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TWO KINDS OF UNFROZEN WATER IN MITOCHONDRIAL SUSPENSION DETECTED BY ^{1}H MAGNETIC RESONANCE

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SUMMARY: Presence of two kinds of "unfrozen water" in mitochondrial suspension, i.e., "loosely" and "tightly" bound water, was demonstrated in the line width and T_1 values of the $^{1}H_2O$ nmr signal. There is a large difference between the intact and sonicated mitochondrial suspensions in the state of "loosely" bound water. The results imply that "loosely" bound water rather than tightly bound water reflects a small change in the state of biomembranes.

The proton magnetic resonance signal of unfrozen water is observable in frozen biological materials, such as muscles (1-6), erythrocytes (6-8) and proteins (9-11). The ^{1}H nmr of water in frozen tissues below -20°C has been shown to give useful information on the state of macromolecules and tissues (1-11). Amount of the unfrozen water changes on the denaturation of proteins (9,10), and tumor contains less amount of unfrozen water than normal tissues (2,3). However, in these studies no information has been deduced from the difference between the proton signal of unfrozen water at -5°C and that at -25°C. We studied the state of water in mitochondrial suspension on freezing, and found a marked change in the nmr spectral properties in the region between -5°C and -25°C.

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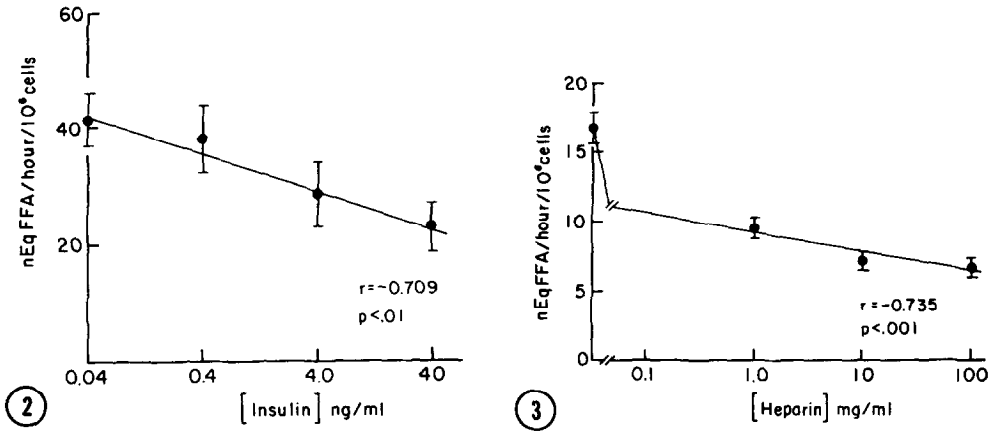

Figure 2: The lipoprotein lipase activity releasable from cellular suspensions during a 45 min incubation with heparin following a prior 2 hr incubation of cells in insulin is plotted on the ordinate. The insulin concentrations utilized are shown on the abscissa. Data points represent the mean±SE of triplicate measurements.

Figure 3: The lipoprotein lipase activity releasable from cellular suspensions during a 45 min incubation with heparin following a prior 2 hr incubation of cells in heparin is plotted on the ordinate with the heparin concentration in mg/ml on the abscissa. The results represent the mean±SE of triplicate measurements.

The lipoprotein lipase releasable with heparin from cellular suspensions decreased with increasing prior insulin concentrations (Fig. 2). This decrease over the range of insulin concentrations utilized during the 2 hr incubation is compatible with a direct effect of insulin on the cell membrane. Cells incubated in 1.0, 10 and 100 mg/ml of heparin for 2 hrs instead of insulin showed a similar decrease in heparin releasable activity (Fig. 3), and results very similar to those shown for insulin (Fig. 2) were obtained. Like insulin, heparin also enhanced a release of lipoprotein lipase into the culture medium, but unlike insulin, heparin had no effect on intracellular activity (data not shown). Similar effects of insulin on lipoprotein lipase were found in 5 experiments (Fig. 4); increases in acetone ether powder and culture medium activity were associated with decreases in activity releasable with heparin from cellular suspensions.

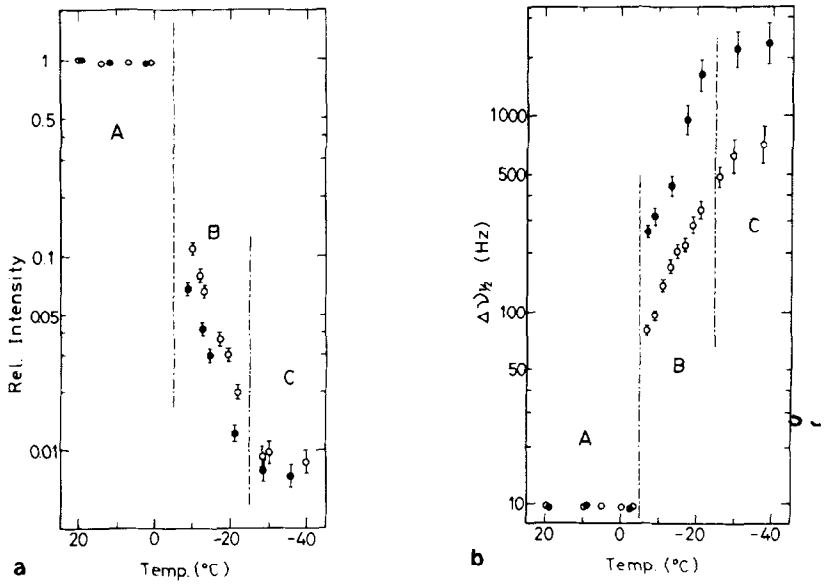

Figure. 1. Temperature dependence of ^1H_2O nmr signals in mitochondrial suspensions. (a) Relative intensities, (b) the line width at half-height, $\Delta\nu_{1/2}$. o: intact mitochondria, ●: sonicated mitochondria. Spectra were taken as described in the section of "Materials and Methods". The experiment, where the sample tube was once frozen by liquid nitrogen and then warmed to the observed temperature, was also performed. There was no difference between the results of both experiments.

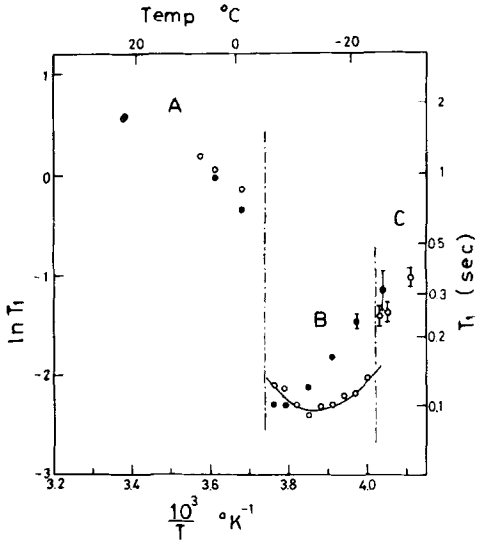

Figure 2. Changes in T_1 of the proton of water with temperature. o: intact mitochondria, ●: sonicated mitochondria. The continuous line in the figure was calculated as described in the text.

intact mitochondria reaches a minimum, $(T_1)_{min}$, at about -14°C. In contrast, $(T_1)_{min}$ of sonicated mitochondria is observed at around -5∿-10°C. Moreover, as shown in Figure 2, with intact mitochondria there is a discrete increase of T_1 at about -25°C, but with sonicated mitochondria such abrupt change was not observed.

Provided that the water protons undergo a single motion with a characteristic correlation time, τ_c, which can be related to T_1 as shown in eq.(I)(ref. 14), where ω_o is the Lamor frequency and K is a constant.

$$(T_1)^{-1} = 2K\left(\frac{\tau_c}{1 + \omega_o^2\tau_c^2} + \frac{\tau_c}{1 + 4\,\omega_o^2\tau_c^2}\right) \qquad (I)$$

From eq.(I), the τ_c at the temperature of $(T_1)_{min}$ is calculated to be 1.0×10^{-9} sec at 100 MHz. The temperature dependence of τ_c is assumed to be represented as,

$$\tau_c = \exp(-E_a/RT) \qquad (II)$$

where E_a is the activation energy and τ_o is a constant. Combining eqs. (I) and (II), the temperature dependence of T_1 can be calculated. The continuous line in Figure 2 which is the calculated change of T_1 with temperature when E_a is assumed 14.7 Kcal /mol, fits well with the experimental values for intact mitochondria. It should be particulary noted from the difference of the temperatures of $(T_1)_{min}$ in Figure 2 that the mobility of the water in phase B is larger in intact mitochondria than in sonicated mitochondria.

The above results indicate that there are two kinds of "unfrozen water" in mitochondria: the waters in phases B and C.

The water in phase C should be the one directly bound to proteins and lipids ("bound water"), its correlation time being estimated to be about 10^{-6}sec, as has been reported by many workers (1-3,6-10). The water in phase B moves faster than that in phase C and is probably located near the membrane surface, or in the matrix space, or in the space between the inner- and outer-membranes of the mitochondria. The presence of this kind of water in biological systems has been suggested from analysis of the temperature dependence of ^1H_2O T_1 values in erythrocytes (7) and of the dielectric relaxation of water adsorbed on lysozyme (15).

In our study the presence of this "loosely bound water" was demonstrated directly for the first time. From the finding that there are large differences between intact and sonicated mitochondrial suspensions in the line width and T_1 value of the ^1H_2O nmr signals in phase B, the water in this phase probably reflects a small change in the state of biomembrane. Thus the nmr signal of ^1H_2O in phase B should be a better probe for the state of biomembranes than that in phase C.

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