ON THE STATE OF WATER IN AQUEOUS SOLUTIONS OF PROTEINS: A MODEL OF CONTINUOUS DISTRIBUTION OF CORRELATION TIMES

By M. Rydzy

Institute of Physics, Jagellonian University, Cracow*

(Received May 19, 1980)

It is shown that the concept of a broad distribution of correlation times is useful in interpreting NMR relaxation data in aqueous solutions of globular proteins. The model of the log-normal distribution of correlation times, when modified by taking into account the overall tumbling motion of protein molecules, gives satisfactory agreement between the theoretical and experimental dependence of the spin-lattice relaxation rate as a function of Larmor frequency.

PACS numbers: 87.90.+y, 76.60.Es

1. Introduction

The properties of water both in living systems and model systems (macromolecular solutions and gels) have been frequently investigated, particularly by means of pulsed nuclear magnetic resonance (NMR) relaxation measurements [1–20]. One of the most promising possibilities of NMR, namely the measurements of the Larmor frequency dependence of the spin-lattice relaxation rate $1/T_1$ have been used to investigate the protein-water interaction, but the picture emerging from this does not allow a unique interpretation of the structure and dynamics of the water molecules [5–15]. Several quantitative theoretical approaches to the problem have been proposed. On one hand two phases of rapidly exchanging water molecules were assumed, each phase characterized by a single correlation time for molecular motion, but the theory failed to account for the Larmor frequency dependence of the relaxation rates [1, 2, 5]. That theory was modified by assuming that three groups of water molecules — tightly bounded, partially slowed down by interaction with protein and free water — exist in the system [3, 13]. On the other hand a model of continuous distribution of phases giving rise to the log-normal distribution of correlation times was proposed by Blicharska et al. [7, 31], in order to account for the experimental

* Address: Instytut Fizyki, Uniwersytet Jagielloński, Reymonta 4, 30-059 Kraków, Poland.

findings. As it was pointed out by the authors, such a model should be considered as a rough approximation because the results suggest that a substantial part of the water molecules should have their correlation times longer than the rotational correlation time of a protein molecule [7]. Moreover, these slow molecules contribute very strongly to the computed relaxation rate. Recently, a long-range hydrodynamic effect has been proposed as a dominant mechanism responsible for the frequency dependence of $1/T_1$, but no quantitative theory of the effect has been presented as yet [11, 12].

The present paper suggests a modification of the log-normal distribution of correlation times which removes the previously mentioned contradictions of the model. Predictions of the theory are compared with the data presented in the literature for the Larmor frequency dependence of the solvent protons spin-lattice relaxation time T_1 in aqueous solutions of apotransferrin [5].

2. Theory

2.1. General theory

When protons which contribute to the observed T_1 exchange rapidly between two distinct regions, the bulk water and that associated with the protein, then [1, 21]

$$T_1^{-1} = f_w/T_{1w} + f_p/T_{1p}, \tag{1}$$

where f_p and f_w are the molar fractions of protons associated with the protein and in the bulk water, respectively. T_{1p} is the effective proton spin-lattice relaxation time in the first of these regions. T_{1w} is the relaxation time in the bulk water and is independent of the Larmor frequency [9]. In order to obtain an explicit theoretical expression for T_{1p} it is assumed that the associated water can be characterized by a continuous distribution of the correlation times caused by the distribution of phases. It is also assumed that the intramolecular dipolar interaction is the main mechanism of proton magnetic relaxation [34].

2.2. Equations for a continuous distribution in correlation times

According to the Odajima theory [22] for systems characterized by a continuous distribution of correlation times, the nuclear magnetic relaxation times T_1 and T_2 fulfil the following equations:

$$T_1^{-1}(\omega) = \tfrac{2}{3}\sigma_0^2 \int_0^\infty P(\tau)K(\omega, \tau)d\tau, \tag{2a}$$

$$T_2^{-1}(\omega) = \tfrac{1}{3}\sigma_0^2 \int_0^\infty P(\tau)\,[3\tau + L(\omega, \tau)]d\tau, \tag{2b}$$

where T_1 and T_2 are, respectively, the spin-lattice and spin-spin relaxation times, σ_0^2 is the effective rigid-lattice second moment of the system and it can be expressed as a multiple, n, of the intramolecular Van Vleck second moment σ_{0m}^2 [23, 24]. $K(\omega, \tau)$ and $L(\omega, \tau)$ are

the characteristic functions of the correlation time τ and the resonant frequency ω from BPP theory [25, 34]

$$K(\omega, \tau) = \frac{\tau}{1+(\omega\tau)^2} + \frac{4\tau}{1+(2\omega\tau)^2}, \tag{3a}$$

$$L(\omega, \tau) = \frac{5\tau}{1+(\omega, \tau)^2} + \frac{2\tau}{1+(2\omega\tau)^2}. \tag{3b}$$

In order to apply the model sketched above, a from of the distribution function for correlation times $P(\tau)$ must be assumed. As it was shown by Resing [24], the log-normal form of the distribution function is compatible with the commonly accepted models for molecular motion in solids and liquids [26, 27]. The normalized distribution function $P(\tau)$ is given by [28]

$$P(\tau)d\tau = (\beta\sqrt{\pi})^{-1} \exp\left(-\frac{z^2}{\beta^2}\right) dz, \tag{4}$$

where

$$z = \log(\tau/\tau^*), \tag{5}$$

τ^* is the centre and β is a parameter describing the width of the distribution. The standard deviation in z, σ_z, is

$$\sigma_z = \beta/\sqrt{\pi}. \tag{6}$$

It is reasonable to assume that the water molecules adsorbed onto proteins have the log-normal distribution of jump times [7, 16, 29, 30]. However, in the case of protein--water solutions the overall tumbling motion of the macromolecules must be taken into account. Assuming that the diffusion of water on the protein molecules' surface and the overall browinian motion of the macromolecules are independent, the correlation time τ of the water molecules in the hydration shell is given by

$$\tau^{-1} = \tau_d^{-1} + \tau_r^{-1}, \tag{7}$$

where τ_r is the correlation time of the brownian tumbling motion of the protein molecules and τ_d is the correlation time of the water diffusion on the surface of the protein. (An assumption was made that protein molecules can be characterized by a single rotational correlation time, which is a good aproximation only for globular proteins.)

Combining (2a, b) with (7) one can obtain the following expressions for the spin--lattice (T_1) and sin-spin (T_2) relaxation times

$$T_1^{-1}(\omega) = \tfrac{2}{3}\sigma_0^2 \int_0^\infty P(\tau_d)K(\omega, \tau)d\tau_d \tag{8a}$$

$$T_2^{-1}(\omega) = \tfrac{1}{3}\sigma_0^2 \int_0^\infty P(\tau_d)[3\tau + L(\omega, \tau)]d\tau_d, \tag{8b}$$

where $K(\omega, \tau)$ and $L(\omega, \tau)$ are given by (3a, b), and τ is a function of the rotational correlation time τ_r of macromolecules and the diffusion correlation time τ_d, given by (7). The distribution of τ is caused by the log-normal distribution of τ_d, described by (4).

3. *Application to experimental data*

Koenig and Schillinger [5] measured the magnetic field dependence of the nuclear spin-lattice relaxation rate T_1^{-1} of solvent protons in the solution of the diamagnetic protein apotransferrin as a function of temperature, pH and protein concentration. The experimental findings were qualitatively interpreted in terms of the two-state model [1], though the theory failed to reproduce closely the experimental variation of T_1^{-1} as a function of the Larmor frequency.

The experimental T_1^{-1} relaxation dispersion curves were analyzed in terms of the theory presented in the preceding sections. Fig. 1 shows a typical example of the NMR relaxation dispersion of solvent protons for an apotransferrin solution in distilled water (from Koenig and Schillinger, [5]) and the theoretical curve predicted by the theory.

The parameters of the fit were obtained by minimizing the sum of squares of differences between the experimental and theoretical values of T_1^{-1}. Values of $\beta = 5.80$, $\tau^* = 3.72$

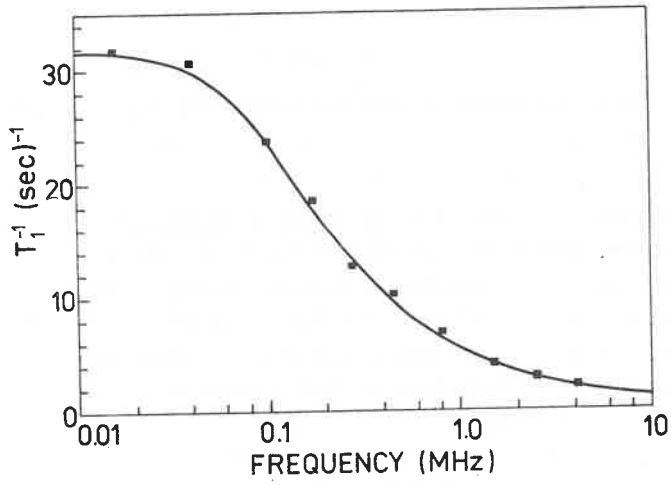

Fig. 1. The Larmor frequency dependence of the solvent protons spinlattice relaxation rate T_1^{-1} in the aqueous solution of apotransferrin; ■ experimental points obtained by Koenig and Schillinger [5]; ——— theoretical fit to these data obtained by assuming the modified log-normal distribution of the correlation times (see text)

$\times 10^{-10}$ sec and $\tau_r = 9.67 \times 10^{-7}$ sec resulted from this analysis for a 20% (w/w) solution of apotransferrin in distilled water, pH = 4.9, at 25°C. The value of $\beta = 5.80$ is similar to that obtained by Lynch et al. [29] for water adsorbed by albino hair keratin and equal to 5.36. Also the value of $\tau_r = 9.67 \times 10^{-7}$ sec is in reasonable agreement with the value of the rotational correlation time of apotransferrin obtained assuming a spherical shape of the protein molecules and using Stokes' law. The longer value of τ_r in comparison with $\tau_r = 2.0 \times 10^{-7}$ sec [5] resulting from Stokes' law could be accounted for by a hydrodynamical interaction between adjacent protein molecules [15].

Comparison of the experimental values of T_1^{-1} with those predicted by the theory allows one to estimate the amount of water associated with the protein. From equations

(1) and (8a), assuming the value of $\sigma_0^2 = 2.54 \times 10^{10}$ sec^2 [29–32] one can obtain the fraction of associated water $f_p = 9.65 \times 10^{-3}$. Taking into account the molecular weight of apotransferrin (equal to 82,000 [33]) and the protein concentration in the solution (20% (w/w)) this value of f_p corresponds to $n \sim 170$ water molecules per protein molecule, or $\sim 26\%$ of the number of water molecules expected to be in the first hydration shell [5]. The corresponding values which resulted from the two-state model were: $f_p = 4 \times 10^{-4}$ and $n \sim 13$ [5], and were difficult to interprete.

REFERENCES

[1] O. K. Daszkiewicz, J. W. Hennel, B. Lubas, T. W. Szczepkowski, *Nature* **200**, 1006 (1963).
[2] K. Caputa, O. K. Daszkiewicz, J. W. Hennel, B. Lubas, T. W. Szczepkowski, in: Proc. XIII Coll. Ampere, North Holland Publ., Amsterdam 1965.
[3] K. Caputa, J. W. Hennel, T. W. Szczepkowski, in: Proc. XIV Coll. Ampere, North Holland Publ., Amsterdam 1967.
[4] G. J. Krüger, G. A. Helcke, in: Proc. XIV Coll. Ampere, North Holland Publ., Amsterdam 1967.
[5] S. H. Koenig, W. E. Schillinger, *J. Biol. Chem.* **244**, 3283 (1969).
[6] S. H. Koenig, W. E. Schillinger, *J. Biol. Chem.* **244**, 6520 (1969).
[7] B. Blicharska, Z. Florkowski, J. W. Hennel, G. Held, F. Noack, *Biochim. Biophys. Acta* **207**, 381 (1970).
[8] M. E. Fabry, S. H. Koenig, W. E. Schillinger, *J. Biol. Chem.* **245**, 4256 (1970).
[9] F. Noack, in: *NMR, Basic Principles and Progress*, Vol. 3, Ed. by P. Diehl, E. Fluck, R. Kosfeld, Springer-Verlag, Berlin 1971.
[10] T. R. Lindstrom, S. H. Koenig, *J. Magn. Resonance* **15**, 344 (1974).
[11] S. H. Koenig, K. Hallenga, M. Shporer, *Proc. Natl. Acad. Sci. USA* **72**, 2667 (1975).
[12] K. Hallenga, S. H. Koenig, *Biochemistry* **15**, 4255 (1976).
[13] L. Grösch, F. Noack, *Biochim. Biophys. Acta* **453**, 218 (1976).
[14] S. H. Koenig, R. G. Bryant, K. Hallenga, G. S. Jacob, *Biochemistry* **17**, 4348 (1978).
[15] S. H. Koenig, to be published in the ACS Symposium Series.
[16] J. Clifford, B. Sheard, *Biopolymers* **4**, 1057 (1966).
[17] D. E. Woessner, B. S. Snowden, Jr., *J. Colloid Interf. Sci.* **34**, 290 (1970).
[18] R. K. Outhred, E. P. George, *Biophys. J.* **13**, 83 (1973).
[19] B. M. Fung, J. Witschel, jr., L. L. McAmis, *Biopolymers* **13**, 1767 (1974).
[20] I. D. Duff, W. Derbyshire, *J. Magn. Resonance* **17**, 1 (1974).
[21] J. R. Zimmerman, W. E. Brittin, *J. Phys. Chem.* **61**, 1328 (1957).
[22] A. Odajima, *Progr. Theor. Phys.* (Kyoto) Suppl. **10**, 142 (1959).
[23] J. H. Van Vleck, *Phys. Rev.* **74**, 1168 (1948).
[24] H. A. Resing, *J. Chem. Phys.* **43**, 669 (1965).
[25] N. Blombergen, E. M. Purcell, R. V. Pound, *Phys. Rev.* **73**, 679 (1948).
[26] M. H. Cohen, D. Turnbull, *J. Chem. Phys.* **31**, 1164 (1959).
[27] C. Wert, C. Zener, *Phys. Rev.* **76**, 1169 (1949).
[28] K. W. Wagner, *Ann. Physik* **40**, 817 (1913).
[29] L. J. Lynch, K. H. Marsden, E. P. George, *J. Chem. Phys.* **51**, 5673 (1969).
[30] L. J. Lynch, K. H. Marsden, *J. Chem. Phys.* **51**, 5681 (1969).
[31] B. Blicharska, J. S. Blicharski, *Acta Phys. Pol.* **A41**, 347 (1972).
[32] K. Kume, *J. Phys. Soc. Japan* **15**, 1493 (1960).
[33] A. J. Leibman, P. Aisen, *Arch. Biochem. Biophys.* **121**, 717 (1967).
[34] A. Abragam, *The Principles of Nuclear Magnetism*, Oxford University, London 1961.