Dynamics and Environment of Mitochondrial Water as Detected by $^1$H NMR*

(Received for publication, December 1, 1995, and in revised form, February 8, 1996)

Emilio A. López-Beltrán, María J. Matét, and Sebastián Cerdán§
From the Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas,
Arturo Dupond 4, 28029 Madrid, Spain

The dynamics and environment of water in suspensions of isolated rat liver mitochondria have been investigated by $^1$H NMR. NMR longitudinal and transversal relaxation times ($T_1$ and $T_2$) were measured in the resuspension medium (2.65 s and 44.57 ms) and in mitochondrial suspensions (1.74 s and 23.14 ms), respectively. Results showed monoexponential relaxation in both cases, suggesting a fast water exchange across the inner mitochondrial membrane. Ferromagnetically induced shift of the extramitochondrial water with nonpermeant ferromagnetic particles revealed no detectable water signal from the intramitochondrial compartment, confirming the fast exchange case. Simulations on a two-compartment model indicated that the intramitochondrial water residence time has an upper limit of approximately 100 μs. Calculated intramitochondrial relaxation times revealed that the intramitochondrial environment has an apparent viscosity 30 times larger than the resuspension medium and 15 times larger than the cytosol of erythrocytes. The higher apparent viscosity of the mitochondrial matrix could account for reductions of more than one order of magnitude in the diffusion coefficient of water and other substrates, limitations in the rate of enzymatic reactions which are diffusion controlled and a more favorable formation of multienzyme complexes.

Adequate understanding of the dynamics of water at the cellular level involves the determination of the water exchange rate across the different intracellular membranes and the study of the water environment within the different organelles (1–4). Considerable evidence has been accumulated with erythrocytes on the kinetics of water exchange across the plasma membrane (2, 4–10), as well as on the physical properties of the cytosolic compartment (11). However, the transport of water across the inner mitochondrial membrane and the environmental properties of the intramitochondrial space remain poorly understood. These two aspects are of particular metabolic relevance since a general belief indicates that viscosity and slow diffusion of metabolites in the matrix could affect the activity of a variety of intramitochondrial enzymes (12–16). However, to our knowledge no direct measurements on the physical properties of the intramitochondrial environment exist to support these hypotheses.

In this report we study the dynamics of water in mitochondrial suspensions, providing values for the mean residence time (17) of water in the intramitochondrial space and estimates for matrix viscosity. Our methodology is based on comparisons of the $^1$H NMR longitudinal ($T_1$) and transversal ($T_2$) relaxation times of the water protons in the mitochondrial suspensions with those observed in the resuspension medium without mitochondria. This approach is specially suited for the noninvasive study of the water environment in biological systems since the magnetic relaxation properties of the water protons are a direct consequence of their translational and rotational motions.

MATERIALS AND METHODS

Preparation and Characterization of Rat Liver Mitochondria—Mitochondria were prepared by differential centrifugation (18) from the liver of adult male Wistar rats (250–300 g) fed ad libitum using 300 mM mannitol, 0.5 mM EDTA (pH 7.2) as isolation medium and 300 mM mannitol, 20 mM HEPES/KOH (pH 7.2) as resuspension medium. Protein concentration was measured immediately after preparation by the biuret method using bovine serum albumin as a standard. Respiratory control ratios (RCR) were routinely determined polarographically in all mitochondrial preparations prior to NMR experiments using glutamate and malate as substrates (19). Preparations with RCRs smaller than 4 were discarded. Intramitochondrial volume was measured essentially as described by Dawson et al. (20). Mitochondrial suspensions (at 75 mg of protein/ml) were incubated for 3 min at 25 °C in a medium containing $^3$H$_2$O (100,000 cpm/ml) and $^{125}$Ijodocate (100,000 cpm/ml) to determine the total water volume and the extramitochondrial volume, respectively. The difference between both volumes was taken as the matrix space. Electron microscopy was performed on three mitochondrial preparations following the protocol of Lang (21). Mitochondria showed the expected spherical morphology with internal cristae (17, 22).

$^1$H NMR Spectroscopy—Longitudinal ($T_1$) and transversal ($T_2$) relaxation times of the water protons were measured on the resuspension medium without mitochondria and on mitochondrial suspensions prepared as described above. An AM-360 Bruker spectrometer (360.13 MHz, $^1$H frequency) equipped with a 5-mm $^1$H selective probe was used. $T_1$ was determined by the inversion-recovery sequence $\tau$–$\tau$–$\tau$–acquire. $T_2$ was measured by the Carr-Purcell-Meiboom-Gill spin-echo sequence $\pi/2$–$\tau$–$\pi$–$\tau$–acquire. Acquisition conditions were: 7 μs ($\tau$) pulse, 10-ppm spectral width, 8K words data table, and 4 scans per spectrum. The temperature dependence of $T_1$ and $T_2$ was measured using a Bruker variable temperature unit (B-VT 1000, temperature stability ± 0.1 °C). In these experiments, a temperature-calibrated coaxial capillary containing ethylene glycol (100%) was inserted in

*This work was supported in part by Grants PM 92/0011–PB 94/0011 from the Spanish DGICYT and AE-00219/94 from the Community of Madrid. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address: Departamento de Cristalográfica, Centro de Investigación y Desarrollo, C.S.I.C., Avda. J ordi Girona, 18–26, 08031 Barcelona, Spain.

‡ To whom correspondence and reprint requests should be addressed. Tel.: 34-1-585-46-33; Fax: 34-1-585-45-87.

§ This work was supported in part by Grants PM 92/0011–PB 94/0011 from the Spanish DGICYT and AE-00219/94 from the Community of Madrid. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement“ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: $T_1$, spin-lattice relaxation time; $T_2$, spin-spin relaxation time; RCR, respiratory control ratio; CP, centipoise (10⁻² poise); $\tau$, rotational correlation time.
every sample for accurate temperature determination. Activation energies (Ea) were calculated using the Arrhenius equation (k2 = k1 exp (Ea / RT), where R = 2 cal K⁻¹ mol⁻¹, and T is the absolute temperature) from the slope of the linear portions of the plots of log k2 versus 1/T, where k1 = 1/T1 and k2 = 1/T2.

Nonpermeant dextran-coated ferromagnetic iron particles were prepared by alkalizing (pH 11.0) a solution of Cl₂Fe (10 mg/ml) and Cl₃Fe (15 mg/ml) in the presence of dextran (M₁, 40,000, Pharmacia, Uppsala) (23). The ferromagnetic particles coated with dextran were separated from free dextran prior to use using a Sephacryl S-300 column (23). Electron microscopy of these preparations revealed spherical particles with diameters in the range of 30–50 nm. Iron concentration of preparations was determined by the thiocyanate method, measuring the absorbance of the iron-thiocyanate complex at 460 nm (24).

Viscosity Measurements—Model solutions of resuspension medium with increasing concentration of glycerol (0, 10, 30, 60, and 75%) were prepared. The dynamic viscosity of the resuspension medium was measured (22 °C) for three of these samples (i.e., partial substitution by glycerol at 0, 30, and 60%, v/v), using a Brookfield viscometer (model RVT, Brookfield Engineering Laboratories, Stoughton, MA). T₁ and T₂ measurements were performed on all the model solutions and compared with T₁ and T₂ measurements performed in mitochondrial suspensions.

Data Analysis and Model Simulations—T₁ and T₂ values were obtained from non linear three parameter fits of the longitudinal and transversal equilibrium magnetization recoveries to the equations M(t) = M(0) / [1 − k1exp (−t/T₁)] + M(0)exp (−t/T₂), and M(t) = K₀ + K₁ + M(0)exp (−t/T₁) + M(0)exp (−t/T₂), respectively. Curve fitting was performed using a nonlinear least squares regression program (IGOR, WaveMetrics, Lake Oswego, OR). Double exponential magnetization recoveries for T₁ and T₂ measurements were also fitted to evaluate the data for the existence of double exponential as opposed to single exponential behavior. The following equations were used: M(t) = M₁n(1 − K₀exp (−t/T₀)) + M₂n(1 − K₁exp (−t/T₁)), and M(t) = K₀ + K₁ + M₀exp (−t/T₀) + M₁exp (−t/T₁), where the a and b subscripts represent two magnetically different water environments. Chi square values (χ²) obtained from the monoexponential and biexponential fits, were analyzed using the Student’s t test.

A kinetic model was developed to estimate the water residence time in the intramitochondrial environment from measurements of the relaxation times of the mitochondrial suspension and the resuspension medium. For this purpose, we used a package for modeling system dynamics in biology, based on the Euler algorithm for the numerical integration of differential equations (STELLA, High Performance Systems, Lyme, NH). T₁ relaxation curves were simulated for different values of the water residence time in the intra- and extramitochondrial space and compared to the experimentally observed T₁ values of mitochondrial suspensions, using χ² criterion for the goodness of fit. These comparisons were further completed with a test for randomness or trends of residuals in every fit (25).

RESULTS

Fig. 1 summarizes the results of T₁ (A) and T₂ (B) measurements, performed on mitochondrial suspensions (closed circles) and on the resuspension medium without mitochondria (open circles). The water relaxation times observed in these suspensions, are determined by the decay of magnetically labeled water in the intramitochondrial space, in the external resuspension medium, and by the exchange of magnetically labeled water between these two environments. T₁ (T₂) measurements of the resuspension medium (n = 6) and of the mitochondrial suspensions (n = 5) were always monoexponential with values of 2.65 ± 0.2 s (44.57 ± 2.2 ms) and 1.74 ± 0.1 s (23.14 ± 1.6 ms), respectively. These results indicate that the relaxation times of intramitochondrial water are significantly shorter than those of water in the resuspension medium. Furthermore, the results shown in Fig. 1 provide information on the rate of exchange of water between the two environments. A fast exchange rate between the intramitochondrial space and the resuspension medium would yield a single exponential relaxation behavior, while a slow or intermediate exchange would result in a more complex behavior. We fitted the data of Fig. 1 to both a single exponential and a double exponential. There was no significant difference in χ² from both single and double exponential fits, and when double exponential was used, the values of both fitted rate constants were virtually identical. However, there was a consistent tendency for the χ² of the single exponential fit to be slightly lower.

The fast exchange situation is confirmed in the experiment of Fig. 2, which shows the water resonance of the resuspension medium (A and C) and of mitochondrial suspensions (B and D), before (A and B) and after (C and D) the addition of 7.5 mg of iron/ml of nonpermeant, dextran-coated ferromagnetic particles. Ferromagnetic shift of extramitochondrial water revealed no detectable water signal from the intramitochondrial space (D). This result confirms that the exchange of water across the mitochondrial membrane must occur faster than the difference in frequency between the shifted and unshifted resonances (26). Since the frequency difference between these two resonances is 936 Hz, the exchange of water across the mitochondrial membrane must be faster than 1.07 ms.

3 A linear relationship exists between the absolute temperature T (K) and the chemical shift difference (Δppm) between the OH and CH₂ resonances of pure ethylene glycol. In the range of 294–325 K, our measurements gave a straight line, where the following linear equation was fitted: Δppm = 4.74 − 0.0104 × (K) (r = −0.9998).
Water exchange across the mitochondrial membrane was further characterized with a study of the variation of $T_1$ and $T_2$ in mitochondrial suspensions with protein concentration and temperature. The dependence of the relaxation times on the mitochondrial protein content is shown in Fig. 3. In these experiments, the final incubation volume was maintained constant at 0.5 ml and the amount of liver mitochondria increased up to 110 mg/ml. An approximately linear decrease in $T_1$ (Fig. 3A) and $T_2$ (Fig. 3B) was observed for increasing protein concentrations. The decrease is due to the shorter relaxation times of intramitochondrial water and to the progressive increase in the relative contribution of intramitochondrial space to the overall relaxation observed. Measurements of the intramitochondrial water in the mitochondrial suspensions gave values of $1.3 \pm 0.1 \mu l/mg$ of protein ($n = 7$). Thus, the titration shown in Fig. 3 covers an intramitochondrial volume range of up to 143 $\mu l/ml$. Total water content was determined in the same samples used for $T_1$ and $T_2$ measurements, measuring the dry/wet weight ratio. A total H$_2$O content of $8.4 \pm 1 \mu l$ of H$_2$O/mg of protein or $840 \mu l$ of H$_2$O/ml of sample was determined for mitochondrial suspensions of 100 mg of protein/ml. For this protein content, the intramitochondrial volume represents approximately 12-16% of the total water volume, the proportion of mitochondrial matrix space normally found in liver cells (27).

Fig. 4 depicts measurements of the temperature dependence for $T_1$ (A) and $T_2$ (B) of the resuspension medium alone and of a typical mitochondrial suspension. In the resuspension medium, $T_1$ increased linearly, whereas $T_2$ increased in a nonlinear fashion. This result suggests that $T_2$ relaxation in the resuspension medium is dependent on more than one process. The activation energies ($E_a$) calculated from the temperature dependence of $T_1$ and $T_2$ in the resuspension medium, for the 22-50°C interval, were 4.8 kcal/mol and 6.3 kcal/mol, respectively. These activation energies are within the range previously reported for the self-diffusion of water (28, 29). In the mitochondrial suspension, $T_1$ showed a biphasic behavior. It increased up to 32°C and decreased at higher temperatures. Additional experiments with different mitochondrial suspensions ($n = 3$) confirmed this behavior, but the transition temperature varied slightly between the samples (32-35°C). The $E_a$ values calculated below the transition temperature (from 3.8 to 4.8 kcal/mol) for these mitochondrial suspensions are also consistent with the values for the self-diffusion of water, being similar to the $E_a$ of the resuspension medium. In con-
Arrhenius analysis of the linear portion above the transition temperature gave much higher $E_a$ values in the range of 3.8–6.4 kcal/mol (35–57°C). The biphasic trend suggests the presence of two different relaxation mechanisms having dominant effects below or above the transition temperature.

The values of intramitochondrial relaxation times are thought to be dependent mainly on the mitochondrial content of paramagnetic ions and on the viscosity of the matrix microenvironment. We investigated the potential contribution of free paramagnetic ions to the relaxation times observed by measuring the $T_1$ and $T_2$ relaxation times in mitochondrial suspensions prepared in the absence or presence of 0.5 mM EDTA. Values of 1.46 s (29.91 ms) or 1.63 s (33.34 ms) were obtained for $T_1$ or $T_2$, respectively. Increasing the concentration of EDTA to 1 mM did not further increase the values of $T_1$ or $T_2$. These results indicate that free paramagnetic ions contribute at most 10% of the observed relaxation in suspensions of rat liver mitochondria. In addition, EPR measurements on typical mitochondrial suspensions ($n = 2$) showed no free $\text{Mn}^{2+}$ signal detectable at maximum EPR sensitivity (results not shown). Our results confirm previous findings indicating very small contributions of paramagnetic ions to mitochondrial relaxation times (30, 31). Thus, intramitochondrial viscosity may be considered as the main determinant of intramitochondrial relaxation under our experimental conditions. To investigate the influence of viscosity on intramitochondrial relaxation times, measurements of $T_1$ and $T_2$ were performed on model solutions of the resuspension medium containing increasing concentrations of added glycerol (Fig. 5). These measurements were compared with intramitochondrial $T_1$ and $T_2$ values calculated for a fast water exchange situation (see "Discussion"). Calculated intramitochondrial $T_1$ and $T_2$ values were $0.6 \pm 0.06$ s ($5.8 \pm 0.6$ ms) ($n = 6$). Interpolation of these values (arrows) on the calibration curve obtained with model solutions, indicated that the matrix space behaves similarly to a solution of the resuspension medium containing approximately 52–59% glycerol. We performed measurements of the dynamic viscosity of the resuspension medium without glycerol, and with two concentrations of added glycerol, namely 30 and 60% (v/v). The viscosities were (mean $\pm$ S.E., $n = 4$) 1.3 $\pm$ 0.1, 20.0 $\pm$ 1.4, and 44.5 $\pm$ 2.5 cP, respectively. Thus, intramitochondrial viscosity would be similar to that of a 55% glycerol solution.

**DISCUSSION**

Our results indicate that the water exchange time between the intramitochondrial matrix and the external resuspension medium is faster than the NMR relaxation time scales. To provide a more quantitative estimate of the water residence time in the intramitochondrial matrix, we implemented a model consisting of two compartments, A and B, representing the extra- and intramitochondrial environments respectively, separated by a semipermeable membrane (Fig. 6). The model...
considered the $T_2$ relaxation of magnetically labeled protons of free water in the extramitochondrial (M$_b$) and intramitochondrial (M$_a$) compartments, the experimentally observed relaxation of all water molecules in the sample (M$_T$) and the exchange of water across the internal mitochondrial membrane. This exchange is expressed in terms of the water residence times in the external medium and in the mitochondrial matrix, $\tau_a$ and $\tau_b$, respectively. The decay of the NMR signal observed experimentally (M$_T$), contains the addition of the decays of the macroscopic magnetization from both compartments, M$_a$ and M$_b$.

$$dM_T/dt = dM_a/dt + dM_b/dt,$$  
(Eq. 1)

where $dM_a/dt$ and $dM_b/dt$ are given by Ref. 32.

$$dM_a/dt = -M_a/T_{2a} - M_a/\tau_a + M_b/\tau_b,$$  
(Eq. 2)

$$dM_b/dt = -M_b/T_{2b} - M_b/\tau_b + M_a/\tau_a.$$  
(Eq. 3)

$T_{2a}$ and $T_{2b}$ refer to the transversal relaxation times of water in the external medium and in the mitochondrial matrix, respectively. The relaxation times of the water in the external medium ($T_{2a}$) and in mitochondrial suspensions ($T_{2b}$) can be determined experimentally (Fig. 1). The relaxation time of water ($T_{2b}$) in the mitochondrial matrix can be calculated, because of the fast water exchange, using the expression:

$$1/T_{2b} = P_a/T_{2a} + P_b/T_{2b},$$  
(Eq. 4)

where $P_a$ and $P_b$ represent the fractional contributions to the total volume of compartments A and B, determined by the $^2$H$_2$O and $[^1$H$]_2$C$_6$H$_{12}$ distributions, respectively. Using Equation 4 we obtained values of $0.6 \pm 0.06$ s (5.8 $\pm$ 0.6 ms) for intramitochondrial $T_1$ ($T_2$). With these values it became possible to simulate the $T_2$ relaxation behavior of the water resonance in mitochondrial suspensions (d$M_a/dt$) as a function of $\tau_a$ and $\tau_b$ (Fig. 7). Values of $\tau_a$ were varied iteratively to mimic the experimental magnetization recoveries. The corresponding values of $\tau_a$ were calculated for every simulation to satisfy the equilibrium condition $P_a/\tau_a = P_b/\tau_b$.

Fig. 7A shows model simulations of the observed relaxation behavior for the limiting cases of very slow or absent water exchange ($\tau_a, \tau_b \rightarrow \infty$) and of fast water exchange ($\tau_a \ll T_{2a}, \tau_b \ll T_{2b}$). In addition, the figure depicts, superimposed to the simulations, the experimental points for $T_a$ and $T_b$ relaxation of mitochondrial suspensions and their corresponding single exponential fits. The figure shows that the fast exchange simulation resembles closely the experimental data, while the no water exchange situation is clearly different from the observed results. We performed additional simulations in the fast exchange regime, for the interval 0.5 ms $> \tau_a >$ 0.02 ms. In these cases, the plot of residuals of every fit (Fig. 7B) revealed more clearly the goodness of the fit than the direct superposition of simulated data over experimental values. Fig. 7B shows that values of $\tau_a$ smaller than 0.1 ms give very similar residual trends to those of the experimental values, indicating that $\tau_a$ must be smaller than 0.1 ms. To our knowledge, this estimate represents the first approximation to the water residence time in the mitochondrial matrix.

Insight about the mobility of the water molecule in the intramitochondrial space may be obtained from the relationship of the relaxation times with the rotational correlation time ($T_2$) (26). $T_2$ values of the extra (intra) mitochondrial medium were 4.57 $\pm$ 2.15 ms (5.79 $\pm$ 0.59 ms), which led to calculated values of $\tau_a$ of 4.5 $\times$ 10$^{-10}$ s ($6.2 \times 10^{-9}$ s), respectively. Thus, water rotational mobility in the matrix is significantly restricted as compared to the extramitochondrial medium. Moreover, water protons in distilled water at 20 °C have a $\tau_a$ of about 3 $\times$ 10$^{-12}$ s (33, 35), approximately three orders of magnitude shorter than the matrix correlation times.

The dynamic interpretation of intramitochondrial relaxation times deserves further attention. Several reports have shown that water in tissues, cells (34–37), or even in intact mitochondria (38), is heterogeneously distributed in different phases. Phase heterogeneity is thought to be the result of the different physical properties of water molecules in the "bulk solvent" and those "bound or adsorbed" to macromolecules or cellular surfaces (39). While bulk solvent water is able to rotate freely, water bound or adsorbed on macromolecular surfaces is thought to adopt the correlation time of the host macromolecule (33). The exchange of water molecules between these different phases is thought to be fast in the NMR time scales (33, 40). Thus, during the $T_{2b}$ relaxation period, water molecules have a defined probability ($0 \leq p_i \leq 1$) to relax in a variety of intramitochondrial microenvironments, including bulk rotational freedom and an array of restricted macromolecular rotations ($T_{2i}$). Thus, $T_{2b}$ can be expressed as:

$$T_{2b} = \sum_{i} p_i T_{2i}^{-1}$$  
(Eq. 5)

Accordingly, the effective intramitochondrial correlation time for water ($\tau_c$) calculated above from $T_{2b}$, contains the weighted average of the contributions from the different correlation times experienced by the water molecule during its intramitochondrial relaxation. As indicated in the results section, viscosity is thought to be the main determinant of reduced water mobility in the matrix. Intramitochondrial relaxation times were found to be similar to those of glycerol suspensions of 40 cP. This apparent matrix viscosity is approximately 15 times larger than the apparent viscosity of the cytoplasm in human erythrocytes (2.10 cP) (11).
Dynamics and Environment of Mitochondrial Water

kinetics of intramitochondrial reactions. The diffusion coefficient (D) of water and substrates are inversely related to the viscosity (η) by the Einstein-Stokes relationship D = kT/6πηr0, where k is the Boltzmann constant, T the absolute temperature, and r0 the Stokes radius of the molecule under study. Thus, a 15 times increase in the average viscosity of the intramitochondrial environment as compared to the cytoplasm can account for an identical reduction in the diffusion coefficient of water and even for a larger reduction in the diffusion coefficient for larger substrates. These reductions can introduce kinetic limitations in those mitochondrial reactions which are diffusion controlled, mainly hydration-dehydration and proton transfer reactions. Notably, recent evidence indicates that cytosolic and mitochondrial aminotransferases of alanine and glutamate control, mainly hydration-dehydration and proton transfer reactions. Notably, recent evidence indicates that cytosolic and mitochondrial aminotransferases of alanine and glutamate

Simulations with open symbols indicate no water exchange (τm, τs → ∞). The dashed line is a simulation for the case of no water exchange with τm = 0. Closed symbols indicate experimental measurements. A, residuals plot obtained for the experimental results fitted to a single exponential (■—■) and to simulations performed in the interval 0.5 ms < τm < 20 ms. Note that the residuals obtained from the simulations mimic better those of the experimental data for the interval 100 > τm > 20 μs.

Acknowledgments—We thank Dr. Julio San Román and Dr. José Manuel Pereira for their collaboration and facilities in the viscosity measurements, Dr. Paloma Calle for measurements of free Mn2+ by EPR, and Dr. Juan M. Gancedo and Dr. Juan J. Aragón for helpful discussion and critical reading of the manuscript.

REFERENCES

1. Haines, T. H. (1994) FEBS Lett. 346, 115–122
2. Latour, L. L., Svoboda, K., Mitra, P. P., and Sotak, C. H. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 1229–1233
3. Podletner, M., Setz-Beyerl, J., and Heinzinger, H. (1993) Naturforsch. 48c, 654–665
4. Sidman, A. K. (1989) Methods Enzymol. 173, 192–222
5. Benga, G., Pop, V. I., Popescu, O., and Borza, V. (1990) Biochim. Biophys. Methods 21, 87–102
6. Benga, G. (1989) Int. Rev. Cytol. 114, 273–316
7. Chien, D. Y., and Macey, R. I. (1977) Biochim. Biophys. Acta 464, 45–52
8. Andruski, J. (1976) Biochim. Biophys. Acta 428, 304–311
9. Colón, T., and Outhred, R. (1972) Biochim. Biophys. Acta 288, 354–361
10. Shpper, M., and Civan, M. M. (1975) Biochim. Biophys. Acta 285, 81–87
11. Priez, W. S., Kuchel, P. W., and Cornell, B. A. (1989) Biophys. Chem. 33, 205–215
12. Ovádi, J., and Srere, P. A. (1992) Trends Biochem. Sci. 17, 445–447
13. Srere, P. A. (1987) Annu. Rev. Biochem. 56, 89–124
14. Srere, P. A., Sumegi, B., and Sherry, A. D. (1987) in Krebs’ Citric Acid Cycle. Half a Century and Still Turning (Kay, J., and Wetzman, P. D., eds) Vol. 54, pp. 173–182, Cambridge University Press, New York
15. Welch, G. R., and Easterby, J. S. (1994) Trends Biochem. Sci. 19, 193–197
16. Welch, G. R. (1988) Symp. Biol. Hung. 30, 211–226
17. Herbst, M. D., and Goldstein, J. H. (1989) Am. J. Physiol. 256, C1097–C1104
18. Ridwood, D., Wilson, M. T., and Darley-Urman, V. M. (1987) in Mitochondria: A Practical Approach (Darley-Urman, V. M., Ridwood, D., and Wilson, M. T., eds) pp. 1–16, IRL Press, Washington D.C.
19. Nieto, R., Cruz, F., Tejedor, J. M., Barroso, G., and Cerdán, S. (1992) Biochimie (Paris) 74, 903–911
20. Dawson, A., Klingenberg, M., and Krämer, R. (1987) in Mitochondria: A Practical Approach (Darley-Urman, V. M., Ridwood, D., and Wilson, M. T., eds) pp. 35–78, IRL Press, Washington D.C.
21. Lang, R. D. A. (1987) in Mitochondria: A Practical Approach (Darley-Urman, V. M., Ridwood, D., and Wilson, M. T., eds) pp. 17–34, IRL Press, Washington D.C.
22. Scarpa, A. (1979) in Membrane Transport in Biology II: Transport Across Single Biological Membranes (Giebisch, G., Tosteson, D. C., and Ussing, H., eds), pp. 263–355, Springer-Verlag, Berlin
23. Molday, R. S., and Mackenzie, D. (1982) J. Innuond. Methods 52, 353–367
24. Renschaw, P. F., Owen, C. S., Evans, A. E., and Leigh, J. S., Jr. (1986) Magn. Reson. Imaging 4, 351–357
25. Straume, M., and Johnson, M. (1992) Methods Enzymol. 210, 87–105
26. Gadian, D. G. (1982) Nuclear Magnetic Resonance and Its Application to Living Systems, pp. 125–129, Oxford University Press, Oxford, UK
27. LaNoüe, K. F., Strzelecki, T., and Finch, F. (1984) J. Biol. Chem. 259, 4116–4121
28. Wang, J. H. (1951) J. Am. Chem. Soc. 73, 510–513
29. Benga, G., Popescu, O., Pop, V. I., Hodor, P., and Borza, T. (1992) Eur. J. Cell Biol. 59, 219–223
30. Hutson, S. M., Williams, G. D., Berkich, D. A., LaNoüe, K. F., and Briggs, R. W. (1992) Biochemistry 31, 1322–1330
31. Gunter, T. E., and Puskin, J. S. (1972) Biophys. J. 12, 625–635
32. McConnell, H. M. (1958) J. Chem. Phys. 28, 430–431
33. Koening, S. H., and Schilling, W. (1969) J. Biol. Chem. 244, 3283–3289
34. Foster, M. A. (1884) Magnetic Resonance in Medicine and Biology, pp. 137–147, Pergamon Press Ltd., Oxford, UK
35. Cooke, R., and Kurtz, J. D. (1974) Annu. Rev. Biophys. Bioeng. 3, 95–126
36. Cleveland, G. G., Chang, D. C., Hazelwood, C. F., and Rorschach, H. E. (1976) Biophys. J. 16, 1043–1053
37. Neville, M. C., Paterson, C. A., Rae, J. L., and Woessner, D. E. (1974) Science 184, 1072–1074
38. Garlid, K. D. (1979) in Cell Associated Water (Drost-Hansen, W., and Cleg, J., eds), pp. 293–361, Academic Press, New York
39. Israelachvili, J., and Wennerström, H. (1996) Trends Biochem. Sci. 21, 99–126
40. Koening, S. H., Hallenga, K., and Shporer, M. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 2667–2671
41. Fehrst, A. (1977) Enzyme Structure and Mechanism, pp. 126–132, W. H. Freeman & Co., New York
42. Molides, M., Cerdán, S., Erhardt, P., and Seelig, J. (1994) NMR Biomed. 7, 249–262

Fig. 7. Representative model simulations of the kinetics of water exchange across the inner mitochondrial membrane. Simulations of experimental Tm (A) relaxation curves for different mitochondrial preparations (100 mg/ml, n = 4) were obtained using the model of Fig. 6. The continuous line with open symbols is a simulation for the case of no water exchange (τm, τs → ∞). The dashed line is a simulation for the case of fast water exchange with τm < 100 μs. Closed symbols indicate experimental measurements. B, residuals plot obtained for the experimental results fitted to a single exponential (■—■) and to simulations performed in the interval 0.5 ms < τm < 20 ms. Note that the residuals obtained from the simulations mimic better those of the experimental data for the interval 100 > τm > 20 μs.
Dynamics and Environment of Mitochondrial Water as Detected by H NMR
Emilio A. López-Beltrán, María J. Maté and Sebastián Cerdán

J. Biol. Chem. 1996, 271:10648-10653.
doi: 10.1074/jbc.271.18.10648

Access the most updated version of this article at http://www.jbc.org/content/271/18/10648

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 34 references, 5 of which can be accessed free at http://www.jbc.org/content/271/18/10648.full.html#ref-list-1