Evidence of Surface Diffusion of Water Molecules on Proteins of Rabbit Lens by ^{1}H NMR Relaxation Measurements

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In this work, we propose a relaxation model for the interpretation of NMR proton spin-lattice and spin-spin relaxation times of mammalian lenses. The framework for this model is based on nuclear magnetic spin-lattice relaxation measurements as a function of temperature at different Larmor frequencies for whole rabbit lenses and fragments of the lens. According to this model, two different dynamic processes of the water molecules determine the relaxation behaviour, namely rotational diffusion and translational surface diffusion. These dynamic processes in conjuction with a two site exchange model give a good explanation of all the measured relaxation data. From the experimental data, we were able to obtain the activation parameters for rotational and translational diffusion of bound lens water. Correlation times of 2.1×10^{-11} sec and 2.5×10^{-9} sec and activation energies of 20.5 kJ/mol and 22.5 kJ/mol respectively were found at 308K. At low Larmor frequencies (≤ 100 MHz) the longitudinal relaxation is mainly determined by translational surface diffusion of bound water with a mean square displacement of 1.5 nm, whereas at higher frequencies (≥ 300 MHz), rotational diffusion is the main relaxation mechanism. The spin-spin relaxation is determined by translational diffusion over the whole frequency range and therefore shows only a very small dispersion. By our model it is possible to explain: 1) the strikingly large difference between the T_1 value and the T_{2A} and T_{2B} values observed in the lens and 2) the different values of the activation energies measured at different fields for the lens.

Introduction

Studies on the magnetic relaxation properties of protons in water molecules of biological tissues is a subject of increasing interest, mainly due to the rapid progress in medical applications of NMR, as these relaxation properties form the main image-forming mechanism in MRI (Mansfield and Moris, 1982; Higgins *et al.,* 1992). Moreover, since these relaxation processes depend in a characteristic way on the morphological and pathological state of the tissues, a detailed understanding of proton relaxation processes in biological systems might yield a valuable insight into these states. There is, however, a general problem with biological systems, as these systems are usually not very well-defined in the physical sense, but are rather inhomogeneous, thus making it difficult to extract unique information from experiments on these samples. Therefore, one has to use either simple artificial systems, e.g. solutions, gels or powders of proteins (Belton *et al.,* 1988; Grucker *et al.,* 1990; Galier *et al.,* 1987), which can act as a model for real tissue; or a biological system with a well-defined structure must be found. One biological system with a well-defined structure can be found in the lens of the mammalian eye which is transparent and capable of projecting a picture onto the retina, requiring a high state of order in the lens (Delay and Tardieu, 1983; Koretz and Handelman, 1988).

The mammalian lens has a relatively simple and regular structure, which is shaped like a flattened globe and is surrounded by a capsule, under which lies a layer of epithelial cells. These cells reproduce and create elongated lens fibers, which are formed through a lifetime, with new fibers covering the old ones. The oldest fibers in the centre of the lens are "glued" together and form a compact mass known as the nucleus; the rest of the lens

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forms the cortex. The fibers make up the bulk of the lens cortex and nucleus. Each fiber represents an elongated cell with a membrane. The cells lose their nuclei as they grow older (Maisel, 1985). The normal eye lens contains approximately 65% water and 35% organic materials which are mainly structural proteins. With this high protein content (33% of the total weight), the lens has the highest protein concentration of all organs in the body (Maisel, 1985). About 85% of the total mass of the proteins forms crystallins called: α (15% , molecular weight (mw) $\cong 10^6$ Da), β (55%, mw $\cong 4\times10^4$ Da) and γ (15%, mw $\cong 2\times10^4$ Da).

Protons in water-protein systems can be classified into at least three different groups: protein protons, hydration (non-freezable, bound) water protons and bulk (freezable, free) water protons. Molecules belonging to the free water portion should exhibit dynamic behaviour which assumably, should be more or less similar to pure water, because the presence of neighbouring proteins does not impose any severe restrictions on these molecules. The situation however, is different for molecules in the hydration shell of the protein which can be seen in the non-freezable nature of these water molecules, meaning that interactions with polar groups from proteins hinder the formation of a regular structure among these water molecules. The average motion of water molecules in the bound phase is particularly reduced so that the residence time of water molecules in a given volume element increases. Moreover, the presence of the surface of the protein imposes geometrical restrictions on possible reorientations of water molecules which, in particular, may become anisotropic. Also, the translation motions of the water molecules will generally, occur with differing probabilities (normal or tangential) at the surface of the protein. Exchange processes between these various types of proton are visible by ^{1}H NMR. In principle, these can be either the material exchange of proton, or a protonated group by chemical exchange, or the exchange of whole molecules (in which protons are located, e.g. mainly water molecules) by diffusion-like processes, or an exchange of magnetization only, of different protons, i.e. a (immaterial) spin exchange, also known as cross-relaxation or spin diffusion (Edzes and Samulski, 1977; Koenig *et al.*, 1978; Kimmich *et al.*, 1990).

Recent works (Bodurka *et al.*, 1994; Gutsze *et al.*, 1995) have shown that the first type of exchange determines the relaxation properties of the lens. The time constant of exchange is in the fast limit on the T_1 time scale, leading to a single averaged T_1 for the whole lens, but slow on the T_2 scale. Therefore, two distinct T_{2A} ,T_{2B} values are observed. In this case, the spin-lattice relaxation rate of the lens is given by:

$$\left(\frac{1}{T_1}\right)^{\mathrm{Exp}} = \left(\frac{p_A}{T_{1A}}\right) + \left(\frac{p_B}{T_{1B}}\right) \cong \frac{1}{T_{1A}} + \frac{p_B}{T_{1B}} \qquad (1)$$

where p_v, $1/T_{1v}$ v = A,B are the proton fractions and the relaxation rates of water protons in free (A) and bound (B) environments. The following relations have been found for the whole lens and its fragments: $T_{1A} > T_{1B}$ and $p_A > p_B$ (Stankiewicz *et al.*, 1989; Cameron *et al.*, 1988; Lerman *et al.*, 1982).

In order to decide which mechanisms are responsible for the relaxation behaviour, one has to measure the magnetic field dependence of the relaxation rates at various temperatures. From these experiments, a dispersion profile of the relaxation rate can be obtained which is characteristic for the underlying relaxation mechanism (Abragam, 1961; Pfeifer, 1972). Therefore, measurements were executed on several NMR spectrometers with differing magnetic fields. The rest of the paper is organised as follows: after a short introduction into the experimental set up and samples, the results of the spin lattice relaxation rates at different B_0 fields are presented. A discussion on these results in terms of possible relaxation models follows and finally, the most important results are summarised.

Methods and Materials

Measurements of spin lattice relaxation rates were performed at four different B_0 fields (1.409 T, 2.349 T, 7.046 T, 11.744 T), corresponding to proton resonance frequencies (60 MHz, 100 MHz, 300 MHz and 500 MHz). The measurements at 60 MHz were carried out on a pulse NMR spectrometer (PMS-60, Radiopan Poland). The spectrometer was equipped with a temperature control system which allowed a stabilization of the probe temperature to an accuracy of approximately 0.5 K. The measurements at 100 MHz were executed on a pulse Fourier Transform (FT) NMR spectrometer (Tesla BS 561A). Measurements at 300 MHz were performed on a Bruker MSL 300 pulse FT spectrometer which was equipped with a Bruker VT1000 temperature control unit and the measurements at 500 MHz were performed on a Bruker AMX-500 pulse FT spectrometer which was also equipped with a VT1000 temperature control unit. The spin lattice relaxation rates at 60 MHz, 300 MHz and 500 MHz were measured via the saturation recovery technique and the spin lattice relaxation rates at 100 MHz were measured by the inversion recovery sequence (due to technical limitations of the Tesla BS561A spectrometer). Typical 90° pulse widths were 2.5 μsec at 60 MHz, 10 μsec at 100 MHz and 300 MHz, and 10 μsec at 500 MHz. Temperature stability was approximately 0.5 K. Absolute accuracy of the temperature is 1 K.

For the two lower frequencies, the relaxation rates were determined from the intensity of the free induction decay after signal recovery and for the two higher frequencies, for the relaxation rates were calculated from the spectrum. Measurements were carried out on whole lenses and lens fragments of several two month-old rabbits. The deviations in the obtained relaxation rates for different animals were below 15%.

Experimental Results

Fig. 1 compares the results of the saturation recovery T_1 measurements carried out on a slice of the lens with measurements of the bare nucleus. Both curves show an increase of the relaxation time with increasing temperature and a sudden step in the relaxation time at about −16 °C (257 K) which is caused by partial freezing of the water in the lens. The low freezing temperature of the bulk water stems from the fact that the lens can be easily undercooled to temperatures well below the freezing point of water. The achievable maximum undercooling temperature is a function of the cooling rate, i.e. an experimentally adjustable parameter (Gutsze *et al.,* 1995). For temperatures above freezing, the higher amount of freezable water leads to a slower spin lattice relaxation for the whole lens in comparison to the nucleus with its higher protein concentration which causes faster spin-lattice relaxation. In the temperature regime below the freezing point, both rates are equal, because now only non-freezable hydration water of the protein hull is observed. For both temperature regimes, a linear dependence of the logarithm of the relaxation time $\log(T_1)$ on the temperature is found.

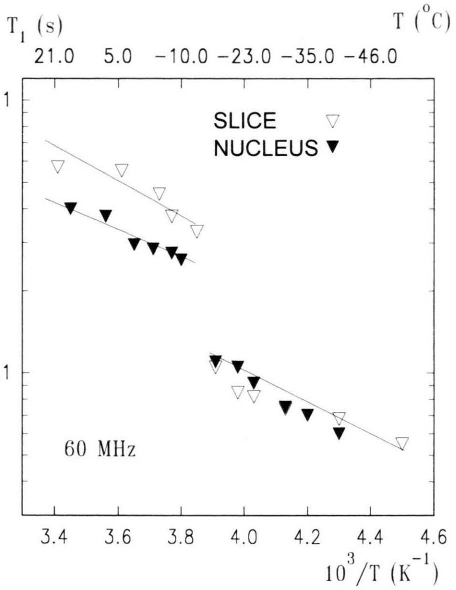

Fig. 1. The dependence of the spin-lattice relaxation time of the lens slice (▽) and the lens nucleus (▼) as a function of temperature measured at a Larmor frequency of 60 MHz.

Fig. 2 depicts the result of the inversion recovery T_1 measurements on a whole lens at 100 MHz. Again, an increase of the relaxation time with increasing temperature is found for temperatures above the freezing point and the sudden step in the relaxation time at about −16 °C (257 K) is caused by partial freezing of the lens water. In the temperature regime below the freezing point, the linear dependence of $\log(T_1)$ on the temperature changes to a more parabolic dependence, indicating the closeness of the T_1 minimum.

Fig. 3 presents the result of the saturation recovery T_1 measurement on a whole lens at 300 MHz, showing a clear minimum in the dependence of $\log(T_1)$ on the temperature in the regime below freezing level (−13 °C). However measurements above the freezing point exhibit still an increase of the relaxation time with increasing temperature.

Fig. 4 shows the result of the saturation recovery T_1 measurement on a whole lens in comparison to

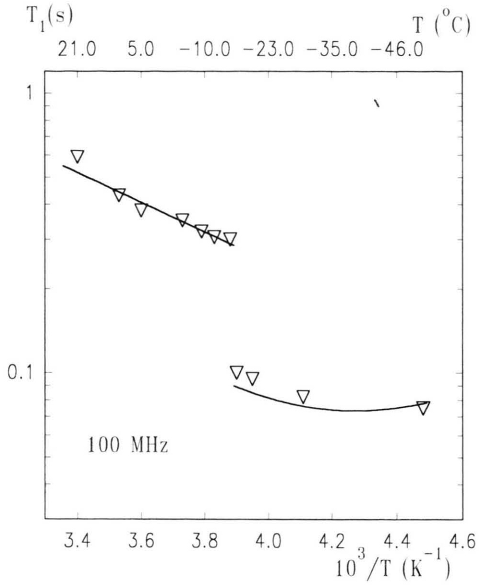

Fig. 2. The dependence of the spin-lattice relaxation time of the whole lens as a function of temperature measured at a Larmor frequency of 100 MHz.

the nucleus alone at 500 MHz. For measurements above freezing point (−8 °C), there is still an increase of the relaxation time with increasing temperature, but in the temperature regime below the freezing point, a decrease of the relaxation times with increasing temperature occurs. In other words, in comparison to previous figures, we are now on the opposite side of the T_1 minimum.

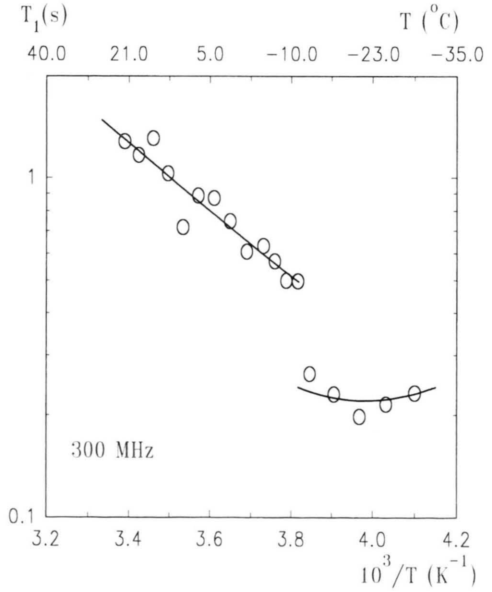

Fig. 3. The dependence of the spin-lattice relaxation time of the whole lens as a function of temperature measured at a Larmor frequency of 300 MHz.

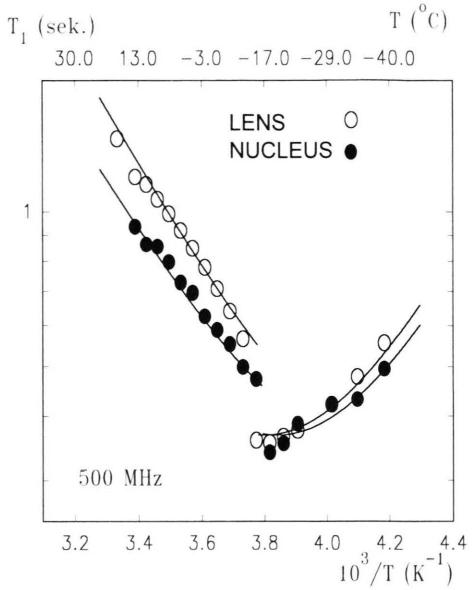

Fig. 4. The dependence of the spin-lattice relaxation time of the whole lens (○) and the lens nucleus (●) as a function of temperature measured at a Larmor frequency of 500 MHz.

Fig. 5 compares the results of the saturation recovery T_1 measurement of a whole lens to measurements of the homogenate of the lens as a function of temperature at 500 MHz. An immediate result obtained from this figure is that the structure of the lens does not influence the spin lattice relaxation time, because the measured rates are equal for both samples.

We fitted all the relaxation data (solid line on figures), assuming a thermally activated Arrhenius process

$$\tau_c = \tau_\infty \exp\left(\frac{E_A}{kT}\right) \tag{2}$$

where: τ_∞ correlation time for infinite temperature; E_A activation energy in kJ/mol.

Taking a possible distribution of the correlation times into account, the log-normal distribution of the correlation times (3.1) from literature (Pfeifer, 1972; Bottomley *et al.*, 1984) is employed.

Table I. Results from fitting the T_1 curves with Eqs. (2, 3.1–3.4) at different B_0 fields, assuming a thermally activated Arrhenius process and the log-normal distribution of the correlation times. The fitting parameters are: the activation energy (E_A); the correlation time for infinite temperature (τ_∞); the width of the distribution (β) of the correlation time.

		Above the freezing point			Below the freezing point		
	ν [MHz]	τ_∞ [sec]	E_A [kJ/mol]	β	τ_∞ [sec]	E_A [kJ/mol]	β
Slice	60	1.9×10^{-13}	12.1	1.2	9.7×10^{-13}	11.8	0.7
Nucleus	60	5.0×10^{-14}	13.9	1.5	3.4×10^{-13}	13.8	0.1
Whole lens	100	9.4×10^{-13}	9.8	0.05	3.4×10^{-13}	14.7	0.21
Whole lens	300	8.3×10^{-15}	18.7	0.01	3.6×10^{-15}	22.6	0.01
Whole lens	500	5.6×10^{-15}	19.6	0.01	1.0×10^{-14}	20.5	0.01
Nucleus	500	1.4×10^{-14}	18.3	0.01	9.4×10^{-15}	20.4	0.05
Lens homogenate	500	3.8×10^{-15}	20.6	0.00	9.4×10^{-15}	20.5	0.01

$$\frac{1}{T_1} = \int_0^\infty \frac{g(\tau_c)}{T_1(\tau_c)}\, d\tau_c \tag{3.1}$$

where: $g(\tau_c)$ is the normalised distribution function of the correlation times and

$$f(s) = g(\tau_c)\,\frac{d\tau_c}{ds} = \frac{1}{\beta\sqrt{\pi}}\exp(s/\beta^2) \tag{3.2}$$

where: $s = \ln(\tau_c/\tau_0)$; τ_0 is the mean correlation time and β determines the width of the distribution.

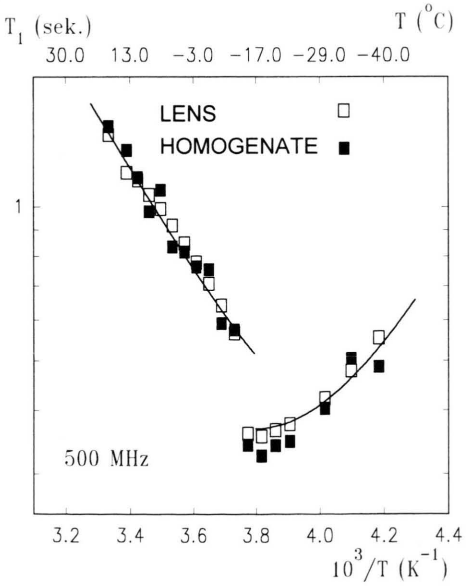

Fig. 5. The dependence of the spin-lattice relaxation time of the whole lens (□) and its homogenate (■) as a function of temperature measured at a Larmor frequency of 500 MHz.

The resulting activation energies (E_A), correlation times (τ_∞) and the distribution width (β) determined from fitting the T_1 curves at the different B_0 fields are summarized in Table I. We found that for all fields the distribution width β is rather small, thus a single correlation time can be safely assumed. The activation energies exhibit a strong dependence on the B_0 field, indicating that the motional processes responsible for relaxation have not yet reached their fast limit.

Discussion

In the following we want to discuss the T_1 relaxation behaviour found in the lens in terms of the underlying relaxation models. As shown by Abragam in chapter VII of his famous book (Abragam, 1961), Bloembergen already pointed out (Bloembergen *et al.,* 1948) , that there are two principal relaxation mechanisms responsible for the spin lattice relaxation in water, namely rotational and translational diffusion of water molecules. Both mechanisms have a characteristic dependence on the B_0 field (dispersion). The overall rate is the sum of these two rates:

$$\left(\frac{1}{T_1}\right)^{\text{Exp}} = \left(\frac{1}{T_1}\right)^{\text{rot}} + \left(\frac{1}{T_1}\right)^{\text{tr}}. \tag{4}$$

The spectral density functions for the spin lattice relaxation rate of the rotational diffusion (Abragam, 1961) are Lorentzian. Since the typical rotational correlation time of water is in the order of 10^{-12} sec (Bloembergen *et al.,* 1948), which is about 3–4 orders of magnitude faster than the inverse larmor frequency (in rad/sec), the denominator of these lorentzian spectral density functions can be neglected ($\omega\tau_c^{rot} \ll 1$). If these results from pure water are compared to the situation in the lens, there is one main difference. In the lens a fraction of the water is bound to the proteins, forming their hydration hull (Bettelheim and Popdimitrova, 1990; Cameron *et al.*, 1988) We assume that in comparison to free water molecules, the rotations of these bound water molecules are not strongly hindered. Similar rotational correlation times are thus expected and $\omega\tau_c^{rot} \ll 1$ is still valid . However, the translational degrees of freedom of these molecules are hindered by this binding to the proteins, so that longer translational correlation times are expected for these molecules. This has been found in experiments on water protein solutions, where translational correlation times in the order of 10^{-9} sec have been found as well as in experiments on several types of tissue where correlation times in the order of 10^{-7} sec were found (Kimmich *et al.*, 1990; Schauer *et al.*, 1988). The correlation time is now of the same order of magnitude as the inverse Larmor frequency ($\omega\tau_c^{tr} \approx 1$) or even slower than the inverse Larmor frequency ($\omega\tau_c^{tr} \ll 1$). As pointed out by Abragam (Chap. X) in his treatise on the diffusion in the crystal lattices, in this case the random walk model of Torrey is appropriate in describing the diffusion process (Torrey, 1953). The spectral density function obtained by Torrey is given as (Torrey, 1953; Pfeifer, 1972):

$$J_1^{tr}(\omega) = \frac{8\pi n}{15a^3\omega} f(\alpha, \mathrm{x}) \tag{5.1}$$

where

$$f(\alpha,\mathrm{x}) = \frac{2}{x^2}\left\{\nu\left[1-\frac{1}{\nu^2+u^2}\right]+\left[\nu\left[1-\frac{1}{\nu^2+u^2}\right]+2\right]e^{-2\nu}\cos(2u)+ u\left[1-\frac{1}{\nu^2+u^2}\right]e^{-2\nu}\sin(2u)\right\} \tag{5.2}$$

and

$$\begin{matrix}u\\(\nu)\end{matrix} = \frac{1}{2}\sqrt{\frac{q(1(\mp)q)}{\alpha}}; \quad q = \frac{\alpha x^2}{\sqrt{1+\alpha^2x^4}}; \quad \alpha = \frac{<r^2>}{12a^2};$$

$$x = \sqrt{\frac{\omega a^2}{D}} \tag{5.3}$$

where: ω-proton resonance frequency , *n*-proton density, *a*-closest possible distance of approach of two protons from adjacent water molecules, $<r^2>$-mean square displacement of water molecule.

The relaxation rates in this case are given as:

$$\left(\frac{1}{T_1}\right)^{tr} = \frac{9}{8}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma^4\hbar^2 \left(J_1^{tr}(\omega)+4J_1^{tr}(2\omega)\right) \tag{6.1}$$

and

$$\left(\frac{1}{T_2}\right)^{tr} = \frac{9}{8}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma^4\hbar^2 \left(\frac{3}{2}J_1^{tr}(0)+\frac{5}{2}J_1^{tr}(\omega)+4J_1^{tr}(2\omega)\right) \tag{6.2}$$

Using equations (1,4÷6), together with the assumption, that the rotational correlation time of the bound water molecules has not changed and is independent of the Larmor frequency, we calculated the dispersion curve of the spin lattice relaxation rate as a function of the Larmor frequency for two different cases: i) the water molecules move in small steps and ii) the water molecules jump in rather large steps. The results of these calculations are presented in Figs 6 and 7 respectively. As can be seen from Fig. 7 (the second model) i.e. diffusion of the water molecules in rather large jumps clearly gives a better description of the experimental data. To obtain the value for the average jump distance the second model was fitted to the experimental data using additional values of T_1 (25 MHz, 80 MHz) taken from the literature (Neville *et al.*, 1974; Stankiewicz *et al.*, 1989). As an example, the result of such a fitting at 308K is shown in Fig. 8. The correlation times for rotational and translational diffusion are $\tau_c^{rot} = 2.1\times10^{-11}$ sec and $\tau_c^{tr} = 2.5\times10^{-9}$ sec respectively. From this, an average jump distance of the water molecule of about of 1.5 nm was calculated, assuming the bound water diffusion coefficient in the lens in the order of 10^{-10} m^2/sec (Kimmich *et al.*, 1990; Wu *et al.*, 1993; Neville *et al.*, 1974). The calculated correlation time for rotational diffusion matches well with values of correlation times obtained at high (300 MHz and 500 MHz) resonance frequencies (Table 1). This indicates that at high magnetic field the spin-lattice relaxation is mainly determined by rotational diffusion of the water molecules. The translational spin lattice relaxation rate increases when decreasing the resonance frequency and at low field it dominates the overall relaxation rates (Fig. 7). Over the whole frequency range discussed here, the condition for the motional narrowing is fulfilled for rotational diffusion at room temperature whereas for translational diffusion this is not the case. This explains the dependency of the activation energies on the resonance frequency (Table I). At a given resonance frequency the minimum of the spin lattice relaxation time due to translational diffusion is shifted to higher temperatures, in comparison to rotational diffusion (Fig. 9). By increasing the Larmor frequency, the T_1 curves are shifted to higher temperatures. At high fields (300 MHz and 500 MHz) the slope of the log(T_1) curve is mainly determined by the rotational diffusion. This causes a steeper slope as compared to the lower fields (100 MHz and 60 MHz) where simultaneously, both contributions, namely rotational and translational diffusion play a role.

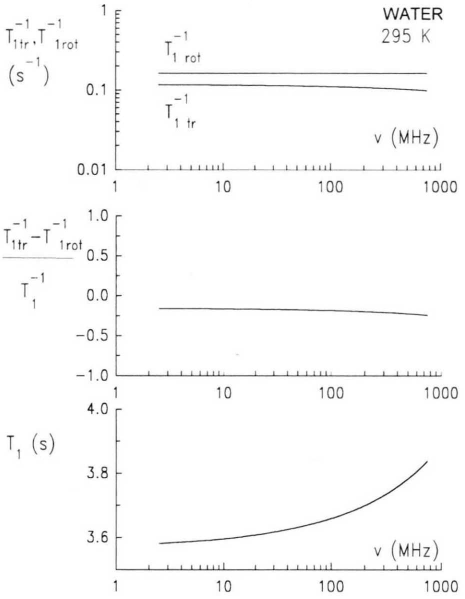

Fig. 6. The results of calculations from (4–6) for diffusion of water molecules moving in small jumps: a) upper figure: the spin-lattice relaxation rates due the translational diffusion $(T_1^{\mathrm{tr}})^{-1}$ and rotational diffusion $(T_1^{\mathrm{rot}})^{-1}$ as a function of the Larmor frequency; b) middle figure: the relative contribution of the spin-lattice relaxation rates normalised to the overall value of the spin-lattice relaxation rate as a function of the Larmor frequency; c) lower figure: the overall value of the spin lattice relaxation rate (Eq. (4)) as a function of the Larmor frequency. Calculations were performed using the following molecular parameters: the water diffusion coefficient equal to $D = 2.5\times10^{-9}$ m²/s (Kärger *et al.*, 1988); the mean displacement of water molecule: <r> = 3×10^{-10} m (Bloembergen *et al.*, 1948); the closest possible distance of approach of two protons from adjacement water molecules: $a = 3\times10^{-10}$ m (Bloembergen *et al.*, 1948); the same correlation times for translational and rotational diffusion were assumed: $\tau_C^{tr} \cong \tau_C^{rot} = 3\times10^{-12}$ sec (Hindman *et al.*, 1973).

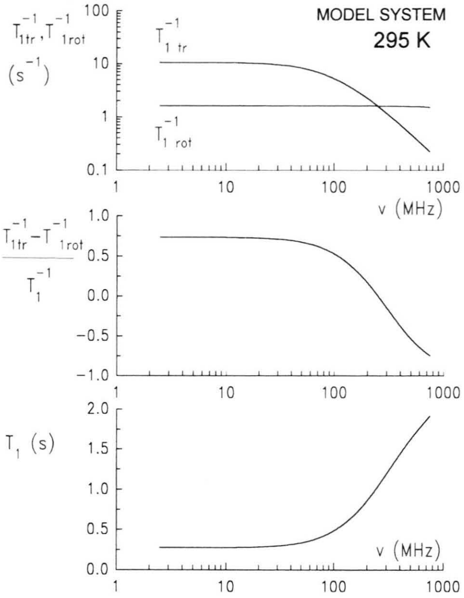

Fig. 7. The results of calculations from (4–6) for diffusion of water molecules moving in large jumps: a) upper figure: the spin-lattice relaxation rates due the translational diffusion $(T_1^{\mathrm{tr}})^{-1}$ and rotational diffusion $(T_1^{\mathrm{rot}})^{-1}$ as a function of the Larmor frequency; b) middle figure: the relative contribution of the spin-lattice relaxation rates normalised to the total value of the spin-lattice relaxation rate as a function of the Larmor frequency; c) lower figure: the overall value of the spin lattice relaxation rate (Eq. (4)) as a function of the Larmor frequency. Calculations were performed using the following molecular parameters: the water diffusion coefficient equal to $D = 1.0\times10^{-9}$ m²/s (Neville *et al.*, 1974; Wu *et al.*, 1993); the mean displacement of water molecule: <r> = 3×10^{-9} m; the closest possible distance of approach of two protons from adjacement water molecules: $a = 3\times10^{-10}$ m (Bloembergen *et al.*, 1948); the correlation times for rotational diffusion was assumed: $\tau_C^{rot} = 3\times10^{-11}$ sec.

In order to calculate the temperature dependence of T_1 in terms of the proposed relaxation model the value of the activation energy for the translational diffusion must be known. The static part of the spectral density function $(J_1^{\mathrm{tr}}(0))$ is the leading term in the expression of the translational spin-spin relaxation time (Eq. (6.2)). The activation energy calculated from the temperature de-

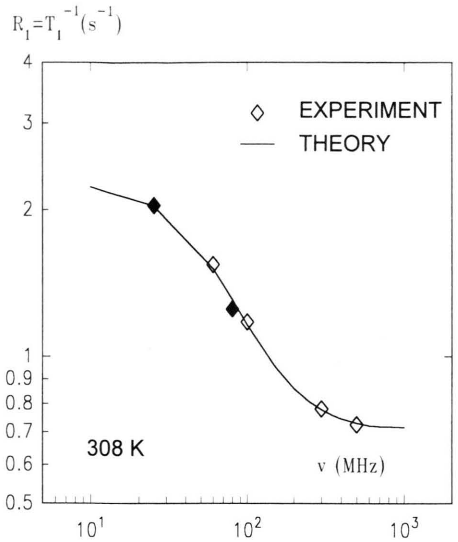

Fig. 8. Calculated (Eqs (4–6); solid line) and measured (◆ literature data (Neville *et al.*, 1974; Stankiewicz *et al.*, 1989); ◇ our data) spin-lattice relaxation time as a function of the larmor frequency (dispersion) measured at 308 K.

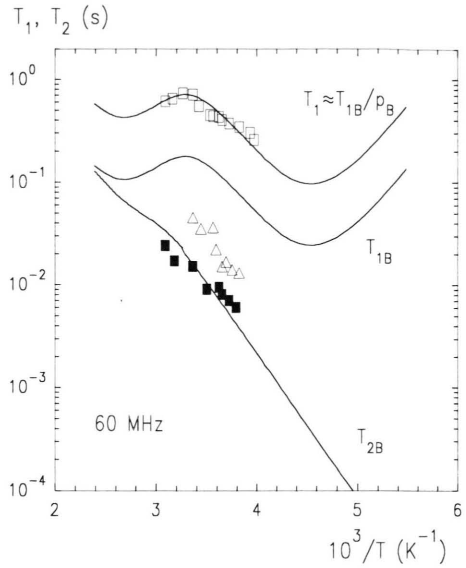

Fig. 9. Calculated (solid lines) and experimental (T_1 □; T_{2A} △; T_{2B} ■) spin-lattice relaxation and spin-spin relaxation times as a function of temperature for the rabbit lens on 60 MHz. The calculation were done with Eqs (1, 2, 4–6), using $\tau_\infty^{rot} = 5.6\times10^{-15}$ sec; $E_A{}^{rot} = 19.6$ kJ/mol for the activation parameters of the rotational diffusion and $\tau_\infty^{tr} = 3.0\times10^{-13}$ sec; $E_A{}^{tr} = 22.5$ kJ/mol for the translational diffusion.

pendence of T_{2B} below the freezing point of bulk water as the value characteristic for the translational diffusion of bound water in the lens may be employed. The value obtained in this way is equal to 22.5 kJ/mol (Bodurka, 1995). We may use the activation parameters for the rotational and translational diffusion to calculate from Equs. (1, 2, 4÷6) the complete relaxation behaviour of the lens and compare this to the experimental data. The result of this comparison is shown in Fig. 9. To summarize these results, we have found that the spin lattice relaxation behaviour of bound, hydration water in the rabbit lens can be described as a superposition of relaxation caused by fast molecular reorientations and relaxation caused by rather slow translational diffusion jumps over long distances.

We now want to interpret these results in terms of a physical picture of the water diffusion in the hydration shell, following the ideas proposed by Kimmich and coworkers (Kimmich *et al.*, 1990; Schauer *et al.*, 1988). As discussed there, in water protein systems, one can not generally expect a three-dimensional diffusion of the water molecules. Due to the existence of polar groups on the surface of the protein molecule, motions of water molecules tangential to the surface will require a different activation energy, in comparison to motions normal to the surface plane. In principle, a tensor has to be used for describing the anisotropy of the diffusive motion of the molecule. In this work, it was shown that the diffusion coefficient for water molecules in this hydration hull is close (within a factor of two or three) to the value of free water, but the average time between two molecular jumps is several orders of magnitude longer, compared to the free water. Using the relationship between diffusion coefficient and correlation time for two-dimensional surface diffusion: $D = \langle r^2\rangle/4\tau_C{}^{tr}$ it follows that the mean squared displacement of the water molecule must have increased with approximately the same factor with which the correlation time decreased. If we compare these findings with our own results, that the spin lattice relaxation in the bound water is caused by a rather slow translational jump over a long distance, we find these two findings in full agreement.

We would like to propose the following physical picture of the motion of the water molecule on the surface of the lens protein. The polar groups of the protein form a network of attractive positions on the surface of the protein for the water molecules. The diffusion of the water molecule along the surface occurs as a sequence of jumps from one of these positions to the next. As mentioned above, the averaged distance of a single jump is about 1.5 nm. This distance is approximately the distance from one turn of the protein helix to the next. One can therefore speculate that the jumps of the water molecule occur in steps from one turn to the next.

In summary, we have presented results of ^{1}H NMR spin lattice relaxation time measurements on whole lenses and fragments of lenses at different temperatures and B_0 fields. We showed that the results of these experiments can be consistently interpreted using the model of a combination of rotational and translational diffusion processes of water molecules. Moreover, we have corroborated results from other authors who reported that the correlation time of the rotational diffusion is practically the same as the value found in free water, but that the correlation time for translational diffusion is several orders of magnitude longer. Furthermore we were able to show that the average jump distance of the water molecules in the hydration shell is about 1.5 nm, which is rather large, and can be interpreted as a anisotropic random walk on the surface of the lens proteins. We have therefore found additional experimental evidence of anisotropic diffusion of water molecules on the rugged surface of the proteins.

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