

Temperature Dependences of T_1 and T_2 of Residual Water in D_2O Determined at 400 MHz 1H -NMR

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Abstract: T_1 and T_2 relaxation times in D_2O were investigated at various temperatures with 400 MHz NMR. It is well known that D_2O is a solvent for investigation of chemical molecules with NMR. Likewise, many biological molecules, particularly proteins and enzymes are studied in D_2O . T_1 was measured at different temperatures by Inversion Recovery Method, while T_2 was measured for the same temperatures by CPMG Method. T_1 relaxation times were found longer than T_2 relaxation times. The rotational correlation time and activation energy values were found as 0.71 ps, 3.93 kcal.mol⁻¹ for T_1 and 2 ns, 3.38 kcal.mol⁻¹ for T_2 , respectively. In conclusion, chemical exchange between protons and deuterons and dipolar coupling may be dominant process for the transverse relaxation mechanism, whereas tumbling reorientation process may dominates the longitudinal relaxation in D_2O . This suggests that, with respect to reorientation dynamics, two distinct molecular species exist in liquid water. In addition, our results are coherent with low frequency studies of D_2O .

Keywords: 1H -NMR, D_2O , T_1 and T_2 relaxation times

1. Introduction

Longitudinal or spin-lattice relaxation is correlated with the overall rotational tumbling of the molecule in solution, because efficient spin-lattice energy transfer requires that the frequencies of rotational tumbling match those of the nuclear spin transitions. The spin-lattice relaxation time (T_1) may be further affected by intramolecular mobility in flexible substructures. Transverse relaxation or spin-spin relaxation accounts for the rapid dephasing of precessing xy-magnetization within the xy-plane at a rate $1/T_2$ by dynamic processes in the molecule. The spin-spin relaxation time (T_2) is shorter than or equal to T_1 in liquid solutions. The principal source of fluctuating magnetic fields in most molecules is molecular motion. We can define a rotational correlation time (τ_c) for a molecule. This is the average time which takes the molecule to rotate through one radian. In biological macromolecules with the rotational correlation times in the nanosecond range, T_2 is considerably shorter than T_1 . The region of small rotational correlation times, where the curves of T_1 and T_2 overlap, is referred to as the extreme narrowing limit ($\omega^2\tau_c^2 \ll 1$) [1-8]. There are several mechanisms by which molecular motions can influence nuclear relaxation; direct interactions with nearby magnetic nuclei (dipole-dipole), chemical shift effects (CSA), quadrupole-electric field gradient interaction (QR) and rapid modulation of J -coupling (SC). In addition to molecular motion, rotational transitions can also be the source of fluctuating magnetic fields (SR).

The dynamics of water has been widely studied using NMR relaxation techniques at low frequencies. Such studies use proton resonance, which has a major drawback [1-5]. At the same time, many biological molecules, particularly proteins and enzymes are studied in D_2O to reduce the water in the environment and to increase the effect of bound water to the protein [9-18].

Residual water in D_2O is in the form of HDO [3,4,19-23]. Therefore, investigation of HDO relaxation is valuable to

understand the relaxation mechanism in the protein-enzyme solutions.

If the proton relaxation of residual water is known, input parameters of chemical change-exchange formula can be calculated easily and so that contribute to investigate the protein and enzyme relaxations.

In this study, we have used proton NMR relaxation to examine residual water in D_2O which is a solvent to investigate chemical molecules with NMR. The spin-lattice (T_1) and the spin-spin relaxation (T_2) times of residual water in D_2O were investigated as a function of temperature by a Avance Bruker 400 MHz 1H -NMR Spectrometer, and also activation energies (E_a) and rotational correlation times (τ_c) have been calculated for T_1 and T_2 .

2. Theory

In D_2O solutions, the detected residual protons are found primarily in HDO molecules. The intramolecular dipolar interactions between H and D are hetero-nuclear and given by Solomon-Bloembergen [7,8].

$$\frac{1}{T_1} = \frac{1}{10} \frac{\gamma_D^2 \gamma_H^2 \hbar^2}{r^6} \left[\frac{\tau_c}{1 + (\omega_D - \omega_H)^2 \tau_c^2} + \frac{3\tau_c}{1 + \omega_D^2 \tau_c^2} + \frac{6\tau_c}{1 + (\omega_D + \omega_H)^2 \tau_c^2} \right] \quad (1)$$

$$\frac{1}{T_2} = \frac{1}{20} \frac{\gamma_D^2 \gamma_H^2 \hbar^2}{r^6} \left[4\tau_c + \frac{\tau_c}{1 + (\omega_D - \omega_H)^2 \tau_c^2} + \frac{3\tau_c}{1 + \omega_D^2 \tau_c^2} + \frac{6\tau_c}{1 + \omega_H^2 \tau_c^2} + \frac{6\tau_c}{1 + (\omega_D + \omega_H)^2 \tau_c^2} \right] \quad (2)$$

where γ_H is the proton magnetogyric ratio, γ_D is the deuteron magnetogyric ratio, \hbar is Planck's constant divided by 2π , r is the distance between the two spins, τ_c is rotational correlation time, ω_H and ω_D are proton and deuteron Larmor frequencies, respectively.

In the extreme narrow case ($\omega^2 \tau_c^2 \ll 1$), as expected, $T_1 = T_2$ so the equations 1 and 2 is reduced to the equation 3 [7,8].

$$\frac{1}{T_1} = \frac{1}{T_2} = \left(\frac{\gamma_D^2 \gamma_H^2 \hbar^2}{r^6} \right) \tau_c \quad (3)$$

To study the temperature dependence of T_1 and T_2 , the rotational correlation time τ_c is regarded to have Arrhenius behavior;

$$\tau_c = \tau_0 \exp(E_a/RT) \quad (4)$$

where E_a is the activation energy of the molecular motions, R is the gas constant, T is the absolute temperature, τ_c is rotational correlation time, and τ_0 is the rotational correlation time constant.

3. Experimental

D₂O (99.9%) used in this study was of the highest purity purchased from Sigma. 1 ml of D₂O solution was placed 5 mm diameter NMR tube, which is degassed three times by freeze-thaw method and sealed for measurements. The experiments were carried out in a Avance Bruker spectrometer operating at 400.132 MHz. T_1 spin-lattice relaxation times were measured at different temperatures (22, 27, 32, 37, 42, 47 °C) by using inversion recovery $[180^\circ-\tau-90^\circ]$ pulse sequence. Sample temperature was increased by steps of 5 °C in each measurement by using variable temperature control unit. Inversion delays were taken from 1 s to 30 s and pulse repetition time was chosen 85 s for T_1 measurements. T_2 spin-spin relaxation times were measured using the CPMG pulse sequence $[90^\circ-\tau-(180^\circ-2\tau)_n]$ for the same temperatures. Inversion delays were taken from 0.01 s to 4 s and pulse repetition time was chosen 5 s for T_2 measurements. The experimental error, based on repeated measurements, was 3% for T_1 and 5% for T_2 . Proton NMR spectrum of HDO, which was found to be a

single narrow line, used for relaxation time measurements was given in Fig. 1.

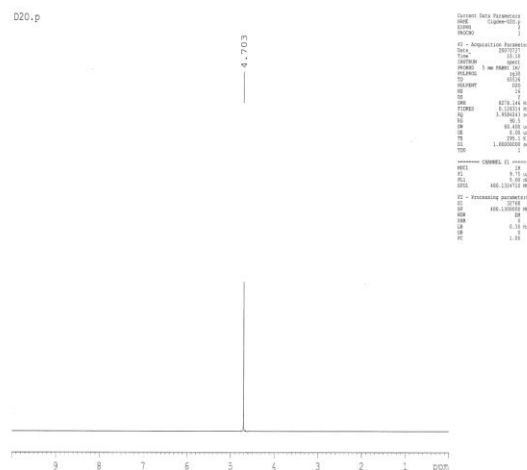


Figure 1. Spectrum of residual water at 22 °C in D₂O.

4. Results and Discussion

Representative IR- T_1 and SE- T_2 curves are given in Fig. 2, and Fig. 3, respectively.

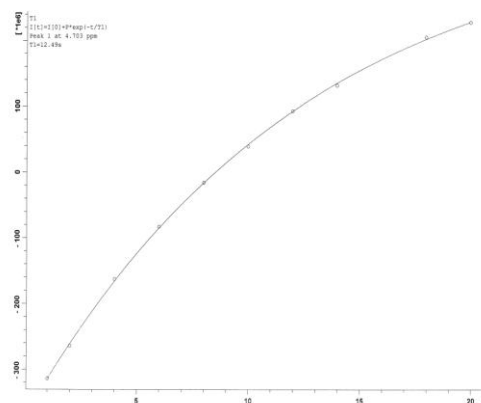


Figure 2. IR- T_1 curves of HDO at 22 °C.

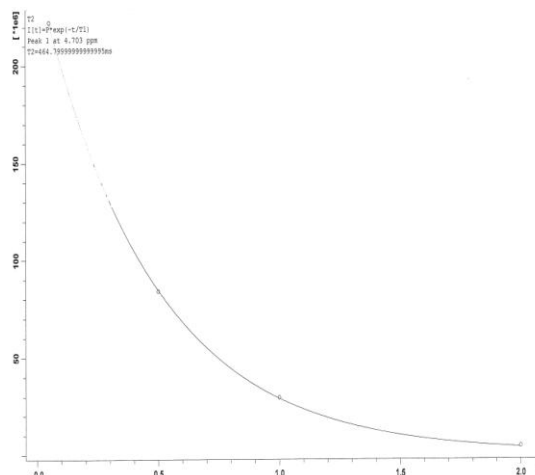


Figure 3. SE- T_2 curves of HDO at 22 °C.

All of the obtained IR- T_1 curves exhibit pure exponential behavior which demonstrates that there is only one type of T_1 relaxation for solution. SE- T_2 curves also

exhibit exponential decay, so that T_2 has one type relaxation. FID spectrums support this idea. T_1 and T_2 relaxation time values of HDO peak measured at different temperatures are given in Table 1.

Table 1: T_1 and T_2 relaxation times of HDO peak in Fig.1

T (K)	T_1 (s)	T_2 (ms)
320	21.66	734
315	19.44	672
310	17.41	605
305	14.63	563
300	13.45	515
295	12.49	465

T_1 and T_2 values are increased depending on the temperature as shown in table 1. Also it is seen that for deuteron $T_1 \gg T_2$ was observed even for $\omega\tau_c \ll 1$ (2). The fact that $T_1 \gg T_2$ clearly indicates that the water molecule dynamics are complicated and that water molecule motions with $\omega\tau_c > 1$ are probable also present in this system [2,6].

In order to find the E_a activation energies and τ_c rotational correlation times, the temperature dependence of the $\ln T_1$ and $\ln T_2$ values in aqueous solutions was plotted against the reciprocal temperature (Fig. 4).

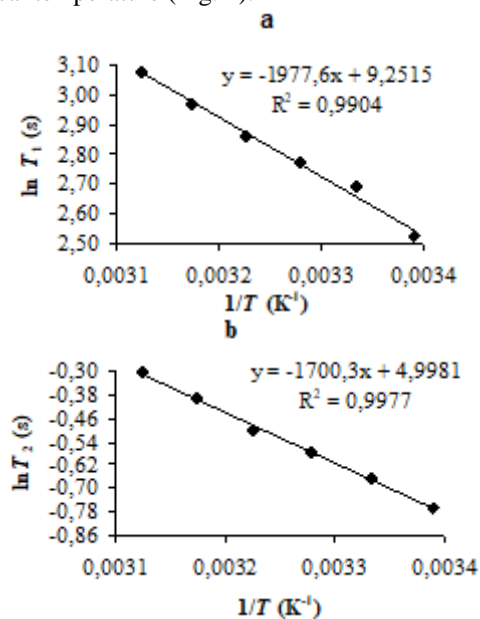


Figure 4: (a) $\ln T_1$ and (b) $\ln T_2$ vs. reciprocal temperature ($1/T$) for HDO peak measured at 400 MHz.

Both of the T_1 and T_2 decreased linearly with increasing $1/T$. Therefore, T_1 and T_2 in the aqueous solution are assumed to be under the high mobility. Then, T_1 and T_2 are proportional to the reciprocal of the rotational correlation time, τ_c , for the molecular motion. T_1 decreases with decreasing temperature. This would suggest that the relaxation is on the high temperature side of the T_1 minimum and that the correlation time τ_c characterizing the reorientation of the nucleus satisfies $\omega\tau_c \ll 1$. This condition must be applicable to some of the water molecules, or at least to a component of their dynamics [5,24].

The activation energies (E_a) were found from the slopes of the curves in fig. 4. The rotational correlation times (τ_0, τ_c) were calculated using Eqs. 3 and 4. The E_a, τ_0 and τ_c values for HDO peak are given in Table 2.

Table 2: E_a, τ_0 , and τ_c values of HDO peak at 22 °C

	T_1	T_2
E_a (kcal.mol ⁻¹)	3.93	3.38
τ_0 (s)	8.73×10^{-14}	6.23×10^{-12}
τ_c (s)	7.12×10^{-11}	2.00×10^{-9}

The relaxation rate of HDO is found shorter than the relaxation rate of H₂O. One reason may be greater strength of H-bonds which would tend to increase the interaction between H-D protons in D₂O than in H₂O. Another reason may be the greater strength of dissolved oxygen in D₂O than in H₂O.

5. Conclusion

The rotational correlation time and activation energy values were found as 0.71 ps, 3.93 kcal.mol⁻¹ for T_1 and 2 ns, 3.38 kcal.mol⁻¹ for T_2 , respectively. This small value of τ_c for T_1 confirms that molecular motion is very fast, and provides extreme narrowing condition. The value of τ_c for T_2 satisfies $\omega\tau_c > 1$. This can be attributed to two reasons. i. intermolecular proton exchange that has the effect of shortening the correlation time. ii. Spin-spin coupling which causes T_2 to become shorter than T_1 [2, 4, 6, 22-28]. Activation energy values, which suggest the isotropic rotational motion of unbonded water molecules, are coherent with the rotational activation energy of water given in literature (1-3, 29). In conclusion, chemical exchange between protons and deuterons and dipolar coupling may be dominant process for the transverse relaxation mechanism, whereas tumbling reorientation process may dominates the longitudinal relaxation in D₂O. This suggests that, with respect to reorientation dynamics, two distinct molecular species exist in liquid water [17, 27-33]. In addition, our results are coherent with low frequency studies. Therefore, it appears that there is no any difference between low frequency and high frequency studies of D₂O.

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