Biochimica et Biophysica Acta 860 (1986) 155–158
Elsevier

BBA 70334

BBA Report

The uptake of manganese ions and the apparent thermal transition of the erythrocyte membranes

Vasile V. Morariu [a,*], Mihai S. Ionescu [b], Maria Frangopol [b], Radu Grosescu [b], Mihaela Lupu [b] and Petre T. Frangopol [b]

[a] *Institute of Isotopic and Molecular Technology, P.O. Box 700, R-3400 Cluj-Napoca and*
[b] *Institute of Physics and Nuclear Engineering, P.O. Box MG-6, R-76900 Măgurele-Bucharest (Romania)*

(Received April 4th, 1986)

Key words: Erythrocyte membrane; Water exchange; Mn^{2+}; Phase transition; NMR

The nuclear magnetic resonance manganese doping technique is currently used for the determination of the water diffusional exchange time through human erythrocyte membranes. An apparent thermal transition at 26°C was noticed at 18–30 mM manganese doping in the suspending solution. An analysis in terms of a two-phase nuclear spin exchanging system revealed that apparent thermal transitions are expected to occur in the upper and lower temperature regions. They represent a shift from intermediate exchange rates where water diffusion through the membrane is dominant to either fast or slow exchange rates where proton relaxation is the controlling process. The lower temperature apparent transition may be altered by the intracellular manganese concentration; the lower the Mn^{2+} concentration the lower the transition. Also according to this interpretation only a fraction of the erythrocytes are significantly permeated by Mn^{2+}. The upper transition depends on the Mn^{2+} concentration in the extracellular volume; it decreases with decreasing Mn^{2+} concentration.

Nuclear magnetic resonance investigations of the water diffusional exchange through human erythrocyte membrane in the presence of Mn^{2+} suggest a thermal transition at about 26°C [1,2]. The presence of Mn^{2+} in the suspending solution or plasma is imposed by the NMR method originally proposed by Conlon and Outhred [3]. The paramagnetic Mn^{2+} is added in order to label magnetically the extracellular environment. It is further assumed that the manganese ions do not penetrate the cells so that the two compartments (the extra- and the intracellular volume) can be distinguished in the NMR relaxation experiment.

However, non-NMR investigations and other authors applying the NMR-Mn^{2+} doping method revealed no thermal transition [4]. A subsequent more systematic investigation has shown that the thermal transition was apparent only in samples of washed blood doped with a relatively high amount of Mn^{2+} [5]. It has further been suggested that the thermal transition as revealed by the NMR-doping method is due to different processes which are operative within different temperature ranges: the water diffusion in the upper range and in the lower range the water proton relaxation by an electron-nuclear spin coupling process. This latter mechanism of the water proton relaxation is due to the presence of a significant amount of the divalent paramagnetic manganese ions within the cells which is obviously contrary to the ideal requirements of the method.

Our previous work does not explain how the thermal transition actually occurs and does not reveal its meaning. Two hypothesis may be considered: (a) the ions penetrate faster into the cell below transition or, (b) the presence of Mn^{2+} is

detected by the water proton relaxation times in only the lower range of temperature and not above transition. The first hypothesis may be excluded on the ground that no hysteresis was detected when cycling the temperature at least within about 10–20 min. Therefore hypothesis b should further be considered. It is the purpose of this report to show that breaks in the Arrhenius plot of the apparent water diffusional exchange are due to a shift from intermediate exchange rates where water diffusional exchange is dominant, to fast or slow nuclear spin exchange conditions where nuclear relaxation is dominant.

The experimental details have been described previously [5].

The apparent thermal transition can be explained in terms of the nuclear spin exchange theory [6]. The main features of this theory will be recalled briefly and illustrated schematically in Fig. 1. Consider a two-phase system of nuclear spins characterized each by the spin-spin relaxation time T_{2a} and T_{2b}, respectively. The apparent T_2' will in fact depend on the rate of exchange between phases. If very fast exchange conditions prevails, than:

$$1/T_2' = (P_a/T_{2a}) + (P_b/T_{2b})$$

where P_a and P_b are the fraction of phases a and b, respectively. When $T_{2a} \gg T_{2b}$, $1/T_2' = P_b/T_{2b}$. This fast exchange condition is fulfilled at higher temperatures (Fig. 1). At low temperatures where the lifetime in each phase is greater than its own relaxation time, in other words slow rate of exchange prevails, both T_{2a} and T_{2b} are detected (Fig. 1).

At intermediate exchange rates,

$$1/T_{2a}' \approx 1/T_{2a} + 1/\tau_a, \text{ if } T_{2b}/\tau_b \ll 1$$

This intermediate region is of interest for measuring the water proton life time in phase a, τ_a (or the diffusional exchange time as frequently called). It should be noted that a temperature t_a exists where an apparent break in the Arrhenius plot is expected to occur due to a shift of the system to slow exchange conditions where a nuclear relaxation process (T_{2a}) is dominant. Also a break occurs at t_b in the upper range of temperatures where the T_{2b} relaxation process becomes dominant.

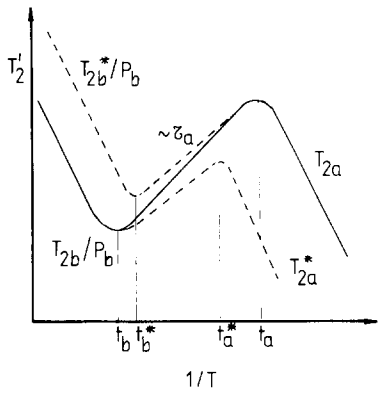

Fig. 1. The temperature dependence of the longer apparent nuclear spin-spin relaxation time T_2' in a two-phase relaxation system characterized by T_{2a} and T_{2b}, respectively. Apparent thermal transitions are evident between slow-exchange to intermediate-exchange rate at t_a and from intermediate to fast-exchange rates at t_b. Decrease of T_{2a} to T_{2a}^* is associated with an increase of t_a to t_a^*. Increase of T_{2b} to T_{2b}^* will decrease t_b to t_b^*.

A significant shift of t_a to higher temperatures can be achieved if T_{2a} becomes shorter. If we assume for simplicity that the activation energy of the nuclear relaxation remains essentially the same than a t_a^* will be expected for the shorter T_{2a}^* (Fig. 1). Such a case can be operative for erythrocytes where shortening of T_{2a} is due to the uptake of Mn^{2+}, a well known relaxation enhancement agent [7]. This effect is concentration dependent (Fig. 2). Therefore we may expect that t_a will in fact depend on the value of T_{2a} which in turn is controlled by the Mn^{2+} concentration within the cells. The higher the Mn^{2+} content, the lower the T_{2a} value and therefore the higher the value of t_a.

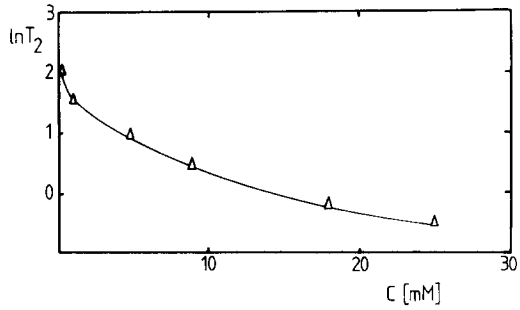

Fig. 2. The water proton spin-spin relaxation time vs. Mn^{2+} concentration in a buffered solution pH 7.4, albumin 0.1%.

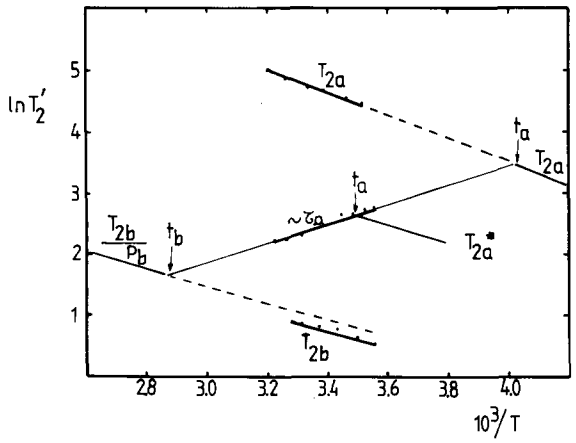

Fig. 3. The temperature dependence of: (a). Water proton spin-spin relaxation of packed erythrocytes T_{2a}. (b). Albumin 0.1%, buffered solution pH 7.4, 5 mM $MnCl_2$, 140 mNaCl, T_{2b}. (c). The water diffusional exchange time through erythrocyte membranes. All values have been extrapolated in order to reveal the expected values of t_a and t_b. The straight line T_{2a}^* corresponds to a protein solution with $1 \cdot 10^{-4}$ M $MnCl_2$, simulating erythrocytes loaded with Mn^{2+}.

The value of t_b may be changed by altering either T_{2b} or P_b or both. This can be achieved for example by decreasing the amount of Mn^{2+} in the suspending solution. As a result an increase of T_{2b} will occur and a shift of t_b to lower temperatures will be noticed.

Therefore we may expect that loading of the cells with Mn^{2+} will reveal breaks in the Arrhenius plot in the lower range of temperatures while doping of plasma of the suspending solution with a relatively low amount of Mn^{2+} might show up as a break in the higher range of temperatures. Both these qualitative predictions are born out by experiments.

The results obtained for a moderately doped washed blood sample is illustrated in Fig. 3 (5 mM Mn^{2+}, $P_a = 0.8$) for 0–40 °C. Only the intermediate exchange rate is evident within this range ($T_2' \approx \tau_a$). Also included is the temperature dependence of T_{2b}/P_b which is normally observable within the same temperature range. The value of T_{2a} and T_{2b}, respectively, were measured for the isolated phases (packed erythrocytes and plasma, respectively). The values of T_{2a} and T_{2b}/P_b are extrapolated to lower or higher temperatures. The extrapolation is fully justified from a theoretical point of view [8] although it is virtual by its nature. However, it serves to show that t_a and t_b are located far away from the range of temperatures which do not damage the cells. No breaks have been noticed in whole blood samples up to 30 mM of Mn^{2+} in plasma while breaks have been noticed in washed samples doped with 18–30 mM Mn^{2+} [5]. We have therefore suggested that only plasma prevents the uptake of Mn^{2+} while suspending solutions prepared with albumin do not [5]. A clue to this peculiar effect of plasma has been recently offered by a systematic investigation of manganese binding by plasma components [9]. It has been reported that other plasma proteins than albumin represent the major binding ligand in plasma. We suggest that Mn^{2+} ligand complex does not penetrate the cell and therefore shortening of T_{2a} is avoided. Consequently the value of t_a will remain low and no apparent thermal transition is likely to occur within the range of temperatures normally explored for cells.

However, in the absence of these binding proteins the uptake of Mn^{2+} will proceed normally by its slow passive diffusion [10]. Obviously a higher amount of Mn^{2+} in the suspending solution will bring a higher uptake of ions. This may explain qualitatively while breaks occur at only higher Mn^{2+} content in the suspending solution. It is possible to predict the value of t_a^* by evaluating the Mn^{2+} content of the cells. We have estimated an intracellular Mn^{2+} concentration of about $1 \cdot 10^{-4}$ M after 3 h of incubation in our experimental condition by using the radiotracer data [10]. Considering the data in Fig. 2 and the fact that the apparent activation energies of proton relaxation in manganese doped plasma and erythrocytes, respectively, is much the same [5], it is possible to draw the line T_{2a}^* in Fig. 3. A break will therefore be apparent at about 14 °C, and the apparent water exchange time will show a maximum. Such a case has been reported for fetal erythrocytes [5]. Obviously this is not a real thermally induced structural transition of the membrane. It is, instead and artifact caused by the particular values of the NMR parameters.

On the other hand the human erythrocytes from adult donors showed results resembling to curves a and b in Fig. 4. The curve T_2'-T_{2a}^* represents the limiting case when all cells have a similar Mn^{2+} content. Intermediate situations (a, b, c) can

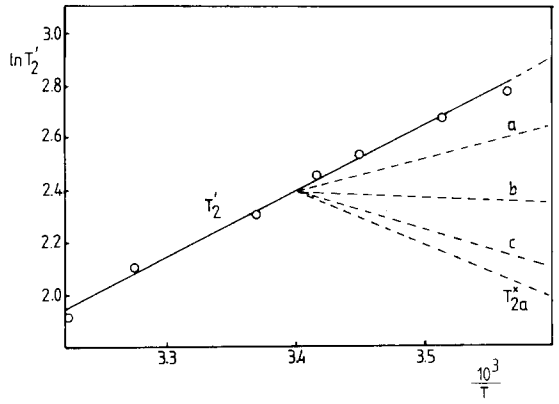

Fig. 4. The longer apparent water proton relaxation vs. temperature in a whole blood sample doped with 30 mM $MnCl_2$. Its value is controlled by the diffusional exchange through the erythrocyte membrane. The broken line T_{2a}^* corresponds to the water proton relaxation ina n albumin 0.1%, buffered pH 7.4, izoton solution containing 1.25 mM $MnCl_2$. Lines a, b and c correspond to weighted averages of T_2' and T_{2a}^* ($P_a' = 0.2$, 0.5 and 0.8, respectively).

be generated if we assume that a only a fraction of the erythrocytes have been penetrated by a significant amount of Mn^{2+}. Therefore we deal with two systems characterized by different sets of relaxation times: (a) T_{2a}, T_{2b}, and (b) T_{2a}^*, T_{2b}. In this case the observed long relaxation rate $1/T_2'$ will represent the weighted average of the two systems. In the temperature range below t_b, the fraction P_a' will be characterized by $T_2' \approx \tau_a$, while the fraction P_a'' by $T_2' = T_{2a}^*$, where $P_a' + P_a'' = P_a$. The observed $1/T_2'$ will be

$$\frac{1}{T_2'} = \frac{P_a'}{\tau_a} + \frac{P_a''}{T_{2a}'}$$

The value of T_2' was calculated for different fractions of P_a' and P_a'' (Fig. 4). It can be seen that $E \approx 0$ (below t_a) when $P_a'/P_a'' = 1$. Such a case was noticed in our earlier report [1]. Therefore the NMR relaxation experiment reveals that not all erythrocytes are loaded by Mn^{2+} to a similar extent and this is an information which cannot be obtained by other direct spectroscopic analysis. It should be further stressed that the present NMR estimation of the Mn^{2+} loading of erythrocytes by using the value of t_a is only semiquantitative.

On the other hand a low doping manganese experiment (3.5 mM) revealed a minimum at about 40°C (not shown). Therefore at temperatures above 40°C the system is already in the fast exchange range of temperatures. A semiquantitative evaluation based on data in Fig. 2 (and $P_b = 0.7$) predicts a $t_b \approx 45$°C which is in satisfactory agreement with experiment.

In conclusion apparent thermal transitions are expected to occur when the water diffusional exchange is measured by the NMR doping technique. These are due to a shift either from an intermediate rate of water proton spin exchange to a slow or fast rate of exchange, respectively. The transition to slow exchange conditions could be of some use for evaluating the manganese loading of erythrocytes and various chemical or pathological effects on it.

References

1 Morariu, V.V., Pop, V.I., Popescu, O. and Benga, G. (1981) J. Membrane Biol. 62, 1–5
2 Morariu, V.V. and Petrov, L. (1986) Cancer Biochem. Biophys., in the press
3 Conlon, T. and Cuthred, R. (1972) Biochim. Biophys. Acta 288, 354–361
4 Brahm, J. (1982) J. Gen. Physiol. 79, 791–819
5 Morariu, V.V., Ionescu, M.S., Frangopol, M., Grosescu, R., Lupu, M. and Frangopol, P.T. (1985) Biochim. Biophys. Acta 815, 189–195
6 Woessner, D.E. (1963) J. Chem. Phys. 39, 2783–2787
7 Dwek, R.A. (1973) Nuclear Magnetic Resonance in Biochemistry: Applications to Enzyme Systems, Clarendon Press, Oxford
8 Farrar, T.C. and Becker, E.D. (1971) Pulse and Fourier Transform NMR, Academic Press, New-York
9 Scheuhammer, A.M. and Cherian, M.G. (1985) Biochim. Biophys. Acta 840, 163–169