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Water Exchange through Erythrocyte Membranes: Nuclear Magnetic Resonance Studies on Resealed Ghosts Compared to Human Erythrocytes

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Summary. The water diffusion across human erythrocyte membrane has been studied on intact cells and resealed ghosts by a doping NMR technique. Although the water exchange time of ghosts was longer than that of erythrocytes, no significant differences in their diffusional permeability were noticed for temperatures in the range 2–43°C. Contrary to what was previously noticed in erythrocytes, no significant increase in the water exchange time of ghosts in the acid range of pH occurred.

Key Words erythrocyte membranes · water permeability · resealed ghosts · NMR · pH effects

Introduction

Because of its relatively simple structure the red blood cell has been a favorite object for studying water permeability. A great deal of work has been done on osmotic and diffusional water permeability in human red blood cells. The information on osmotic permeability of erythrocyte membranes has been recently reviewed by Sha'afi (1981) and that on diffusional water permeability by Morariu and Benga (1984).

During recent years methods of preparing red blood cell ghosts by hypotonic hemolysis have very much improved. Since such ghosts are devoid of intracellular structure and consist primarily of the cell membrane, they are widely used in studies of composition, structure and function of the red blood cell membrane. It is assumed that hypotonic ghosts have a membrane composition very similar to that of the intact cell (Wood & Passow, 1981). Moreover, two types of hypotonic ghosts have been prepared having a membrane permeability very close to that of the intact cell: (a) the resealed (pink) ghosts (Schwoch & Passow, 1973), which retain a small amount of the original hemoglobin, and (b) white ghosts (Bjerrum, 1979), which are free from visible contamination with intracellular components including hemoglobin.

In spite of potential advantages offered by hypotonic ghosts as an object for investigating various processes, there are few studies, performed by the radio-tracer method, on the water permeability of ghosts (Bjerrum, 1979; Brahm, 1982). Comparative studies of water diffusion in erythrocytes and ghosts would give us a better understanding of the molecular mechanisms of this transport process. Moreover, such studies could help in interpreting the effect of pH on water diffusion.

The aims of our paper were: (a) to compare the water diffusion in erythrocytes and ghosts by the doping NMR method; (b) to investigate the effect of pH in ghosts; and (c) to establish the characteristics of water permeability of human red blood cell membrane on a relatively large number of subjects at various temperatures.

Materials and Methods

Blood Sample Preparations

Human blood was obtained by venipuncture in heparinized tubes and used within 4 hr. The donors were healthy male or female subjects, 20 to 40 years old. The erythrocytes were isolated by centrifugation, and washed three times in 166 mM NaCl. For the preparation of resealed (pink) ghosts the procedure of Bodemann and Passow, as described by Wood and Passow (1981) has been used. In some experiments white ghosts were prepared as described by Bjerrum (1979). Finally the erythrocytes or the ghosts were suspended in 150 mM NaCl, 5.5 mM glucose, 5 mM HEPES (pH 7.4) and 0.5% bovine serum albumin at a cytocrit of 50%.

NMR Measurements

Samples for NMR[1] measurements were prepared by carefully mixing 0.2 ml erythrocyte or ghost suspensions and 0.1 ml dop-

[1] *Abbreviations:* NMR = nuclear magnetic resonance; T_1 = longitudinal (spin-lattice) relaxation time; T_2 = transversal (spin-spin) relaxation time.

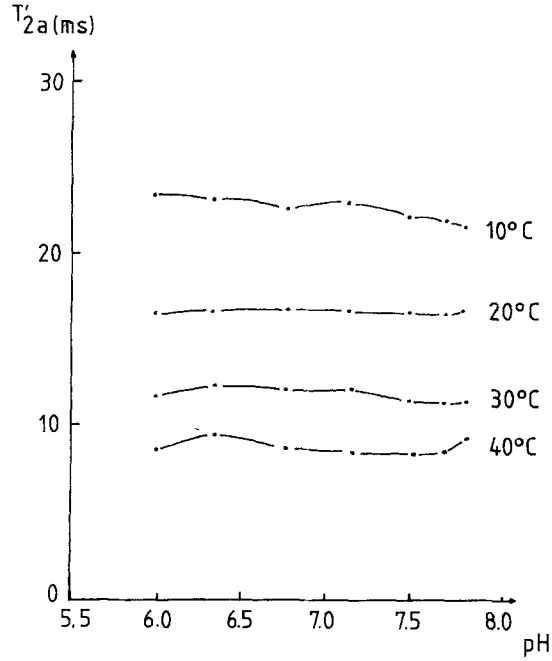

Fig. The values of the water proton relaxation time (T'_{2a}) in ghosts as function of pH at different temperatures

ing solution (40 mM $MnCl_2$, 100 mM NaCl). The water proton relaxation time (T'_{2a}) was evaluated by the spin-echo method (Conlon & Outhred, 1972), as previously described (Morariu & Benga, 1977; Benga et al., 1983). T'_{2a} is dominated by the exchange process through erythrocyte membrane and is related to the water diffusion exchange time (T_e) by the equation (Conlon & Outhred, 1978)

$$\frac{1}{T_e} = \frac{1}{T'_{2a}} - \frac{1}{T_{2i}} \tag{1}$$

where T_{2i} = the transverse relaxation time of the cell interior.

T_{2i} was measured by the 90–180° method using the Carr-Purcell-Meiboom-Gill sequence (Farrar & Becker, 1971), on packed cells or ghosts from which the supernatant, with no added Mn, had been removed by centrifuging at 50,000 × g for 60 min.

The membrane permeability for water diffusion, P, is related to $1/T_e$, the cell water volume, V, and the cell surface area, A, by

$$P = \frac{V}{A} \cdot \frac{1}{T_e}. \tag{2}$$

Since different authors have used different values of V and A, in order to compare our results with previous ones, we have used two sets of values. On one hand, we have taken a value of 65 μm^3 for the intracellular solvent volume of erythrocytes and 86 μm^3 for that of resealed ghosts and a value of 1.42×10^{-6} for the membrane area, after Brahm (1982). These give V/A ratios of 4.58×10^{-5} and 6.06×10^{-5} cm for erythrocytes and ghosts, respectively. On the other hand, we have used a slightly higher V/A ratio, after Dix and Solomon (1984), e.g. 5.33×10^{-5} cm for erythrocytes and the corresponding value for ghosts.

The NMR measurements were performed with an AREMI-78 spectrometer (manufactured by the Institute of Physics and Nuclear Engineering, Bucharest-Măgurele, Roumania) at a frequency of 25 MHz. The temperature was controlled to ±0.2°C by air flow over an electrical resistance using the variable temperature unit attached to the spectrometer. The actual temperature in the sample was measured with a thermocouple connected to a microprocessor thermometer (Comark Electronics Limited, Rustington, Littlehampton, England).

Other Procedures

Hemoglobin was estimated spectrophotometrically (Antonini & Brunori, 1971). The calculations of the correlation coefficients of the lines obtained with the sets of data points in the Arrhenius plots have been performed with a HP-41 CV computer (Hewlett-Packard, USA).

Results

Comparison of Diffusional Water Permeability of Erythrocytes and Ghosts

Comparative values of parameters characterizing the diffusional water permeability in erythrocytes and ghosts at various temperatures are listed in the Table. The temperature values were chosen such as to enable comparison with values reported by other authors for measurements performed at various temperatures. It is obvious that for all temperatures the values were higher in ghosts than in erythrocytes. However, when the permeability values were estimated it appeared that resealed ghosts have a permeability similar to erythrocytes. The longer values of T_e in ghosts are thus due to a higher intracellular solvent volume caused by the removal of hemoglobin.

We have also compared the diffusional permeability of pink and white ghosts. No significant differences in their water permeability were found.

The Effect of pH on the Water Exchange Time of Ghosts

A marked increase of the water exchange time values in the acid range of pH was previously noticed with erythrocytes (Morariu, Pop, Popescu & Benga, 1981), and this was considered to be due to a decrease of the intracellular solvent volume, produced by the protonation of hemoglobin, in the acid range of pH (Brahm, 1982). Consequently, if ghosts relatively free of hemoglobin are used, one would expect a much smaller variation of T_e with pH. As shown in the Figure, for all temperatures explored there was indeed no significant increase in T_e of ghosts in the acid range of pH.

Table. Diffusional permeability of the human erythrocytes and resealed ghosts[a]

Temperature (°C)	Erythrocytes			Resealed ghosts			T_e (msec)	Statistical significance of the difference	
	T_e (msec)	P (cm · sec × 10^3)		T_e (msec)	P (cm · sec^{-1} × 10^3)			P	
		I	II		I	II		I	II
15	15.4 ± 1.8	2.8 ± 0.2	3.2 ± 0.3	23.4 ± 4.3	2.6 ± 0.1	3.0 ± 0.1	$P < 0.001$	NS	NS
20	13.3 ± 1.3	3.4 ± 0.3	4.0 ± 0.5	19.3 ± 3.3	3.1 ± 0.1	3.6 ± 0.2	$P < 0.001$	NS	NS
25	11.4 ± 1.3	4.0 ± 0.3	4.7 ± 0.5	17.0 ± 2.9	3.6 ± 2.9	4.2 ± 0.2	$P < 0.001$	NS	NS
30	9.6 ± 0.8	4.8 ± 0.6	5.6 ± 0.8	13.5 ± 2.1	4.5 ± 0.2	5.2 ± 0.3	$P < 0.001$	NS	NS
37	7.4 ± 0.7	6.2 ± 0.7	7.2 ± 0.9	10.4 ± 1.4	5.8 ± 0.4	6.8 ± 0.4	$P < 0.001$	NS	NS

[a] The measurements have been performed on duplicate or triplicate blood samples from 12 donors as described in Materials and Methods. Results are expressed as mean ± SD. The permeability was calculated from T_e using a V/A ratio of 4.58×10^{-5} cm for erythrocytes and 6.06×10^{-5} cm for ghosts (Brahm, 1982) in column I, and the slightly higher value for the V/A ratio (e.g. 5.33×10^{-5} cm) for erythrocytes given by Dix and Solomon (1984) in column II. The statistical significance was calculated using unpaired t test. NS = statistically not significant.

Discussion

The ease with which erythrocytes can be isolated and their relatively simple structure have made them a favorite object for studying water permeability. Since ghosts prepared by hypotonic hemolysis consist primarily of the red cell membrane that has a composition very similar to that of the cell (Schwoch & Passow, 1973) they should be used in studies aimed to characterize the diffusional water permeability of this type of membrane. However, there are few NMR data on the water permeability of erythrocyte ghosts.

Our data (Table) show that resealed ghosts have a slower exchange rate compared to erythrocytes. A similar finding was reported by Brahm (1982), who has followed the diffusional water permeability of erythrocytes and ghosts by an isotopic technique. The slower exchange rate in ghosts is due to the fact that the cellular solvent volume was increased after removal of the cell hemoglobin. The hemoglobin content of resealed ghosts in our preparation was reduced to 4–7% of that of erythrocytes.

In the white ghosts prepared according to Bjerrum (1979) the hemoglobin content was even more reduced. However, their water permeability was similar to that of erythrocytes, a finding in agreement with data of Bjerrum (1979). It appears that the isotopic and NMR techniques are in agreement in revealing that both erythrocytes and ghosts have a similar water diffusional permeability.

The values of the water exchange time and of the diffusional permeability reported in this paper are in excellent agreement with the values previously reported by doping NMR (Conlon & Outhred, 1972; Chien & Macey, 1977; Morariu & Benga, 1977; Fabry & Eisenstadt, 1978), isotopic (Vieira, Sha'afi & Solomon, 1970) or bulk diffusion (Osberghaus, Schönert & Deuticke, 1982) measurements. With regard to pH there was no significant variation of T_e in ghosts in the acid range of pH (Figure), in contrast to a marked increase of the same parameter in erythrocytes (Morariu et al., 1981; Brahm, 1982). Since the ghosts are largely devoid of hemoglobin it is clear that the pH dependence of T_e in erythrocytes is due to the decreased intracellular solvent volume in the acid range of pH caused by the protonation of hemoglobin (Brahm, 1982).

The NMR method, having some important advantages (relative technical simplicity, speed of data collection, and reproducible results), appears as a useful tool for further studies of water diffusion in erythrocytes and ghosts, aiming to characterize the molecular mechanisms of this transport process, both in normal and pathological conditions (Benga & Morariu, 1977; Serbu et al., 1984).

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