–58 kJ mol^{-1}, as observed for artificial lipid bilayer membranes.[3,29] A possible reason for incomplete inhibition might be that *p*-MB could not readily penetrate to the functionally important SH-groups in the protein sites deeper in the membrane or that, in addition to complete inhibition, new leaks are created in the membrane.

CONCLUSION

Based on the observed temperature dependence of the diffusional water exchange time τ_{exch} for normal and *p*-MB treated bovine erythrocytes, the following statements can be made:

(i) The semi-log plots of τ_{exch} *versus* inverse absolute temperature for both normal and *p*-MB treated erythrocytes are straight lines, implying that one activation energy E_a determines the rate of transmembrane diffusional water transport in the whole temperature range studied (5–40 °C);

(ii) For normal erythrocytes the value E_a = 20.4 kJ mol^{-1} is similar to that for self-diffusion of water (E_a = 19.3–20.1 kJ mol^{-1});

(iii) The value τ_{exch} = 12.5 ms, obtained for normal bovine erythrocytes at 20°C, corresponds to the cell membrane diffusional water permeability coefficient P_d of 3.6×10^{-3} cm s^{-1}, which conforms to the range of room temperature P_d between 3.3 and 4.7×10^{-3} cm s^{-1}, deduced recently[23] for fresh normal human erythrocytes;

(iv) The data for *p*-MB treated erythrocytes display a lengthening of τ_{exch} and increased E_a of 29.0 kJ mol^{-1};

(v) The value E_a = 29.0 kJ mol^{-1} and the permeability coefficient P_d = 2.6×10^{-3} cm s^{-1} at 20 °C (obtained from τ_{exch} = 17.3 ms at this temperature) for *p*-MB treated erythrocytes, if compared to the corresponding permeability data for artificial lipid bilayer membranes, indicate only partial inhibition of the mercurial sensitive part of the membrane diffusional water permeability, possibly as a consequence of poor accessibility of the functionally important transmembrane protein SH-groups to *p*-MB, or due to »complete« inhibition plus new leaks.

REFERENCES

1. J. Singer and G. L. Nicolson, *Science (Wash. DC)* **175** (1972) 720.
2. H. Träuble, *J. Membrane Biol.* **4** (1971) 193.
3. R. Fettiplace and D. A. Haydon, *Physiol. Rev.* **60** (1980) 510.
4. R. I. Macey, *Am. J. Physiol.* **246** (Cell Physiol. 15) (1984) C 195.
5. A. K. Solomon, B. Chasan, J. A. Dix, M. F. Lukacović, M. R. Toon, and A. S. Verkman, *Ann. N. Y. Acad. Sci.* **414** (1984) 79.
6. F. L. Vieira, R. I. Sha'afi, and A. K. Solomon, *J. Gen. Physiol.* **55** (1970) 451.
7. J. H. Wang, *J. Phys. Chem.* **69** (1965) 4412.
8. C. V. Paganelli and A. K. Solomon, *J. Gen. Physiol.* **41** (1957) 259.
9. A. Cass and A. Finkelstein, *J. Gen. Physiol.* **50** (1967) 1765.
10. T. C. Terwilliger and A. K. Solomon, *J. Gen. Physiol.* **77** (1981) 549.
11. J. Brahm, *J. Gen. Physiol.* **79** (1982) 791.
12. T. Conlon and R. Outhred, *Biochim. Biophys. Acta* **288** (1972) 354.
13. M. E. Fabry and M. Eisenstadt, *Biophys. J.* **15** (1975) 1101.
14. J. L. Pirkle, D. L. Ashley, and J. H. Goldstein, *Biophys. J.* **25** (1979) 389.
15. R. I. Macey and R. E. L. Farmer, *Biochim. Biophys. Acta* **211** (1970) 104.
16. R. I. Macey, D. M. Karan, and R. E. L. Farmer, *Properties of Water Channels in Human Red Cells*, in: F. Kreutzer and J. F. G. Slegers (Eds.), *Biomembranes*, Vol. 3, New York, Plenum, 1972, pp. 331-340.
17. A. K. Solomon, B. Chassan, J. A. Dix, M. F. Lukacović, M. R. Toon, and A. S. Werkman, *The Aqueous Pore in the Red Cell Membrane: Band 3 as a Channel for Anions, Cations, Nonelectrolytes, and Water,* in: F. Kummerow, Gh. Benga, and R. P. Holmes (Eds.), *Biomembranes and Cell Function,* Vol. 414, Annals of the New York Academy of Sciences, 1983, pp. 97-124.
18. G. Benga, O. Popescu, V. Borza, V. I. Pop, A. Muresan, I. Mocsy. A. Brain, and J. M. Wrigglesworth, *Eur. J. Cell Biol.* **41** (1986) 252.
19. M. Shporer and M. M. Civan, *Biochim. Biophys. Acta* **385** (1975) 81.
20. J. Andrasko, *Biochim. Biophys. Acta* **428** (1976) 304.
21. H. Y. Carr and E. M. Purcell, *Phys. Rev.* **94** (1954) 630.
22. S. Meiboom and D. Gill, *Rev. Sci. Instrum.* **29** (1958) 688.
23. M. D. Herbst and J. H. Goldstein, *Am. J. Physiol.* **256** (Cell Physiol. 25) (1989) C 1097.
24. D. Y. Chien and R. I. Macey, *Biochim. Biophys. Acta* **464** (1977) 45.
25. P. Maček, I. Zupančič. and G. Lahajnar, *Period. Biol.* **93** (1991) 291.
26. G. Lahajnar, P. Maček, M. Anžur, and D. Lebez, *Period. Biol.* **88** (1986) 150.
27. G. Lahajnar, P. Maček, and M. Koren, *Vestn. Slov. Kem. Drus.* **34** (1987) 361.
28. W. R. Redwood and D. A. Haydon, *J. Theor. Biol.* **22** (1969) 1.
29. H. D. Price and T. E. Thompson, *J. Mol. Biol.* **41** (1969) 443.
30. E. Ponder, *Hemolysis and Related Phenomena*, New York, Grune & Stratton, 1971, pp. 18-80.

SAŽETAK

Difuzijska permeabilnost vode u goveđim eritrocitima. Studij pulsnom nuklearnom magnetskom rezonancijom

Gojmir Lahajnar

Pulsna tehnika nuklearne magnetske rezonancije (NMR) primijenjena je na studij temperaturne ovisnosti vremena difuzijske izmjene vode τ_{exch} za normalne goveđe eritrocite, kao i one obrađene *p*-hidroksimerkuribenzoatom (*p*-MB). Metoda se temelji na mjerenju NMR transverzalne releksacijske funkcije protona za izmjenu vode između dijamagnetskog unutarstaničnog prostora i paramagnetski cijepljene vanstanične otopine suspenzije eritrocita. Semilogaritamski grafovi τ_{exch} prema inverznoj apsolutnoj temperaturi i za normalne i za merkurirane eritrocite jesu pravci, čime se podrazumijeva da jedna aktivacijska energija (E_a) određuje brzinu transporta

transmembranske difuzije vode u čitavom temperaturnom području 5–40 °C. Za normalne eritrocite vrijednost E_a od 20,4 kJ mol^{-1} slična je onoj za samodifuziju vode (E_a = 19,3–20.1 kJ mol^{-1}). Vrijednost τ_{exch} = 12,5 ms, dobivena za normalne govede eritrocite pri 20 °C, odgovara koeficijentu permeabilnosti difuzije vode na staničnoj membrani (P_d) od $3{,}6\times10^{-3}$ cm s^{-1}. To je slično rasponu P_d za sobnu temperaturu od 3,3 i $4{,}7\times 10^{-3}$ cm s^{-1}, koji je nedavno izveden za svježe normalne ljudske eritrocite. Podaci za eritrocite obrađene s *p*-MB pokazuju produljenje τ_{exch} i povišenu E_a od 29,0 kJ mol^{-1}. Ta vrijednost E_a, kao i koeficijent permeabilnosti P_d od $2{,}6\times10^{-3}$ cm s^{-1} pri 20 °C, ako se usporede s odgovarajućim podacima za permeabilnost dvoslojnih membrana s umjetnim lipidima, ukazuju na djelomičnu inhibiciju onog dijela permeabilnosti koji je osjetljiv na merkuriranje. To može biti rezultat slabe dostupnosti skupina SH u transmembranskom proteinu prema *p*-Mb, ili pak zbog »potpune« inhibicije pojačane novim pukotinama.